The Ecology of Orally Ingested Parasites in Ungulates of Etosha National Park

by

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Abstract

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Wildlife populations contain a diverse assemblage of parasites; hosts are often infected by multiple parasite species and parasites may infect multiple host species. I studied parasites infecting herbivorous mammals of Etosha National Park, Namibia, and examined how host-parasite relationships are modulated by host ecology, parasite interactions and environmental variability. The focal parasites were the orally ingested gastrointestinal strongyles (Nematoda, Strongylida) and *Eimeria* spp. (Protozoa, Eimeriidae).

Variation in host foraging behavior influences parasite prevalence and transmission. I used estimates of host intake rates and diet to examine variation in strongyle prevalence among host species. Species that require increased intake rates per metabolic weight exhibited higher strongyle prevalence, because either they are unable to combat these infections or the fitness cost of mounting a stronger immune response is too high.

Ecological questions about host-parasite systems often require estimates of parasite intensity. The main indirect method for quantifying gastrointestinal parasite intensity uses weight of fecal matter, and systematic variation in fecal water content could bias interpretation of propagule intensity patterns. From examination of springbok (*Antidorcas marsupialis*) and zebra (*Equus quagga*) feces, water variation influenced interpretation of sex differences in intensity, but not seasonal or age differences.

Disease dynamics are affected by seasonality, host age and immunity. I assessed temporal and host demographic variation in parasite prevalence and intensity of zebra, springbok, wildebeest (*Connochaetes taurinus*) and gemsbok (*Oryx gazella*). Age-related parasite patterns provided evidence of variable protective immunity against parasite types. Strong seasonal differences in parasitism suggest a long dry season may limit survival of parasite environmental stages.

Finer taxonomic resolution in parasite identifications enhances the study of host-parasite relationships. I described three new species of *Eimeria* from springbok and then examined GI parasite interactions within hosts and among study areas. I found significant positive associations in parasites intensities and co-occurrences and that variation in host condition was best explained by combining the effects of multiple parasite types. Hosts from the study area with the least rainfall generally had reduced parasite prevalence or intensity. Understanding how parasites respond to environmental variability will provide critical insight into how climate change could alter host-parasite dynamics.

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Chapter 1: Summary of the dissertation

All organisms, even bacteria, have parasites, making parasitism one of the most fundamental interspecific ecological relationships. In planning my dissertation, I set out to study host-parasite relationships within an assemblage of herbivorous mammals in Etosha National Park, Namibia, and, specifically, to investigate how orally ingested gastrointestinal (GI) parasites may influence host mortality from another orally ingested parasite, the anthrax bacterium, *Bacillus anthracis*. What started as gathering "background data" on GI parasites for an anthrax study turned into a parasite odyssey, with more than enough material for a single dissertation. Ultimately, research into connections between GI parasites and host anthrax mortality still lies in my future.

In this dissertation, I focused on host-parasite relationships in an assemblage of herbivorous mammals and how these relationships are modulated by host ecology, parasite interactions and environmental variability. The outcome of a single host-parasite relationship is influenced by the surrounding assemblage of other parasites and hosts (Gilbert *et al.* 2001; Holt *et al.* 2003; LoGuidice *et al.* 2003; Lello *et al.* 2004; Keesing *et al.* 2006) and can vary seasonally and spatially (Bertolino *et al.* 2003; Altizer *et al.* 2006). Of particular relevance to my dissertation work reported here is accumulating evidence that environmental change is very likely to impact parasite transmission, host susceptibility, and parasite-induced species extinctions (Wilson 2000; Harvell *et al.* 2002; Kutz *et al.* 2005; Hall *et al.* 2006; Poulin & Mouritsen 2006; Pounds *et al.* 2006). It is therefore important to describe the larger assemblage of hosts and parasites if we want to tease apart their interactions with each other and how these are modulated by the environment.

Comparative assessments of parasitism are generally difficult to perform, because the methods for quantifying parasitism vary considerably among studies (Couvillion 1993). Thus I pay considerable attention to standardizing methods while assessing parasitism among different hosts and environments within Etosha. I focused on an assemblage of thirteen host species and various generalist and specialist organisms that parasitize the GI tracts of hosts within this assemblage. I focused on two groups of directly transmitted, orally ingested parasites: strongyle nematodes (Nematoda, Strongylida) and *Eimeria* spp. coccidia (Protozoa, Eimeriidae). Both of these parasite groups are generally pathogenic and responsible for widespread production losses in livestock (Bowman 2003). These type of parasites are known to play an important role in the dynamics of wildlife host populations (Dobson & Hudson 1992; Gulland 1992; Hudson *et al.* 1992b; Murray *et al.* 1997; Hudson *et al.* 1998; Stien *et al.* 2002; Newey *et al.* 2005).

The research reported in this dissertation was conducted in Etosha between July 2005 and April 2008, during which time I collected 3090 fecal samples from individual hosts. Strongyle nematodes and *Eimeria* spp. parasites release propagules into the lumen which are released into the environment through host defecation, allowing an indirect assessment of GI parasitism from host fecal matter. Direct measures of parasitism would be preferable for precise enumeration of parasitic nematode loads and their effects on hosts. Short of lethal sampling of hosts, however, the only way to assess GI parasitism in free-ranging wildlife hosts is indirectly. When working within protected areas and with protected species, indirect measures of parasitism are the only acceptable option. Of the host species examined, one is IUCN red listed as critically endangered (black rhino,

Diceros bicornis), two species are vulnerable (Hartmann's zebra, *Equus zebra harmannae* and black-faced impala, *Aepyceros melampus*) and one near-threatened (elephant, *Loxodonta africana*) (IUCN 2008).

Given the use of indirect measures in this study, in this dissertation I have not used the term "parasite load," meaning an estimate of the number of nematodes in a host (this term does not apply to intracellular parasites like *Eimeria* spp.). I work with the following measures: parasite prevalence and the intensity of propagule shedding. Prevalence is the proportion of individuals examined who are shedding parasite propagules in feces, and intensity is the estimated number of parasite propagules shed per gram of feces in infected individuals. Prevalence is a population-level measure of how commonly hosts are infected with a parasite during a particular time period. Parasite intensity is a measure of parasite propagules released into the environment, which will affect the number of infectious stages in the environment and subsequent parasite transmission. Intensity is also an estimate of an individual's ability to control infection given that parasites are present and actively reproducing, providing an indication of relative differences in parasitism between hosts.

