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Nematode–coccidia parasite co-infections in African buffalo: Epidemiology and associations with host condition and pregnancy

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ABSTRACT

Co-infections are common in natural populations and interactions among co-infecting parasites can significantly alter the transmission and host fitness costs of infection. Because both exposure and susceptibility vary over time, predicting the consequences of parasite interactions on host fitness and disease dynamics may require detailed information on their effects across different environmental (season) and host demographic (age, sex) conditions. This study examines five years of seasonal health and co-infection patterns in African buffalo (*Syncerus caffer*). We use data on two groups of gastrointestinal parasites, coccidia and nematodes, to test the hypothesis that co-infection and season interact to influence (1) parasite prevalence and intensity and (2) three proxies for host fitness: host pregnancy, host body condition, and parasite aggregation. Our results suggest that season-dependent interactions between nematodes and coccidia affect the distribution of infections. Coccidia prevalence, coccidia intensity and nematode prevalence were sensitive to factors that influence host immunity and exposure (age, sex, and season) but nematode intensity was most strongly predicted by co-infection with coccidia and its interaction with season. The influence of co-infection on host body condition and parasite aggregation occurred in season-dependent manner. Co-infected buffalo in the early wet season were in worse condition, had a less aggregated distribution of nematode parasites, and lower nematode infection intensity than buffalo infected with nematodes alone. We did not detect an effect of infection or co-infection on host pregnancy. These results suggest that demographic and seasonal variation may mediate the effects of parasites, and their interactions, on the distribution and fitness costs of infection.

1. Introduction

In free ranging populations, hosts are commonly co-infected with more than one parasite species. Co-infecting parasites may interact, and mounting evidence suggests that these interactions are critical to understanding the dynamics of host and parasite communities in the wild (Telfer et al., 2008; Ezenwa and Jolles, 2011). Co-infecting parasites have been shown to alter infection risk (Telfer et al., 2010), infection intensity (Pedersen and Antonovics, 2013), and the fitness consequences of infection (Graham et al., 2005). For example, competition between co-infecting parasites may result in decreased infection severity (Dobson and Barnes, 1995); alternatively, suppression of the host immune response by one parasite may increase the likelihood or severity of infection with another parasite (Su et al., 2005; Bandilla et al., 2006). These studies highlight the importance of parasite interactions, yet our ability to predict the dynamics and host fitness consequences of interacting parasites in the wild remains limited.

Gastrointestinal (GI) parasites are ubiquitous, taxonomically diverse, and cause mortality, or declines in condition and/or reproduction in a variety of livestock (Larsson et al., 2006; Larsson et al., 2011; Thumbi et al., 2013) and wildlife systems (Gulland, 1992; Stien et al., 2002; Pedersen and Greives, 2008). GI parasites can affect their hosts by directly consuming host resources or indirectly by damaging intestinal function (Stewart and Penzhorn, 2004), altering host behavior (Adelman and Martin, 2009), or disrupting the control of co-infecting parasites (Jolles et al., 2008). The magnitude of these direct and indirect effects, and thus their

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impact on host fitness, is likely to be dependent on the host immune response (e.g. Adelman et al., 2013). Seasonal resource availability is directly related to the host immune response (Demas, 2004; Altizer et al., 2006) and mediates the intensity of infection (Ezenwa, 2004) and host survival (Pedersen and Greives, 2008). In young animals or sub-optimal environmental conditions, hosts may prioritize growth and self-maintenance over immunity (Eraud et al., 2008; Cotter et al., 2011), potentially resulting in seasonal increases in infection severity. Thus, predicting the consequences of parasites and parasite interactions on host fitness (Thumbi et al., 2014) and disease dynamics (Telfer et al., 2010) may require detailed information on the effects of GI parasites on individual condition and fitness across different environmental (season) and host demographic (age, sex) conditions.

Interactions between coccidia and nematode parasites, two common components of GI parasite communities, have been well documented in laboratory systems (Stewart et al., 1980; Rose et al., 1994, reviewed in Cox, 2001). Nematode parasites are a diverse group of macroparasitic (extracellular parasites) worms and coccidia are highly immunogenic protozoan microparasites (intracellular parasites). Coccidia replicate within the epithelial cells of the intestinal mucosa, often resulting in physical damage and activation of the mucosal immune system (Stewart and Penzhorn, 2004). The host immune system likely plays an important role in the interactions between nematodes and coccidia because intracellular and extracellular parasites invoke opposite and cross-regulating immune responses (Morel and Oriss, 1998). As a result, hosts may have difficulty simultaneously mounting a strong response to co-infection by intracellular and extracellular parasites, leading to increased disease severity in co-infected animals (Graham, 2008; Jolles et al., 2008). Parasites are also often indirectly linked through host resources, which could result in either facilitation or competition (Randall et al., 2013). For parasites within the gastrointestinal tract, resource mediated interactions are reported more frequently than immune mediated interactions (Griffiths et al., 2014), although the relative strength and consequences of these interactions requires further investigation. Thus, interactions between coccidia and nematodes are likely because of their shared resources and location within the host gastrointestinal tract and the opposing immune responses they invoke.

This study explores how coccidia–nematode co-infection influences the prevalence, egg/oocyst counts (a proxy for parasite intensity), and fitness costs of infection in African buffalo (*Syncerus caffer*), a host commonly co-infected with both parasites (Penzhorn, 2000). Like most ruminants, buffalo body condition (Ezenwa et al., 2009; Parker et al., 2009) and some immune responses (Beechler et al., 2012) vary seasonally with resource quality. Buffalo body condition mirrors resource quality at a 2–4 month lag (based on fecal nitrogen; Ryan et al., 2012) and thus, buffalo reach peak condition in the late wet season/early dry season and are in poorest condition in the late dry season/early wet season (Caron et al., 2003). Further, both coccidia and nematodes have a fecal-oral transmission route that results in seasonal variation in exposure (Horak et al., 2004; Stewart and Penzhorn, 2004). Exposure, interactions among parasites, and mortality may all influence the distribution of parasites among hosts, or parasite aggregation (Wilson et al., 2002). Because host immune responses and parasite exposure vary over time, we predict that the strength parasite interactions, and thus the fitness costs of co-infection, will be exacerbated during times when animals are in poor condition. If host mortality is dose-dependent, then reduced parasite aggregation may be indicative of mortality in highly infected individuals. We use a 5-year dataset on seasonal health and infection patterns of 1375 African buffalo to establish seasonal patterns of infection and co-infection. We then examine three proxies for host fitness—host pregnancy, host body condition, and parasite aggregation — to evaluate how co-infection and season interact to influence host fitness.

2. Materials and methods

2.1. Study Site and population

African buffalo (*S. caffer*) were sampled at Hluhluwe-iMfolozi Park (HIP), located in KwaZulu-Natal, South Africa (28°14′S, 31°54′–32°03′N), as part of a test-and-cull program aimed at limiting the spread of bovine tuberculosis. HIP is home to approximately 3000 buffalo in fairly stable herds of 70–180 individuals across its 900 km^2 area. Rainfall is seasonal, occurring from September/October to March/April and varies geographically throughout the park (Berkeley and Linklater, 2010). Buffalo were captured annually from 2002 to 2006 between April and October. Different areas of the park were targeted each year, resulting in data from 1375 sampled buffalo. Buffalo were herded using a helicopter into a capture corral within the park and all captured buffalo were marked with brands to allow identification in subsequent captures. Each animal was monitored for parasite infections, age, sex, body condition, and pregnancy status. Because HIP's captures were conducted to control bovine tuberculosis (bTB) spread through the park, bTB positive animals were culled. Branded individuals were released and if recaptured, were not included in this study's analysis to avoid pseudoreplication.

2.2. Coccidia and nematode infection status

We determined coccidia and nematode infection status by examining fecal samples collected rectally while the buffalo were immobilized (Jolles et al., 2008). We performed oocyst and egg counts using a modification of the McMaster Fecal egg counting technique (MAFF, 1980). Fecal oocyst/egg counts were conducted at the KwaZulu-Natal State Veterinary Laboratory (Allerton, South Africa) until 2005 and subsequently performed in our field laboratory in HIP following protocols described previously (Ezenwa, 2003a,b). Counts are expressed as eggs or oocysts/gram feces. The species of coccidia affecting buffalo remain unknown, but work to identify them is currently underway. Studies in South African cattle have found 12 *Eimeria* species, including the highly virulent, *Eimeria bovis* and *Eimeria zuernii* (Matjila and Penzhorn, 2002). Gastrointestinal nematode infections in buffalo at HIP are caused primarily by the genera *Haemonchus* and *Cooperia* (Jolles et al., 2008) although others have also been identified in buffalo in the region (Hoberg et al., 2008; Budischak et al., 2012; Taylor et al., 2013). For this analysis, the fecal egg/oocyst data analyzed are taxonomically coarse, and for our purposes are lumped into all strongle nematode species and coccidia species. We use the counts of nematode eggs and coccidia oocyst as a proxy for parasite intensity (Penzhorn, 2000; Ezenwa and Jolles, 2008). Nematode egg counts reflect adult worm burdens in African buffalo (Budischak et al., in preparation) and both oocyst and egg counts remain valuable noninvasive means of assessing the relative infection levels across hosts (Bryan and Kerr, 1989; Ezenwa, 2003a,b).

2.3. Buffalo age, body condition, and pregnancy

To assess host age, we used a combination of tooth emergence and wear patterns as described in (Jolles, 2007). We estimated age in juveniles without permanent incisors (<2 years) based on body size and horn development. For animals between ages 2–5 years old, we assessed age from incisor emergence patterns (Grimsdell, 1973) and we estimated age with tooth wear of the first incisor for buffalo >6 years old (Jolles, 2007). Body condition was assessed

on a scale of 1–5 by palpation of four main areas where fat is stored on buffalo: ribs, spine, hips, and the base of the tail. Details of this method and validation based on kidney fat have been described previously (Ezenwa et al., 2009). Pregnancy status was determined by rectal palpation of the uterus. To minimize variation during data collection, pregnancy testing was conducted by a veterinarian at Hluhluwe-iMfolozi Park and body condition assessments were conducted according to a standard protocol (see Ezenwa et al., 2009 for details).

2.4. Statistical analysis

We examine coccidia and nematode infections using two important and related indices of infection: parasite prevalence (number positive/total sampled) and the counts of eggs or oocysts/gram feces in infected animals as a proxy for parasite intensity. To determine the main predictors of both parasite infections, we used generalized linear models and performed model selection on four dependent variables: (1) nematode egg counts, (2) coccidia oocyst counts, (3) nematode prevalence, and (4) coccidia prevalence. All model selection considered the effects of age, sex, season, and co-infecting parasite egg or oocyst counts. As an independent variable, co-infecting parasite burdens were modeled as log (fecal egg counts +1) for nematodes and log (oocyts counts +1) for coccidia. Host age was included in the models as a categorical variable (calf, juvenile, sub-adult, adult, and senescent, represented as 0, 1–2, 3–4, 5–13, 14+) because this provided a better fit than representing age as a linear predictor. Sampling year was included as a categorical covariate in all models to account for annual variation in capture areas and environmental conditions. Based on the biology of the region and the structure of the data, season was included in the models as a binomial variable with two options: the late dry/early wet season (September–October) and the late wet season/early dry season (April–June). These categories encompass the transitional times between the wet and dry season. In the late dry/early wet season, buffalo are in poor condition but forage quality is improving from early wet season rainfall while in the late wet/ early dry season buffalo are in better condition, but forage availability is diminishing. For brevity, we refer to these seasons as the early and late wet season, respectively.

We analyzed parasite prevalence using generalized linear models with binomial error structure and a logit link function. Because gastrointestinal parasites are commonly aggregated across hosts, we tested the fit of the binomial models, which assumes the variance is associated with the mean (Wilson and Grenfell, 1997). Parasite egg and oocyst counts were analyzed using generalized linear models with quasipoisson error structure and a log link function. Quassipoisson error structures were chosen over the more traditional method of log transforming egg/oocyst counts and assuming normality because it is more robust to highly overdispersed data, such as count data (Hoef and Boveng, 2007). All two-way interactions among host age, host sex, season, and co-infection were considered. Nematode parasites play an important role in the dynamics of bovine tuberculosis (Jolles et al., 2008; Ezenwa et al., 2010). Although the focus of this work is on nematode–coccidia co-infections, we used model selection to ensure our results were robust to the effects of tuberculosis. Model selection for models predicting parasite prevalence was based on the AIC values of all potential models and model selection for models predicting parasite counts was based on qAIC (Anderson et al., 1994; Richards, 2008). We report the top six models for each dependent variable and because models within 2 AIC values of the model with the lowest AIC value provide similar fit to the data (Burnham and Anderson, 2002), we report the most parsimonious model within 2 of the minimum AIC value (Table S1).