Beyond this introductory chapter, my dissertation is broken into five additional chapters, each written as a stand alone paper. Some chapters have already been submitted for publication, while others await additional editing before they are ready to submit. For this reason, some of the material in each chapter is duplicated, particularly some of the methods. As publications, these component chapters will have various coauthors, some of whom have helped with project design or data acquisition, or participated to an extent to justify co-authorship. On all these component studies,

however, I have taken the lead in designing the methods, carrying out the field work, and writing up the text and will be lead author on all papers emerging from this dissertation.

In Chapter 2, I report on how foraging ecology and metabolism of host species correlates with strongyle nematode prevalence at the population level in an ungulate assemblage. Foraging behavior may be extremely important to understanding disease transmission and occurrence of orally ingested parasites. However, in the context of interspecific comparisons, variation in foraging ecology has yet to be examined beyond the use of diet to differentiate parasites into transmission modes. In this study, I used estimates of host intake rates and diet to examine variation in GI parasite prevalence across an assemblage of African ungulates. The host species examined have similar social organization, utilize overlapping habitats and share water sources, but vary in their diet composition, intake rates, digestive physiologies and body sizes. Given that intake rates scale with metabolic weight (*i.e.*, $mass^{3/4}$) across taxa, if immune function also scales with metabolism, then variation in metabolically normalized intake rates (i.e. intake per $mass^{3/4}$) across foraging guilds may explain variation in parasite prevalence among species.

Across all species studied, as well as just among the ruminants themselves, the metabolically normalized intake rate emerged as a much stronger predictor of strongyle nematode prevalence than daily intake rate or diet. This suggests that across species it is not the quantity or type of food consumed that matters most, but how the quantity consumed deviates from a predicted intake rate based solely on a species' metabolic weight. To explain the dependence of parasite prevalence on the residual between actual intake rate and the predicted intake rate predicted solely from metabolic considerations, I

propose that an individual's ability to mount an effective immune response depends upon how much contact that individual has with infectious stages in relation to what that individual can metabolically afford to allocate to its immune function. Therefore, species that require increased intake rates per metabolic weight to fulfill their energy requirements may exhibit higher parasite prevalence, either because they are unable to combat these infections or the cost to fitness of mounting a stronger immune response is too high.

Many ecological, veterinary or conservation questions relating to host-parasite systems require estimates of parasite intensity within particular hosts, and of the variation in parasite intensity among hosts in a population of interest (Anderson & May 1978). However, the main indirect method for quantitatively estimating the intensity of parasitic infection for the types of parasites I studied is the McMaster technique, which is based on the wet weight of fecal matter. If the water content of fecal samples varies systematically in relation to the ecological or demographic factors of interest (e.g, season or host age) then this unexplained variation could bias intensity estimates and confound statistical analyses.

In Chapter 3, the seasonal, age- and sex-related variability in fecal water content is evaluated for two wild ungulate species, springbok (*Antidorcas marsupialis*) and plains zebra (*Equus quagga*). This chapter has been accepted to the Journal of Helminthology with co-authors Carrie Cizauskas and Wayne Getz. The focus of this chapter is an assessment of whether or not fecal water content biases conclusions regarding differences in strongyle intensity by season, age or sex. I found evidence of significant variation in fecal water content by season and age for both species, and by sex in springbok. For

season and age, differences in water content were swamped out by much greater differences in strongyle egg counts for these same variables. Analyses of fecal egg counts demonstrated that sex was a significant factor in explaining variation in strongyle parasite infection rates in zebra and springbok using wet-weight fecal samples. However, once these intensity estimates were re-scaled by the percent of dry matter in the feces, sex was no longer a significant factor. These results demonstrate that systematic variation in fecal water content may confound analyses and could produce spurious conclusions, as was the case with host sex as a factor in the analysis. I thus recommend that researchers assess if water variation could be a confounding factor when designing and performing research using fecal indices of parasite intensity.

In Chapter 4, the focus on host diversity (from those examined in Chapter 2) is narrowed down to an assemblage of four host species that share a common habitat in central Etosha. In this reduced host assemblage I expanded the scope of factors considered to assess temporal and host demographic variation in parasite prevalence and intensity of propagule-shedding intensity in plains zebra, springbok, wildebeest (*Connochaetes taurinus*) and gemsbok (*Oryx gazella*). These species co-occur in an extensively grazed short grassland and dwarf shrub savanna habitat surrounding the Etosha Pan.

Most GI parasites are transmitted between hosts via a reproductive stage excreted with feces that is not immediately infectious, but requires a developmental period in the environment. Therefore, by affecting development and survival of free-living parasites, such factors as seasonal changes in rainfall, temperature and resource availability may have a strong influence on disease dynamics (Altizer *et al.* 2006). In addition to

seasonality, describing disease dynamics may require an understanding of the effects of host age structure and immunity to the host-parasite system (Cattadori *et al.* 2005; Cornell *et al.* 2008). These factors are not mutually exclusive, however, for host immunity may vary seasonally in relation to changes in reproduction, stress, nutrition, and photo period (Martin *et al.* 2008). Seasonal environmental changes can also affect transmission of gastrointestinal parasites by affecting the abundance of parasite propagules produced, the development and survival of those propagules in the environment, and host contact with infectious free-living parasite stages (Stromberg 1997; Altizer *et al.* 2006).

I observed strong seasonal differences in parasite prevalence and intensity of propagule shedding for most parasites in the four host species, with both measures higher in wet seasons than dry seasons. The strong seasonal relationship was further related to monthly rainfall, with peaks in parasite prevalence and propagule shedding intensity occurring 1-2 months after peak rainfall. As this time frame is sufficient for both *Eimeria* and strongyles to complete their life cycles, the marked increase in parasitism may be due to a resumption of parasite transmission, or parasite activity, in the case of arrested strongyle larvae. Age-related patterns of parasitism provided evidence of protective immunity against *Eimeria* spp., and a weaker immune response against strongyle parasitism. The seasonal patterns further demonstrated the potential limiting effect a long dry season may have on the survival or transmission of parasite infectious stages in the environment. In fact, strong variation in rainfall may be the main constraining factor affecting parasite dynamics in semi-arid systems by severely limiting transmission (Chiejina *et al.* 1989). Understanding how parasites respond to variation in rainfall will

provide critical insight into how environmental change could alter host-parasite dynamics.