To test the hypothesis that infection and co-infection will affect host fitness, we used generalized linear models of host condition and pregnancy. Host body condition was modeled using a generalized linear model assuming normally distributed errors and host pregnancy was modeled with a binomial error structure and a logit link function. We tested their associations with coccidia oocyst counts, nematode egg counts, and season after accounting for age, sex, and sampling year. Model selection was conducted as described above based on AIC values (Table S1). Because we were interested in the costs of co-infection in different seasons, we considered all potential 2-way interactions and tested if model fit was significantly improved with a three-way interaction between season, coccidia burdens and nematode burdens with a drop-in-deviance test. To ensure that the results were robust to uncertainties inherent in using egg and oocyst counts as a proxy for adult parasite intensity, we then refit the condition and pregnancy models with infection represented categorically as parasite presence or absence (Table S2). We also account for seasonal variation in our ability to detect host pregnancy by including season in our statistical models. This is because the sensitivity of palpation is limited in the first month after conception (Karen et al., 2011). January–March are the peak months of birthing (Ryan et al., 2007) and conceptions are likely occurring throughout the late wet season (Jolles, 2004), which could result in more in false-negative pregnancy test results during the late wet season.

Aggregation was assessed based on the corrected moment estimate, k (Wilson et al., 2002). The corrected moment estimate is calculated with the mean (m) and variance (s^2) of the parasite distribution, $k = (m^2 - s^2/n)/(s^2 - m)$ and estimates the shape parameter of the negative binomial distribution (Elliot, 1977). This estimate was chosen over other estimates (Wilson et al., 2002) because it partially corrects for differences in sample size and is more robust to variation in mean burdens compared to variance-to-mean ratios (Gregory and Woolhouse, 1993). However, with any estimate of aggregation, caution must be used when comparing aggregation among groups (Gregory and Woolhouse, 1993). We, therefore, interpret the results of this analysis in concert with patterns of mean parasite burdens and present variance-to-mean ratios to show that the results are robust to the metric of aggregation used.

Less aggregated distributions of parasites are associated with higher estimated values of k and tend to be truncated compared to highly aggregated distributions (i.e. they include fewer hosts with very heavy parasite burdens). A drop in parasite aggregation in a host population can thus be indicative of mortality of highly infected individuals (Wilson et al., 2002; Jolles et al., 2008). We compare aggregation among groups of buffalo to test for infection-related mortality among groups of buffalo, assuming that increased mortality with season or co-infection will result in a less aggregated (higher k) or more even distribution of parasites. We compared nematode aggregation among coccidia positive and negative buffalo in both seasons. Because we were concerned that high egg/oocyst counts of both infections in calves could be responsible for this pattern, we repeated the analysis on calf and non-calf buffalo. We used bootstrap tests to evaluate the observed differences in aggregation (k) among groups. For example, when testing how nematode aggregation differs in singly infected vs. co-infected buffalo, we randomized coccidia infection status among all observed worm burdens, and then compared the observed difference in worm aggregation to the distribution of differences from 10,000 bootstrap runs to assess the likelihood of observing our results if there was no difference in worm aggregation between coccidia-positive and coccidia-negative buffalo. In these analyses, we included only infected animals so our measure of aggregation avoids additional variability due to differences in parasite prevalence (Cattadori et al., 2008). All data analyses were conducted in R version 2.15.2 (R core development team).

3. Results

3.1. Gi parasite prevalence and intensity in African buffalo

Of the 1375 buffalo sampled, the overall coccidia prevalence was 30.8% and coccidia oocyst counts ranged from 11 to 65,600 oocysts/gram. The nematode prevalence was 70% and nematode egg counts ranged from 10 to 9700 eggs/g. Infection prevalence and egg or oocyst counts were associated with age, sex, season, and co-infecting parasites (Table 1).

3.1.1. Age, sex, and seasonal correlates of infection

The prevalence of both nematodes and coccidia was lower in older age categories (Table 1; drop-in-deviance test, nematode: $p < 0.0001$, coccidia: $p < 0.0001$), but coccidia prevalence had a more pronounced reduction with age than nematode prevalence (Fig. 1a). Nematode prevalence decreased from 86% in calves to 63% in senescent buffalo while coccidia prevalence decreased from 64% to 9%. The intensity of both parasites was also higher in calves and juveniles compared to older age classes (Fig. 1b). In models predicting nematode egg counts, the significant interaction between age and season indicates that nematode egg counts were higher in calves in the early wet season (Table 1).

Coccidia and nematode parasites had different association patterns with sex. Nematode prevalence was higher in female buffalo compared to male buffalo, but nematode egg counts did not vary between sexes (Fig. 1c and d). Conversely, coccidia prevalence was not different between sexes but coccidia oocyst counts were higher in male buffalo. The significant interaction between season and sex indicates that sex differences in coccidia oocyst counts were limited to the late wet season (season × sex, $\beta = -1.352$, $p = 0.002$).

Parasite prevalence was highly season-dependent. Nematode prevalence and coccidia prevalence were both higher in the early wet compared to the late wet season (Fig. 1e). The average nematode prevalence after accounting for other predictors was estimated to be 73.75% (95% confidence interval from 65.03% to 80.81%) in the late wet season and 86.12% (95% confidence interval from 78.52% to 91.33%) in the early wet season. The average coccidia prevalence was estimated to be 2.92% (95% confidence interval from 1.47% to 5.73%) in the late wet season and 15.59% (95% confidence interval from 8.24% to 27.53%) in early wet season. Coccidia oocyst counts were also significantly higher in the early wet season (mean oocysts/gram in the early wet = 47.23; late wet = 21.84), but there was no main effect of season on nematode egg counts (Fig. 1f).

3.1.2. Co-infections

Coccidia and nematode parasites were positively associated in analyses of infection prevalence (Table 1). Based on models of nematode prevalence, each log unit increase in coccidia oocyst counts was associated with an estimated 1.194 times higher odds of nematode infection (95% confidence interval from 1.117 to 1.276). This pattern was consistent across sexes, seasons, and age categories (Fig. 2a). Models explaining the odds of coccidia infection showed a similar but context dependent association with nematodes egg counts. Increasing nematode egg counts in young (calf, juvenile, subadult) and old (senescent) but not prime-aged buffalo (adult) were associated with increased odds of coccidia infection (Fig. 2b).

Coccidia and nematode parasites were also positively associated in analyses of egg and oocyst counts (Fig. 3a and b). In models explaining coccidia oocyst counts, there was no main effect of nematode egg counts although there was suggestive evidence for a positive association in calves (calf × coinfection $\beta = 0.434$, $p = 0.059$). In models explaining nematode egg counts, the effect of coccidia oocyst counts was highly significant. In addition to the positive main effect of coccidia oocyst counts, co-infection also mediated associations with age category and season. Irrespective of coccidia infection status, calves always had higher nematode egg counts in the early wet season compared to the late wet season (Fig. 3c). By contrast, in non-calf buffalo, seasonal patterns of nematode egg counts were different in single and co-infected buffalo (Fig. 3d). Nematode egg counts did not differ between seasons in coccidia negative buffalo, but the interaction between season and coccidia oocyst counts shows that in buffalo co-infected with coccidia, nematode egg counts were lower in the early wet season than in the late wet season.

3.2. Gi parasites and host fitness

3.2.1. Associations with host body condition and pregnancy

After accounting for age and yearly variation, neither nematode egg nor coccidia oocyst counts were associated with host

Table 1
Demographic, seasonal, and co-infection associations with parasite prevalence and intensity. Associations are shown between infections and host demographics (age category and sex), season, and co-infecting parasite burdens (coinf). The age categories presented include, calves (0 years), juveniles (1–2 year), subadults (3–4years), adults (5–13years) and senescent buffalo (>14 years). The reference group is singly infected, adult, female buffalo in the late wet season. Arrows represent the direction of significant associations. Parameter values and standard errors are displayed for interactions with p-values $\leqslant 0.05$, non-significant associations are represented with a dash. P-values are not shown for terms that were removed based on AIC/qAIC model selection. All models accounted for sample year. The significance of each parameter was not altered in models accounting for bovine tuberculosis and inclusion of tuberculosis infection status did not improve model fit (drop in deviance tests; p-value = 0.157, 0.766, 0.411, 0.920, for nematode prevalence, coccidia prevalence, nematode intensity, and coccidia intensity).

	Nematode prevalence		Coccidia prevalence		Nematode intensity		Coccidia intensity	
	Estimate (SE)	p-Value	Estimate (SE)	p-Value	Estimate (SE)	p-Value	Estimate (SE)	p-Value
Age (Calf)	↑ 1.041 (0.344)	0.002	↑ 2.622 (0.665)	<0.0001	–	0.673	–	0.759
(Juvenile)	↑ 1.112 (0.171)	<0.0001	↑ 1.014 (0.367)	0.006	↑ 0.396 (0.155)	0.011	↑ 1.139 (0.825)	0.092
(Subadult)	–	0.376	–	0.899	–	0.955	–	0.917
(Senescent)	-	0.326	↓ −3.367 (1.359)	0.013	–	0.616	–	0.819
Season (Early)	↑ 0.791 (0.210)	<0.001	↑ 1.815 (0.213)	<0.0001	–	0.129	↑ 1.102 (0.485)	0.023
Sex (Male)	↓−0.359 (0.135)	<0.001	–	–	–	–	↑ 1.119 (0.263)	<0.0001
Co-infections	↑ 0.177 (0.034)	<0.0001	–	0.157 [a]	↑ 0.110 (0.022)	<0.0001 [b]	–	0.501 [c]
Interaction terms	-	-	Age × coinf		Age × season Season × coinf		Age × coinf season × sex	

[a] Age × coinf: Coccidia prevalence was positively associated with nematode burdens in juvenile, subadult, and senescent buffalo, but only suggestively associated in adults ($p = 0.0157$).

[b] Age × season and season × coinfeciton: This pattern is explained in detail in Fig. 3c and d. Briefly, calves had higher nematode egg counts in the early wet season but season was not significant in other age groups when buffalo were infected with nematodes alone (calf × season, $\beta = 1.595$, $p < 0.0001$). Adults co-infected with coccidia had increased loads in the late wet season (season × coinfection, $\beta = -0.080$, $p = 0.011$).

[c] Age × co-infection and season × sex: Coccidia intensity was suggestively associated with nematode burdens in calves but there was no association in other age categories (calf × coinfection $\beta = 0.434$, $p = 0.059$). Males were associated with higher coccidia intensities in the late wet season only (sex × season, $\beta = 1.352$, $p = 0.002$).