Generally, an understanding of host-parasite relationships is strengthened by a finer taxonomic resolution in parasite identifications. Chapter 5 works toward this goal by describing three new species of *Eimeria* from springbok: *E. antidorcasi, E. lammekia* and *E. gasawayi*. Species of *Eimeria* are generally very host-specific parasites and no *Eimeria* have yet been described from this host species. These new species were distinctly different in the size, shape and phenotypic characteristics of the sporulated oocysts. *Eimeria antidorcasi* was more prevalent in hosts than was *E. lammekia*, and the prevalence of *E. gasawayi* is unknown. Both *E. antidorcasi* and *E. lammekia* were more prevalent in wet seasons than dry seasons, although this difference was far more pronounced for *E. antidorcasi* than for *E. lammekia*. The prevalence and intensity of *E. lammekia* was greater in juveniles than older age classes. *Eimeria antidorcasi* had greater intensity in juveniles than other age classes, but the prevalence was not significantly different among age classes.

In Chapter 6, I further narrowed the diversity of hosts examined to focus on springbok, the most abundant of the host species in Etosha, which also showed the greatest diversity of parasites. I examined parasite interactions within springbok hosts, how parasitism and parasite interactions varied among three study areas within Etosha, and how parasite types individually, and in concert, related to host body condition. GI parasites in wildlife populations are associated with reduced host physical condition or body mass (Stien *et al.* 2002; Holmstad *et al.* 2005; Lello *et al.* 2005; Newey *et al.* 2005; Craig *et al.* 2008). Multiple parasite infections often co-occur in hosts and parasite

interactions may be direct (e.g., competition for space) or indirect (e.g., mediated by host immune responses). The type of interaction and its directionality tend to vary depending on the type of parasites involved. Indirect parasite interactions via host immunity can occur through cross-immunity between similar types of parasites (a negative interaction) or through suppression of the acquired immune response (a positive interaction) (Cox 2001).

Parasite interactions were generally positive, with increased intensity or presence of one parasite type associated with increased intensity or presence of a second parasite. Among study areas, hosts from the study area receiving the lowest rainfall generally had reduced parasite prevalence or intensity. The drier conditions in this study area may limit transmission of infective larvae or survival of free-living stages in the environment. Models of variation in host body condition were best fit combining the effects of multiple parasite types. Strongyle nematodes were most strongly related to host condition of the parasite groups examined. In both wet and dry seasons, individuals with higher strongyle nematode intensities had poorer body condition than individuals with lower strongyle intensities. Relationships between other parasites and host body condition were only observed when the parasite assemblage was examined together. When the parasites were examined independently, I only observed a significant relationship between host condition and strongyle intensity. These results indicate the importance of examining how the parasite assemblage may relate to host condition. Further research into the diversity of species within the strongyle nematodes could identify which parasite species have the largest impact on springbok body condition.

Chapter 2: Parasite prevalence and intake rates in African ungulates

Introduction

Although food consumption provides the nutrients necessary for survival and reproduction, it comes with an associated risk of exposure to parasites ingested while foraging (Lima & Dill 1990; Yearsley *et al.* 2002; Hall *et al.* 2007). Individuals can mitigate this foraging risk by avoiding foods associated with parasites (Hutchings *et al.* 1999; Hutchings *et al.* 2000), or by selecting food items that improve host resistance or contain antiparasitic compounds (Lozano 1991). They can also adjust their foraging intake rate to reduce the risk of parasitism (Hutchings *et al.* 2001; Yearsley *et al.* 2002), though, ultimately, they will be constrained by their basal metabolic requirements. Foraging behavior may be important to our understanding of parasite diversity and abundance among host species because many parasites have orally ingested infectious stages. Across various groups of vertebrate taxa, factors that influence foraging rates, such as host body size, diet, and metabolism have been related to parasite species richness and abundance (Kennedy *et al.* 1986; Sousa 1994; Poulin 1995; Gregory *et al.* 1996; Morand & Harvey 2000; George-Nascimento *et al.* 2004). Surprisingly, however, few if any interspecific comparisons have attempted to directly assess the relationship between intake rates and parasitism.

Gut capacity and metabolic rate both increase with body mass (m) for mammalian herbivores. However, since metabolic rate scales allometrically with $m^{3/4}$ and gut capacity scales isometrically with m, larger animals require more total energy and smaller animals more energy per unit body mass (Demment & Van Soest 1985). In particular, daily intake rates (x) for herbivorous mammals scale with metabolic weight (*i.e.*, $m^{3/4}$), though some variation is expected based on digestive physiology (Clauss *et al.* 2007).

Additionally, the function of the immune system is predicted to scale with metabolism (Wiegel & Perelson 2004), if basal metabolic rate in fact scales with $m^{3/4}$ from the subcellular to the cellular to the organismal levels (West *et al.* 2002). Furthermore, it has been demonstrated that quickly dividing cells, like those of the immune system, have invariant cell size (when grouped by cell type) and therefore their cellular metabolic rates scale with body size (Savage *et al.* 2007). Given these scaling relationships, and under the assumption that parasite ingestion increases with forage ingestion, we expect to see a positive relationship between GI parasite prevalence and host intake rate normalized by metabolic weight.

To isolate the effects of a species' metabolism versus mass on foraging rates *per se*, and determine which factors related to foraging ecology best explain parasite prevalence (w), we selected the following host factors for analysis: 1) *daily intake rate* (x kg of forage ingested per day); 2) *metabolically normalized intake rate (MNIR, y* kg of forage ingested per day per $kg^{3/4}$ of host body mass); 3) *diet*, as represented by the percent of C4 plants (*i.e.*, grasses, as opposed to C3 plants or browse) consumed (z); and 4) *type of digestive system* (non-ruminant versus ruminant). Diet was included since the type of foods consumed may increase or decrease the chance of parasite ingestion (Kennedy *et al.* 1986; Bell & Burt 1991; Aho & Bush 1993; Poulin 1995; Vitone *et al.* 2004) depending on the transmission mode. The host species examined included those with ruminant and non-ruminant digestive systems, an important distinction for comparison because ruminants require relatively lower ingestion rates compared with similarly sized non-ruminant herbivores to achieve their metabolic needs (Janis 1976).

Therefore, ruminant digestion, as an evolutionary feeding adaptation, should strongly influence GI parasitism.

Methods

Sample collection and parasitological analysis

In this study we estimated gastrointestinal parasite prevalence across 13, co-occurring African ungulate species in Etosha National Park, Namibia. Etosha is a 22,915 km^2 reserve in northern Namibia located between 18˚30'-19˚30'S and 14˚15'-17˚10'E. The vegetation is classified as arid savanna and the mean annual rainfall at Okaukuejo station in the center of the park was 384mm from 1934-2007. Rainfall generally occurs between November and April, with the greatest monthly totals in January and February (Engert 1997). Sampling for parasitism was conducted across Etosha, to target the areas of highest density for each host species. Parasitism was determined using fecal indices, and we collected a total of 767 fecal samples from 13 ungulate species during the period July-September in 2005 and 2006.