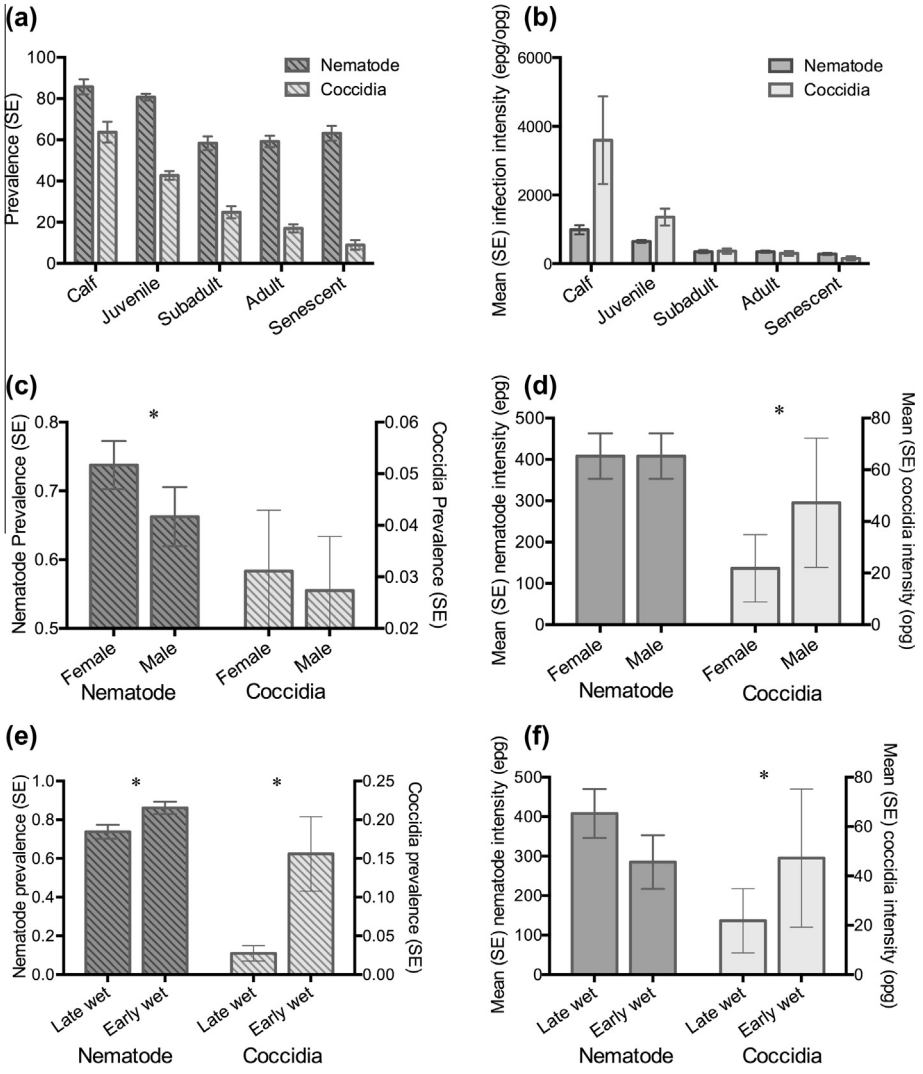

Fig. 1. Age, sex and seasonal patterns of infection. Both parasites had the highest (a) prevalence (sample size for calf, juvenile, subadult, adult, and senescent respectively: $N = 91, 555, 221, 326, 182$) and (b) mean intensity in calves and juveniles (nematode $N = 78, 448, 129, 208, 100$; coccidia $N = 58, 237, 55, 60, 14$). (c) Males had lower estimated nematode prevalence and (d) higher estimated coccidia intensity compared to female buffalo. (e) The estimated nematode prevalence, coccidia prevalence, and (f) mean coccidia intensity were all increased in the early wet season compared to the late wet season. *Indicates significant differences at $p < 0.05$.

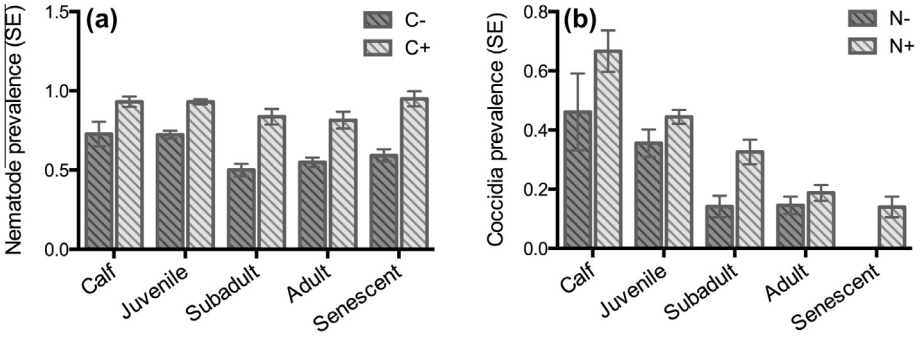

Fig. 2. Age specific patterns of parasite prevalence with co-infection. (a) Prevalence of nematodes is higher in buffalo co-infected with coccidia (C+) compared to coccidia negative buffalo (C−) in all age categories ($N = 33, 318, 166, 272, 162$ for calf, juvenile, subadult, adult and senescent C− buffalo; $N = 58, 237, 55, 54, 20$ for C+ buffalo). (b) Prevalence of coccidia is higher in buffalo co-infected with nematodes (N+) compared to nematode negative buffalo (N−) in calf, juvenile, subadult, and senescent buffalo but not adult buffalo ($N = 13, 107, 92, 144, 56$ for calf, juvenile, subadult, adult and senescent N− buffalo; $N = 38, 448, 129, 208, 100$ for N+ buffalo).

pregnancy. After accounting for sampling year, season was the only significant predictor of pregnancy. The odds of pregnancy were estimated to be higher in the early wet season than in the late wet season by 5.254 (95% confidence interval from 4.150 to 6.651; $F_{2,\ 531} = 5.336$, $p < 0.0001$).

Host body condition was associated with season, gastrointestinal nematode egg counts, and their interactions with other environmental, demographic, and infection variables. Buffalo were in poor condition in the early wet season as were older buffalo and buffalo with higher nematode egg counts. There was no main effect

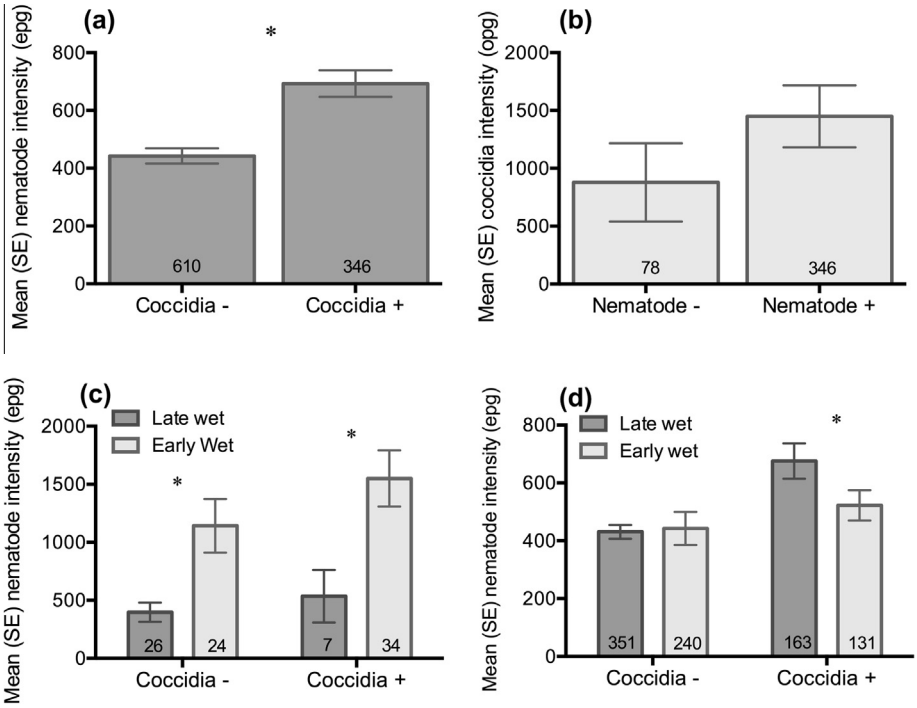

Fig. 3. Patterns of parasite egg/oocyst counts with co-infection for (a) nematodes and (b) coccidia. (c), the mean nematode intensity in calves is higher in early wet season than in the late wet season independent of co-infection with coccidia. (d) Co-infection with coccidia alters the seasonal patterns of nematode intensity in non-calf buffalo (>1 year, juvenile through senescent). Calf vs. non-calf division is based on model paramters (Table 1).

of sex on body condition (Table 2). After accounting for host demographic and environmental variables, uninfected buffalo had the highest mean body condition. Increasing nematode egg counts were associated with reduced body condition although this effect was minimal in the late wet season. In the late wet season, one log unit increase in nematode egg counts was associated with a minimal decrease in body condition by −0.018 (95% confidence interval from −0.034 to −0.002). We did not observe a main effect of coccidia, although its interactions with other variables are discussed below.