The parasites examined were GI nematodes in the order Strongylida, hereafter referred to as "strongyles", with a fecal-oral transmission route and without intermediate hosts or vectors. The strongyles comprise superfamilies Strongyloidea, Trichostrongyloidea and Ancylostomatoidea and are a relatively homogenous group of mammalian parasites (Arneberg *et al.* 1998). Although little is known of their effects on wildlife, they are pathogenic and responsible for widespread production losses in livestock (Bowman 2003). The prevalence of other GI parasites observed is presented in

Chapter 5 (*Eimeria* spp.) and Appendix I (*Strongyloides* spp. nematodes and Anoplocephalid cestodes).

The ungulate host species in the assemblage are social to the degree that they have familiar or herd structures, aggregate periodically into larger groups, utilize overlapping habitats and share water sources. Across the assemblage they vary in their intake rates, diet composition, and body sizes. Ungulate study species included red hartebeest (*Alcelaphus buselaphus*, *AB*), springbok (*Antidorcas marsupialis*, *AM*), black-faced impala (*Aepyceros melampus petersi*, *AMP*), blue wildebeest (*Connochaetes taurinus*, *CT*), black rhino (*Diceros bicornis*, *DB*), plains zebra (*Equus quagga*, *EQ*), mountain zebra (*Equus zebra*, *EZ*), giraffe (*Giraffa camelopardalis*, *GC*), elephant (*Loxodonta africana*, *LA*), gemsbok (*Oryx gazella*, *OG*), common warthog (*Phacochoerus africanus*, *PA*), eland (*Taurotragus oryx*, *TO*) and greater kudu (*Tragelaphus strepsiceros*, *TS*) (Figure 1). Rare or furtive ungulate species for which adequate samples sizes were not easily acquired were excluded from this study.

Fecal samples were collected from the host species only during dry seasons, when animals congregate around point water sources along the road network. This allowed sampling of a greater diversity of ungulate species than was possible during wet seasons when animals disperse into inaccessible areas. We stratified the time of sampling for each species: samples from most species were collected in the morning (7:00-13:00) when individuals came to water excepting giraffe, which drink in the late afternoons, and elephant and black rhino, which drink at night. We collected samples that we either saw deposited or fresh fecal piles from areas recently utilized by observed groups. For elephant and rhino, we searched near waterholes at dawn, collecting any fresh samples

deposited during the night. During the study period, night temperatures are cool without freezing, naturally inhibiting egg development. To reduce inadvertent resampling of individuals, we sampled an area no more than once per month and only collected discrete fecal piles. After collection, all fecal samples were immediately placed in a refrigerator mounted on the vehicle.

Fecal samples were evaluated for strongyle nematodes within 48 hours of collection using a modification of the McMaster method for fecal egg counts (FAO 2005), a commonly used non-invasive method for quantifying parasitism (Bowman 2003). These data were used to assess the presence or absence of strongyle nematodes and to calculate the prevalence, or proportion of individuals infected within a species.

Host trait variables

Foraging intake rates may vary among individuals of a species based on physiological state, diet, seasonality and among populations based on system productivity, but we expected interspecific variation to be greater than intraspecific variation. To reduce the effect of intraspecific variability on species-level intake estimates, we used experimentally determined intake rates recorded in an alfalfa feeding trial on zoo animals (Foose 1982) as surrogates for foraging rates in the wild and as the best available estimates for our study species. This trial controlled for forage type and quality, climate variability and activity levels, and for reproductive state and age of individuals selected as experimental animals. In this trial Foose directly measured intake rates for seven of our species (*DB, EQ, EZ, GC, LA, OG* and *TO*), and we estimated the intake for the other six species based on his mean intakes reported by category of

digestion type (ruminant vs. non-ruminant), feeding type (grazer vs. non-grazer) and mean female body mass (< and >1000kg).

A species' diet, determined as the percent of grass (C4 plants) versus browse (C3 plants) consumed was taken from stable carbon isotope studies of ungulate diets in southern Africa (Sponheimer *et al.* 2003; Codron *et al.* 2006; Codron *et al.* 2007). For the calculation of MNIRs (y), body mass was either measured by Foose (1982) where possible or taken from the estimated mean adult female body mass for the species compiled by Owen-Smith (1988). We did not explicitly include body mass in analyses because daily food intake rate (x) and body mass (m) were strongly correlated (r=0.95, t=14.2, p<0.0001, calculated using only body mass data compiled by Owen-Smith (1988) for consistency), with daily intake rate being the more salient variable of the two. This correlation has a slope estimate of 0.79±0.06, which encompasses the theorized scaling exponent of 0.75 for intake rate by body mass (Clauss *et al.* 2007).

Data analysis

A comparison of heritable traits across species using species values as though they were independent (*i.e.*, non-phylogenetic methods) runs the risk of increased Type I error: the more closely related two species are, the less they will fit the assumption of independence (Felsenstein 1985). Therefore, species-level data should either be tested prior to analysis for phylogenetic independence (Freckleton *et al.* 2002) or analyzed using the method of independent contrasts to account for non-independence (Felsenstein 1985). We chose to perform independent contrasts for all analyses. For completeness,

however, we present results of statistical analyses based on both species values without accounting for dependence and the method of independent contrasts.

For species values, the relationship between parasite prevalence and host variables was analyzed using logistic regression in R 2.7.0 (R Development Core Team 2008), because prevalence is a proportional variable, bounded between zero and one. The host traits were checked for collinearity, and all had variance inflation factors less than 10 (VIF_{max}=2.1). Logistic regressions were weighted by sample size to control for the influence of sample size on variance estimates and, hence, model fit. Prevalence data were overdispersed (Dispersion=8.4), and failed to conform to the assumption of Dispersion=1 for the binomial family. Therefore we fitted a quasibinomial distribution that includes an additional parameter to account for overdispersion (MacCullagh & Nelder 1989).

Independent contrasts for each variable were calculated in the PDAP module (Midford *et al.* 2003) of Mesquite 2.01 (Maddison & Maddison 2007) using a phylogeny of the host species with branch lengths extracted from the "best dates" mammal supertree of Bininda-Emonds et al. (2007) (tree and branching lengths shown in supplementary materials). Daily intake rate was log transformed to conform with the assumption that there is no relationship between the absolute value of the standardized contrast and the square root of the sum of the corrected branch lengths (Garland *et al.* 1992). All other variables were in accordance with the assumptions of this method. The soft polytomy (unresolved branch) was not accounted for through a reduction in degrees of freedom, because simulation studies show that a small number of polytomies have negligible effects on Type I error rates (Garland & Díaz-Uriarte 1999).

The relationship between the parasite and host independent contrasts was analyzed using multiple regression with the intercept set to zero (Garland *et al.* 1992). Sample size (range 15 to 100) was included as a variable in the analysis, to control for the potential influence of sampling effort on prevalence estimates (Nunn *et al.* 2003). The two major classes of digestive system were evaluated for each statistical method by separately performing fits of the host factors to the parasite data for the whole assemblage and then to the non-ruminant and ruminant guilds on their own. We illustrate the multiple regression for the whole assemblage using partial regression leverage plots (Sall 1990) to show the effects of each foraging variable on strongyle prevalence, while controlling for the effects of the other variables within the full model.

Results

Metabolically normalized intake rate (MNIR) had the greatest power of our primary factors in explaining the strongyle prevalence in analyses that both ignore and account for phylogenetic relationships among host species (Table 1). Increased strongyle prevalence was strongly correlated with increases in MNIR (Figure 2). Traditional statistical analysis detected a significant positive relationship between log(daily intake) and strongyle prevalence; however, there was no correlation between these variables once phylogenetic relationships were taken into account. Contrary to expectations, the proportion of grass in the diet was negatively correlated with strongyle prevalence, although the estimated slope was close to zero (Table 1; β=-0.03 for species values and β=-0.42 for independent contrasts). As a control, we also included sampling effort as a factor, but it turned out to be insignificant.

Strongyle prevalence varied from 5.3% in red hartebeest to 100% in mountain zebra, and the species with the highest strongyle prevalences were the non-ruminant herbivores (Supplementary materials). In light of the observed prevalence differences between ruminant and non-ruminant herbivores, the relationship found between MNIR and strongyle prevalence could arguably be driven by inherent differences in the digestive anatomy and physiology for these particular host species. To assess this we tested the relationship between parasite prevalence and host foraging traits within each digestive type separately. Among non-ruminant species there was no statistical relationship between parasite prevalence and any of the host variables because, with the exception of warthogs, the non-ruminant hosts had parasite prevalence near maximum, between 90-100% (Figure 3). Among ruminant hosts, however, the pattern was consistent, with increased MNIR strongly correlated with increased strongyle prevalence (Table 1).

Discussion

This study examined relationships between various host foraging factors and the prevalence of strongyle nematodes, an orally ingested group of parasites. Our results indicated that the host factor MNIR was a much stronger predictor of strongyle prevalence than was daily intake rate or diet. This suggests that across species it is not the quantity or type of food consumed that matters most, but how the quantity consumed deviates from a predicted intake rate based solely on a species' metabolic weight (*i.e.,* on $w_i - \overline{w}_i$, where w_i is the observed prevalence for species i and $\overline{w}_i = \overline{w}(y_i)$ is the predicted value from the regression relationship).

Species-level variation in MNIR related to the prevalence of strongyle parasites across all host species studied and among ruminant hosts themselves, this pattern was not simply driven by differences in the type of digestive system. Many authors have highlighted the differences between ruminant and non-ruminant herbivores in their intake rates (e.g., Janis 1976; Foose 1982; Owen-Smith 1988; Illius & Gordon 1992), with non-ruminants requiring higher intake rates to fulfill their metabolic needs than ruminants of similar body size. The persistence of this pattern among ruminant species strengthens our result by making it far less likely that the relationship between MNIR and parasitism is an artifact of the assemblage of species in our study.

Ruminant digestion may have evolved to enable greater digestive efficiency in systems characterized by resource scarcity (Illius & Gordon 1992), such as occurs seasonally in African savannas. A possible secondary benefit of this evolution may be decreased parasitism, as greater digestive efficiency allows reduced intake while still achieving nutritional requirements. A reduction in parasite risk compared to non-ruminant herbivores could be an additional factor in the evolutionary success of ruminants.

Host diet did not support the hypothesized pattern of increased parasitism in species with a high proportion of grass in their diets. The lack of a positive relationship between the percentage of grass in the diet and strongyle prevalence is in consonance with the theory that herbivores use behavioral mechanisms such as selective feeding or avoidance of fecal-contaminated areas to offset the potential risk of parasitism posed as a result of foraging near ground-level (Hart 1990). In support of this behavioral theory,

studies have shown that herbivores selectively avoid foraging in areas contaminated with feces (e.g., Hutchings *et al.* 1999; Ezenwa 2004b).

To explain the dependence of parasite prevalence on the residual between actual foraging intake rate and that predicted solely from metabolic considerations, we propose that an individual's ability to mount an effective immune response depends upon how much contact that individual has with infectious stages in relation to what that individual can metabolically afford to allocate to its immune function. Up-regulating the immune system is energetically and nutritionally costly (Lochmiller & Deerenberg 2000) and when parasite contact is generally high, hosts may not be able to sustain an effective immune response. Therefore, species that require increased foraging intake rates per metabolic weight to fulfill their energy requirements may exhibit higher parasite prevalence, because either they are unable to combat these infections or the cost to fitness of mounting a stronger immune response is too high. This argument is only cogent in the context of immunological responses to parasites where protective immunity against future re-infections is weak, and for parasites that have intra-annual life cycles. The age of hosts would become a factor if the parasites had a life history that went beyond a seasonal or annual cycle. Finally, we stress that MNIR explains only differences among species in GI parasite prevalence with respect to a baseline average for the foraging guild in question. Many other ecological factors are needed to explain the baseline average itself.

Table 1. Results of strongyle nematode prevalence and host traits based on species values and independent contrasts. Species values were analyzed using logistic regression weighted by sample size. Independent contrasts were analyzed using multiple regression including *N* as a measure of relative sampling effort. As sampling effort was always non-significant ($p>0.4$) it is not presented here.