We predicted that the fitness costs of co-infection would be exacerbated in the early wet season when buffalo are in poor condition. In support of this prediction, the best-fitting model of host body condition included an interaction between season, coccidia oocyst counts, and nematode egg counts (drop-in-deviance, coccidia × nematodes × season, $p < 0.009$). Similar results were found in models of host body condition when infection was represented as a categorical variable (parasite presence or absence; Table S2). In the late wet season, when buffalo were in higher body condition overall, buffalo free from both coccidia and nematodes displayed the highest body condition, compared to similar condition scores in all other groups (Fig. 4). Co-infected buffalo fared no worse than singly infected buffalo, in terms of body condition. Conversely, in the early wet season, co-infected buffalo had significantly lower body condition than uninfected or singly infected buffalo (Fig. 4). The estimated mean body condition score for uninfected buffalo was estimated to be 3.22 (95% confidence interval from 3.13 to 3.31) compared to 3.01 (95% confidence interval from 2.90 to 3.11) for co-infected buffalo in the late wet season. Thus, buffalo co-infected with both parasites had an estimated mean body condition lower than expected if both parasites reduced condition additively.

Table 2
Summary of the age, season, and infection correlates of host body condition. Parameter values and standard errors of the GLM, *F*-value, and significance are shown for the final model of buffalo body condition ($N = 1375$). Coccidia and nematode parasites are represented as log transformed egg or oocyst counts. Arrows for age categories indicate if body condition is increased or decreased from adult buffalo and arrows for season indicate the difference from April–June. The full model is shown in the Table S3.

Parameter			Estimates (SE)	*F*-value	*p*-value
Calf		↑	0.920 (0.126)	7.304	<0.0001
Juvenile		↑	0.624 (0.056)	11.166	<0.0001
Subadult		↑	0.320 (0.067)	4.756	<0.0001
Senescent		↓	−0.352 (0.067)	−5.899	<0.0001
Season (September–October)		↓	−0.506 (0.074)	−6.836	<0.0001
Coccidia		–	−0.032 (0.067)	−1.287	0.198
Nematode		↓	−0.018 (0.008)	−2.189	0.031
Age × Coccidia	Subadult	↑	0.062 (0.030)	2.109	0.035
	Senescent	↓	−0.072 (0.041)	−1.744	0.081
Season × Coccidia		-	0.044 (0.029)	1.511	0.131
Season × Nematode		-	0.016 (0.013)	1.268	0.205
Nematode × Coccidia		↑	0.006 (0.002)	2.370	0.017
Season × Nematode × Coccidia		↓	−0.013 (0.005)	−2.606	0.009

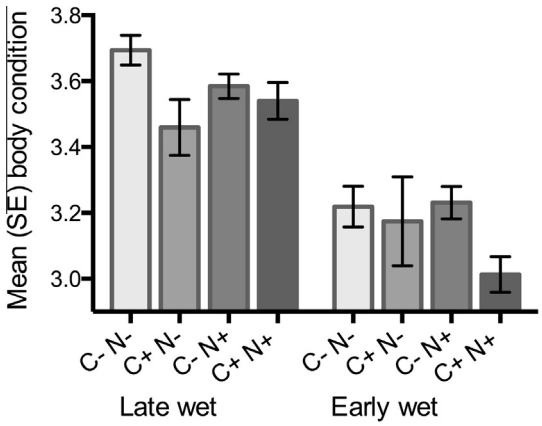

Fig. 4. Predicted mean and standard error body condition scores show associations with infection presence and season, with co-infected buffalo in much lower condition in the early wet season (Table S2). Coccidia infection status is represented with C− and C+; nematode infection status is represented with N− and N+.

3.2.2. Co-infection and seasonal patterns of parasite aggregation

Because host body condition varied with co-infection and season, we used parasite aggregation as a proxy for mortality to test for a role of seasonal mortality in buffalo with heavy nematode infections. We predicted that the fitness costs of co-infection would be exacerbated during times when buffalo are in poor condition (early wet season). For buffalo sampled in the early wet season, we expected a less aggregated (higher k) distribution of nematodes in buffalo co-infected with coccidia than in singly infected buffalo.

Consistent with this expectation, nematode distributions in the early wet season were less aggregated in non-calf buffalo co-infected with coccidia compared to buffalo with nematode infections alone (cocc+ $k = 0.753$, cocc− $k = 0.245$, p-value = 0.020; Fig. 5). There was no significant difference in aggregation in the late wet season (cocc+ $k = 0.747$, cocc− $k = 0.944$, p-value = 0.111). The range of nematode intensity in the early wet season was 50 to 9700 eggs/g in singly infected buffalo and 50–7331 eggs/g in co-infected buffalo, indicating that the right side of the distribution was truncated in co-infected buffalo (variance-to-mean ratio = 1776 and 687 for coccidia negative and positive buffalo, respectively). In calves, coccidia positive buffalo had consistently lower aggregation in all seasons, but no patterns were significant (early wet: cocc+ $k = 1.208$, cocc− $k = 3.383$; late wet: cocc+ $k = 0.243$, cocc− $k = 1.138$). We focused on nematode aggregation in this analysis, because nematodes directly affect host body condition in our study system. Coccidia were not associated with host body condition; as such, we would not expect to see host mortality to be strongly driven by variation in coccidia burdens. Accordingly, we found no difference in coccidia aggregation between seasons or with co-infection status (Fig. S1).