Host species	Predictors	Species values				Independent contrasts			
		β	SE	t	p	β	SE	t	p
All species									
	intercept	-11.21	2.26	-4.953	0.0008	0.00	0.00	-	-
	residual intake	135.55	28.93	4.685	0.0011	1413.65	303.33	4.66	0.0012
	% grass in diet	-0.03	0.01	-2.916	0.0171	-0.42	0.14	-2.90	0.0177
	log(daily intake)	1.70	0.71	2.382	0.0411	13.67	9.91	1.38	0.2009
Ruminants									
	intercept	-7.34	1.76	-4.17	0.0141	0.00	0.00	-	-
	residual intake	89.76	22.47	4.00	0.0162	1379.56	305.10	4.52	0.0106
	% grass in diet	-0.02	0.01	-3.24	0.0317	-0.43	0.14	-3.01	0.0394
	log(daily intake)	0.03	0.62	0.67	0.5395	6.91	10.15	0.68	0.5335

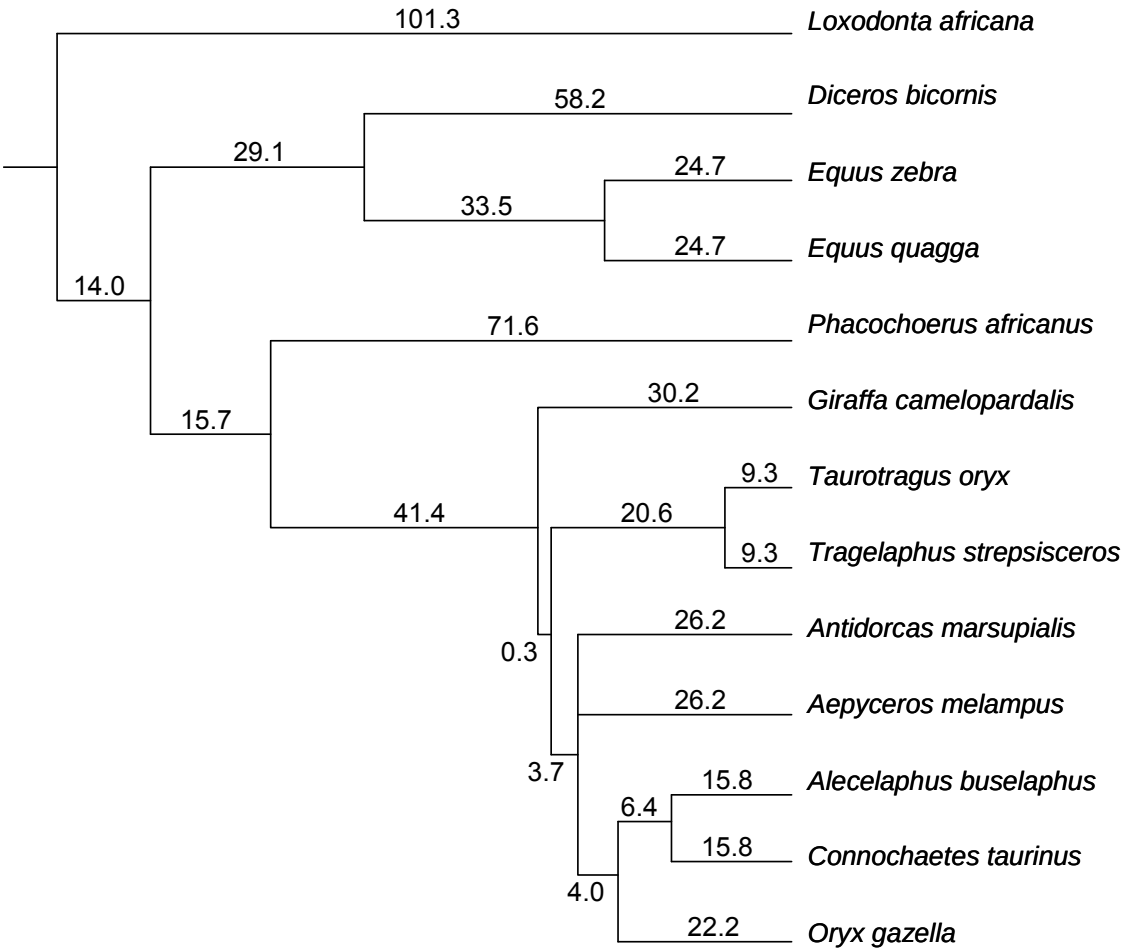

Figure 1. The phylogeny of host species with branch lengths in millions of years before present, based on the mammal supertree of Bininda-Emonds et al. (2007). Branch lengths are not drawn to scale.

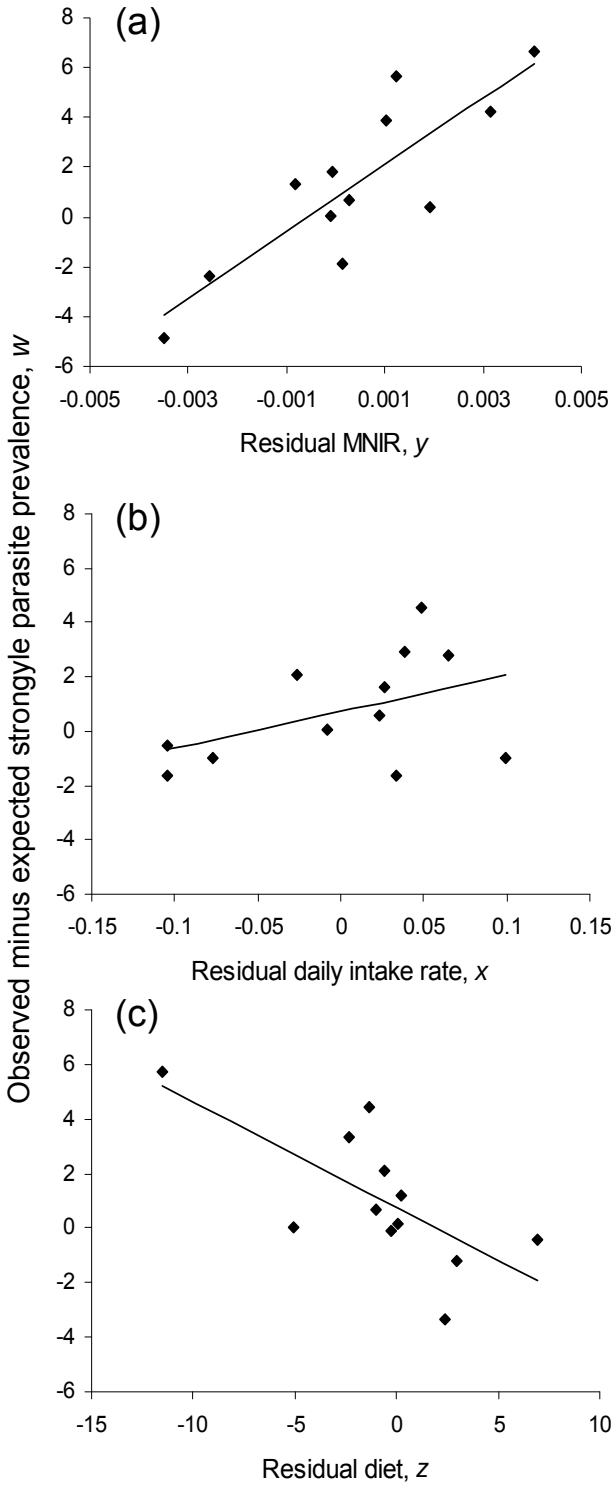

Figure 2. Rescaled partial regression leverage plots showing the effects of residual a) MNIR, *y*, b) daily intake rate, *x*, and c) diet, *z*, on the phylogenetically corrected multiple regression of strongyle prevalence, *w*.