4. Discussion

African buffalo showed similar age and seasonal patterns of infection as other African ungulates (Gwaze et al., 2009; Turner and Getz, 2010) and ungulates in more temperate climates (Pyziel et al., 2011). Young buffalo had the highest prevalence and egg/ oocyst counts for both gastrointestinal parasites, presumably because young animals are immunologically naïve and initially less able to prevent parasite establishment and reproduction (Gasbarre et al., 2001; Stewart and Penzhorn, 2004). The many additional processes that may contribute to age patterns of infection have been reviewed in detail and include age-specific exposure, density dependent parasite establishment, or mortality (Duerr et al., 2003). Our data showed a stronger reduction in coccidia prevalence and oocyst counts with age than in nematodes, suggesting adaptive immunity to coccidia may be more effective than immunity to a relatively long-lived nematode community (Maizels et al., 2004). Similar results were found in free ranging

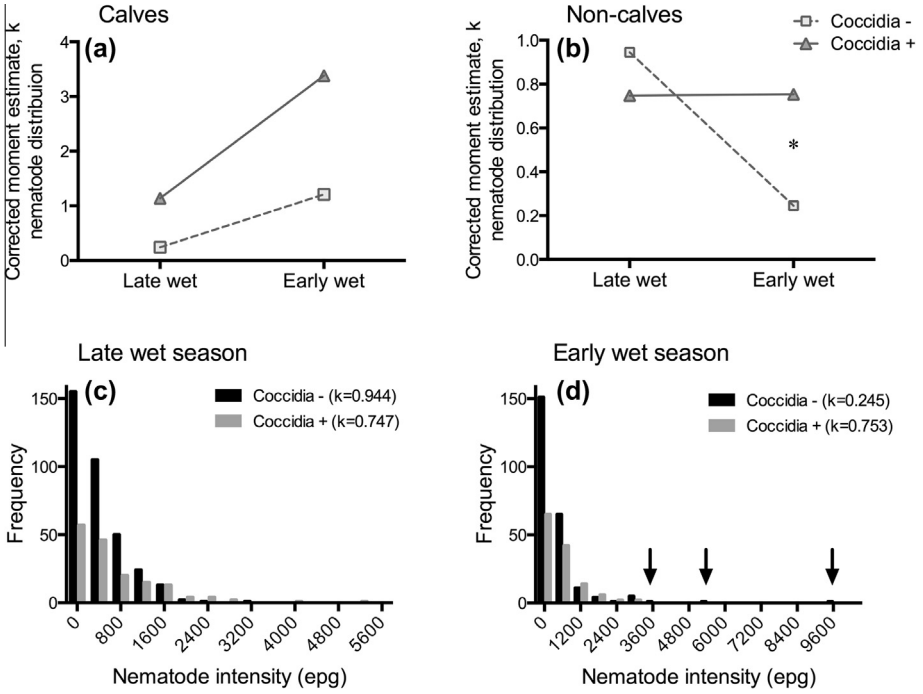

Fig. 5. Season and co-infection differences in nematode aggregation. (a) Aggregation patterns in calves (b) and non-calves. (c) In non-calves, the distribution of nematode parasites in the late wet season shows that k is not significantly different in coccidia positive vs. negative buffalo. (d) In the early wet season coccidia positive buffalo have a truncated distribution, resulting in significantly reduced aggregation. Arrows indicate nematode intensity values in the tail of the distribution of coccidia negative buffalo. Coccidia infection status is represented with C− and C+.

populations of springbok and zebra at Etosha National Park (Turner and Getz, 2010).

Buffalo had higher coccidia prevalence, coccidia oocyst counts, and nematode prevalence in September–October, which corresponds with the early wet season and the end of the dry season. These seasonal patterns may result from differences in egg and oocyst shedding related to variation in host condition, nutrition, and immunity. Parasite egg and oocyst counts may reflect the host's ability to regulate the survival/expulsion (Balic et al., 2002), growth, or reproduction (Rowe et al., 2008) of parasites to which it has been exposed. Parasite egg and oocyst counts in African buffalo has been negatively associated with body condition (Ezenwa and Jolles, 2008), host immunity (Beechler et al., 2012) and diet quality (Ezenwa, 2004). Because forage quality decreases throughout the dry season, buffalo reach their lowest body condition at the end of the dry season and beginning of the wet season (Caron et al., 2003; Ryan et al., 2012). Seasonal differences in egg counts could, therefore, be related to the low body condition observed in the early wet season if animals in poor condition after the dry season are less able to control their parasite infections (Beldomenico and Begon, 2010).

Two alternative but non-exclusive hypotheses are that the seasonal patterns may also be driven by hypobiosis (Rossanigo and Gruner, 1995) or periparturient rise (Agyei et al., 1991). Periparturient rise occurs when females show high parasite intensity following parturition and is thought to occur because hosts invest finite resources into reproduction rather than anti-parasite immunity (Beasley et al., 2010). Hypobiosis has been well characterized and occurs when the development of a proportion of ingested larvae temporarily halts (Eysker, 1993). Hypobiosis often occurs during adverse conditions, including seasons when environmental transmission is unlikely. When favorable environmental conditions or host immunosuppression occurs, parasite maturation and transmission result in high levels of exposure (Armour and Duncan, 1987). Although hypobiosis does not always occur in sub-tropical conditions (El-Azazy, 1995; Gatongi et al., 1998), low levels have been noted in South Africa's Eastern Cape Province (Boomker et al., 1989; Horak et al., 1991) and the Zimbabwean Highveld (Pandey, 1990). In combination, condition-related release of parasites from host immune control and hypobiosis may result in large numbers of infective stages in the environment during a time when many hosts are less able to defend themselves against parasite invasion, leading to a strong seasonal signal in parasite abundance.

Gastrointestinal nematode and coccidia infections were positively associated in buffalo, both in terms of the likelihood of infection and infection intensity, measured by egg and oocyst counts. When interpreting co-infection patterns, caution must be exercised to guard against spurious correlations reflecting commonalities in exposure, susceptibility and age patterns of infection rather than interactions among parasites (Behnke et al., 2005; Beldomenico and Begon, 2010; Fenton et al., 2010). Young buffalo were indeed more prone to both coccidia and nematode infections, but the two remained positively associated when controlling for host age. The similar life cycles of coccidia and nematodes make correlated exposure likely, as both parasites' life cycles include fecal-oral transmission and development of infectious stages in the environment (Stewart and Penzhorn, 2004). It is also possible for the positive association between coccidia and nematodes to be driven by hosts in poor condition being susceptible to both infections (Beldomenico and Begon, 2010). However, the contrasting seasonal nematode egg counts we observed for different age groups of buffalo suggests that common exposure or susceptibility may not be the only process underlying associations between nematodes and coccidia in buffalo. In buffalo less than one year old, co-infected hosts had higher nematode egg counts in the early wet season as predicted if common exposure is important in this system. However, in older buffalo, coccidia infection status made no difference to nematode egg counts in the early wet season, because nematode egg counts in coccidia-positives declined from the late wet season (April–June) to the subsequent early wet season (September–October; Fig. 3d).

Our results may also have been influenced by variation in worm fecundity because our analyses were based on fecal egg counts rather than parasite burdens. Associations of parasite fecundity with immunity and intraspecific competition are well documented (Paterson and Viney, 2002; Chylinski et al., 2009). For example, because nematode egg counts also reflect differences in parasite fecundity, if variance in worm fecundity decreases with co-infection, we may also see decreased aggregation in coccidia-positive buffalo with minimal changes to parasite intensity. Previous work, however, has shown host characteristics like age and sex, which are accounted for in our analyses to be more important than co-infections for nematode fecundity (Luong et al., 2010).

Gastrointestinal parasites have been linked to declines in host condition in livestock (Stewart and Penzhorn, 2004) and wildlife (Stien et al., 2002). Studies also suggest that associations with host condition are often age and sex-specific (Stien et al., 2002; Craig et al., 2008; Turner et al., 2012). For example, in free ranging populations of springbok, strongyle burdens were only associated with reduced body condition only in adult females, while coccidia burdens were not associated with condition in any age category (Turner et al., 2012). In Soay sheep, both coccidia and nematodes were negatively associated with August weight in adults, but only nematodes were negatively associated with condition in yearlings and lambs (Craig et al., 2008). We observed lower body condition with increasing nematode burdens in all ages, and reductions in condition with coccidia burdens only in senescent buffalo. While the direction of causality is often unclear for associations between body condition and infection (Behnke et al., 2005; Fenton et al., 2010), experimental work has shown that gastrointestinal nematodes cause declines in body condition in this buffalo population (Ezenwa et al., 2010) and in other wild (Craig et al., 2008; Pedersen and Greives, 2008) and domestic species (Hoglund et al., 2013).

In our study, the effects of co-infection on host body condition were magnified following the dry season: co-infected buffalo had lower body condition scores than uninfected and singly-infected buffalo in September–October, which corresponds to the early wet and late dry season. The magnitude of reduction, while minimal in the late wet season, was the difference from a body condition score (bcs) of 3.22 in uninfected buffalo to a bcs of 3.01 in buffalo infected with both parasites in the early wet season (Fig. 4). Given that buffalo body condition in the early dry season ranged from 1.6 to 5, co-infection was associated with buffalo in the 25^{th}–50^{th} percentile of the distribution of host body conditions while uninfected buffalo remained above the 50^{th} percentile. This result ties in with previous findings where body condition was lower in buffalo co-infected with gastrointestinal nematodes and *Mycobacterium bovis* (causative agent of bovine tuberculosis; Jolles et al., 2008). During periods of resource restriction, animals may be less able to balance competing metabolic demands, sharpening trade-offs between immunity and self-maintenance, or among different immune functions (Eraud et al., 2008; Ezenwa and Jolles, 2011). These scarcity-driven trade-offs may underlie the observed seasonal association between co-infection and poor body condition.

In this study, we did not detect any effects of parasites, alone or in combination, on host pregnancy. While female ungulates in poor condition are less likely to conceive and carry pregnancies to term in some species (VanRooyen, 1993; Piasecke et al., 2009), the effects of body condition and parasite infection on reproduction are very difficult to disentangle without experimental studies.

Accordingly, other observational studies on fitness effects in ungulates have also failed to find strong evidence for a negative effect of parasites on reproductive fitness (Irvine et al., 2006; Hughes et al., 2009). However, experimental nematode removal in Svalbard reindeer found that nematodes removal increased pregnancy rates through a positive effect on body condition (Stien et al., 2002).

Our data revealed two interesting patterns of nematode aggregation in our study population. First, for buffalo only infected with nematodes, aggregation was far less in the late wet season/ early dry season than in the subsequent early wet season/ late dry season. This decrease in heterogeneity suggests that differences among hosts in susceptibility and /or exposure to nematodes may be accentuated during the dry season. This is consistent with previous work indicating that energetic trade-offs during resource restriction can cause changes in immunity and infection in this and other mammalian study systems (Ezenwa, 2004; Martin et al., 2008). Second, for buffalo co-infected with coccidia, heterogeneity in nematode burdens did not vary between seasons, leading to a higher estimate of k in co-infected buffalo when compared to buffalo with nematodes only, in the early wet season. This difference is likely driven by a truncated nematode distribution rather than a change in mean nematode egg counts because we did not observe a significant difference in the mean egg counts in coccidia-positive and negative buffalo in the early wet season. This contrasts with other studies in wild mammals, where co-infection has been implicated as a cause for heterogeneity in worm burdens (Cattadori et al., 2008), because here heterogeneity in worm burdens was lower for co-infected than singly infected buffalo.

At least two explanations for this unusual pattern are possible: coccidia infection may dampen seasonal changes in condition or immunity that we hypothesize underlie the dramatic increase in aggregation in coccidia negative buffalo after the dry season. An alternatively (but not exclusive) hypothesis is that co-infected buffalo with very high nematode burdens may suffer a disproportionately high mortality risk over the dry season. Loss of these individuals may balance out the increase in worm aggregation we would expect to see based on our findings for buffalo with only nematode infection. We cannot address the first hypothesis with our current data primarily because detailed immune information was not collected. However, the fact that nematode egg counts are lower after the dry season than before for buffalo co-infected with coccidia supports the notion that co-infected buffalo might suffer increased mortality during this period. This interpretation is also consistent with lower body condition in co-infected buffalo in the early wet season/ late dry season. Experimental work is underway to explicitly test how immunity and condition changes over the course of intestinal parasite infection, determine the parasite species responsible, and test how these immune responses alter the survival costs of infection. In combination, these data suggest that coccidia–nematodes co-infections may play an important role in host health, especially if the season-dependent dependent associations with host body condition translate into consequences for host mortality.

5. Conclusions

We examined the hypothesis that co-infecting parasites and season interact to shape parasite infection patterns and effects on host fitness. Our results suggest that co-infecting parasites and season influence the prevalence and intensity of GI parasites. Coccidia prevalence, coccidia intensity and nematode prevalence were sensitive to factors that influence host immunity and exposure (age, sex, and season) but nematode intensity was most strongly predicted by co-infection with coccidia and its interaction with season. We also found that associations of co-infection with both body condition and parasite aggregation were heavily dependent on season, with contrasting patterns in the early and late and dry seasons. This study demonstrates the importance of demographic and seasonal variation in the effects of parasite interactions on the distribution and fitness costs of infection in free-living wildlife populations. Longitudinal and experimental studies including explicit measurement of immune parameters and host nutrition will be essential to continued progress towards understanding the mechanisms underlying parasite infection patterns in wildlife, and their effects on host fitness.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijppaw.2014.05.003.

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