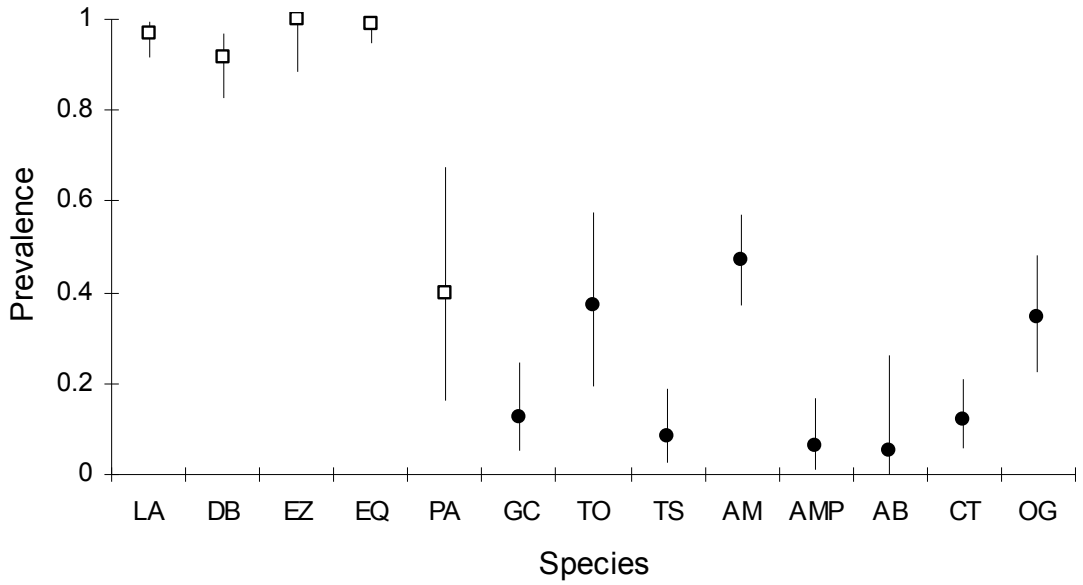

Figure 3. Strongyle nematode prevalence and the 95% binomial confidence intervals (based on sample size) for each study species. AB, *Alcelaphus buselaphus* (red hartebeest); AM, *Antidorcas marsupialis* (springbok); AMP, *Aepyceros melampus petersi* (black-faced impala); CT, *Connochaetes taurinus* (blue wildebeest); DB, *Diceros bicornis* (black rhino); EQ, *Equus quagga* (plains zebra); EZ, *Equus zebra* (mountain zebra); GC, *Giraffa camelopardalis* (giraffe); LA, *Loxodonta africana* (elephant); OG, *Oryx gazella* (gemsbok); PA, *Phacochoerus africanus* (common warthog); TO, *Taurotragus oryx* (eland); TS, *Tragelaphus strepsiceros* (greater kudu). Non-ruminant herbivores are indicated with open squares, ruminant herbivores with filled circles. The sample sizes range from 15 for PA to 100 for LA, EQ and AM.

Chapter 3: Variation in fecal water content may confound estimates of gastrointestinal parasite intensity in wild African herbivores

Introduction

Many ecological, veterinary, or conservation questions relating to host-parasite systems require quantitative estimates of parasitism within particular hosts, and of the variation in parasitism among hosts in a population of interest (Anderson & May 1978). Estimation of gastrointestinal (GI) parasite burdens in living animals, however, is no simple task. In post-mortem examination, it is possible to directly count or estimate the parasite burden within the digestive tract. In live animals, particularly in free-ranging wildlife, estimation of parasite burden is much more challenging. Available non-invasive methods estimate GI parasite intensity indirectly, by quantifying parasite propagules or DNA excreted in fecal matter, or by detecting antigens in feces using ELISA-based tests (Wilson *et al.* 2001). These indirect measures may produce biased estimates of parasitism in hosts and may have unknown or varying specificity and sensitivity (Wilson *et al.* 2001), but without lethal sampling are often the only means available for estimating parasite intensity in wildlife.

To reduce possible biases introduced though these indirect methods, several studies have highlighted factors that can confound estimates of parasite intensity from fecal measures. For example, the time of day in which sampling occurs can affect egg/oocyst output in feces and therefore stratifying sampling times can help reduce within-day variation in intensity estimates (Ezenwa 2003; Villanúa *et al.* 2006). The symptoms of disease associated with parasite infection can also affect quantitative parasite measures. High burdens of GI parasites can cause diarrhea, which increases the ratio of water to dry matter in feces and reduces fecal egg counts (Le Jambre et al. 2007). To correct for the influence of variation in the water content of feces on fecal egg counts,

various adjustment factors have been proposed (Levine and Clark 1956; Gordon 1967; Le Jambre et al. 2007) which rescale fecal egg counts based on visually assessed categorical estimates of fecal consistency.

Our study examined fecal water variation and parasite intensity estimates from fecal egg counts obtained using the McMaster technique, a commonly used non-invasive method for quantifying parasitism (Bowman 2003). This technique is based on the wet-weight of feces, and variation in fecal water content may confound or obscure patterns in relation to the variables of interest. Although we present data in the context of this particular method, our findings have relevance to any estimate of parasite intensity that is obtained from examination of feces.

In this study we examined variation in fecal water content in relation to host age class (juvenile, yearling, adult), sex and seasonality, three factors commonly evaluated in ecological studies. We used quantitative measures of fecal water content to assess these differences for two free-ranging wild ungulate species, springbok (*Antidorcas marsupialis*), and plains zebra (*Equus quagga*, previously *Equus burchelli*). Because researchers are not always able to directly measure fecal water content in the field, we also assessed whether a categorical scale for estimating fecal water content adequately described the measured variation in water content of fecal samples. Finally, we evaluated whether or not variation in fecal water content significantly influenced the outcome of statistical analyses of ecological patterns in strongyle nematode intensity for these two wild ungulates.

Materials and methods

Study site

This study was undertaken in Etosha National Park, a 22,915 km^2 reserve in northern Namibia between 18˚30'-19˚30'S and 14˚15'-17˚10'E. Etosha contains a 4,760 km^2 salt pan, a dominant geological feature which is the remnant of a paleolake (Hipondoka *et al.* 2006). The vegetation is classified as arid savanna (Huntley 1982) with a single wet and a single dry season each year. Much of Etosha National Park is covered by mopane (*Colophospermum mopane*) shrubveld or treeveld, but extensive sweet grassveld plains surround the Etosha salt pan (le Roux *et al.* 1988). The only perennial water comes from boreholes and artesian or contact springs (Auer 1997). Rainfall is strongly seasonal, mainly falling between November and April, with the greatest monthly rainfall occurring in January and February (Engert 1997).

Sample collection

Between February 2006 and April 2008, we collected fecal samples from zebra (N=666) and springbok (N=634) in the central Okaukuejo section of Etosha National Park. Samples were collected between 7:00-13:00 to reduce diurnal variation in parasite egg output. During sample collection, we used binoculars to watch individuals defecate and collected a homogenized sub-sample of the feces within 10 minutes of deposition to avoid desiccation. For each fecal sample collected, we recorded the date, time, species, age, sex, and the fecal consistency (pellets formed, semi-formed, unformed, liquid). We used the aging criteria (juvenile: <1 year, yearling: 1-2 years and adult: 2+ years) used by Gasaway et al. (1996) to characterize individuals. Seasons were defined based on

rainfall, with the wet season from January-April, and the dry season from May-October. No samples were collected in November or December.

Samples were collected in a zip-lock plastic bag and immediately placed in a refrigerator mounted on the vehicle. Each sample was evaluated for parasites within 48 hours of collection using a modification of the McMaster method for fecal egg counts (F.A.O., 2005). In brief, this method requires the combination of 4g of homogenized fresh fecal matter with 56ml of a saturated salt (NaCl) solution, removal of large plant debris via a strainer, and filling of each chamber on a McMaster slide with a separate homogenized aliquot of the filtrate. The number of eggs observed in each chamber using a compound microscope is added together and multiplied by 50 to get the number of eggs per gram of feces. After parasitological analysis, samples were stored frozen (-20°C) in their sealed, original zip-lock bags.

A subset of the frozen samples were selected in 2007 and 2008 for quantitative fecal water content measurement (N=243 for zebra, N=266 for springbok). This subset included samples collected in the wet and dry seasons of 2006-2007, and were chosen to represent the sexes equally and all age classes in both species. Samples were defrosted completely and then mechanically homogenized while still in the bags. We then removed roughly one third of each sample for weighing. Each of these subsamples was weighed on a piece of aluminum foil, sealed loosely into a foil packet and placed in a drying oven (80-100°C) for 48 hours. Each sample was weighed again post-drying. Percent water content (m) was determined by dividing the difference between the wet (w_w) and dry weights (w_d) by the original wet weights and converting to a percentage (*i.e.*, $m=100(w_w - w_d)/w_w$).

Statistical analyses

The relationship between fecal water content and season or sex was evaluated using a *t*-test for each species. We excluded the juvenile and yearling age classes when analyzing the effect of sex on percent water. For the effect of age on fecal water content we used an analysis of variance. We tested whether our categorical fecal consistency scale could adequately describe variation in fecal water content using an analysis of variance. We estimated fecal water content on a four-point scale (1: pellets formed, 2: semi-formed, 3: unformed, or 4: liquid, ranging from viscous to watery) but there were so few liquid samples (3/635 for springbok and 3/666 for zebra) that we combined these samples with the unformed category and used a three-point scale for our analyses.

We evaluated whether or not fecal water content significantly influenced fecal egg counts using a generalized linear model (GLM) that also included season, age and sex as independent variables. We used a negative binomial GLM (Wilson *et al.* 1996), because the count data were overdispersed and could not be transformed for parametric analyses.

For simplicity, we focus this analysis on the strongyle nematodes (Nematoda, Strongylida), although other parasites were observed in fecal flotation including nematodes in the genus *Strongyloides*, coccidia in the genus *Eimeria*, and cestodes in the family Anoplocephalidae (Turner, unpublished data). Parametric analyses were performed using JMP 4.1 (SAS Institute, 2001); GLMs were performed in R 2.7.0 (R Core Development Team, 2008). Means are reported with standard errors unless otherwise stated.

Results

Variation in percent water of feces

The percent water content of springbok feces was significantly higher in the wet season than the dry season (t=-8.2, $d.f.$=264, p<0.0001; Fig. 1a). In zebra, the percent fecal water content was marginally higher in the wet season than the dry season (t=-2.3, $d.f.$=241, p=0.022; Fig. 1a). While this was a statistically significant result, the difference between the means was very small (Fig. 1a), with little of the observed variation in fecal water content described by season.

The percent water of zebra feces was significantly related to age (F=247.6, N=243, p<0.0001; Fig. 1b), with juvenile zebra feces having nearly half the water content of yearling or adult feces. Juvenile springbok feces also had significantly lower percent fecal water than yearling or adult feces (F=10.1, N=266, p<0.0001; Fig. 1b), but the pattern was not as pronounced as for zebra. Adult male springbok feces had significantly higher percent water than adult female feces (t=-3.66, N=171, p=0.0003; Fig. 1c), but there was no significant difference in percent water content between the feces of adult male or female zebra (t=1.79, N=175, p=0.076; Fig. 1c).

Fecal consistency and percent water content

The categorical fecal consistency scale was able to describe 32% of the variation in fecal water observed in springbok (pellets: 52.9±1.0%, semi-formed: 65.2±1.3%, unformed: 71.7±1.7%, F=55.1, N=240, p<0.0001). For zebra, the consistency scale was significantly related to percent water, but the variance explained was only 3% (pellets:

62.5±0.7%, semi-formed: 66.5±3.3%, unformed: 74.9±4.3%, F=4.6, N=228, p=0.011). The poor fit of this model for zebra was perhaps driven by the very low water content of juvenile feces and the homogeneity of fecal consistencies. Juvenile fecal pellets were very dry compared to pellets in other age classes, and when we excluded juveniles from the model, the fit improved but was still low with only 13% of variance explained (F=15.6, N=208, p<0.0001). For zebra, the consistency scale did not successfully capture variation in percent fecal water, as the consistencies observed were very homogenous: 93% of fecal samples were classified as formed pellets.

In light of the low success in describing variation in fecal water content using the categorical scale, we felt it was better to evaluate the effect of water variation on fecal egg counts using only the individual measurements of percent dry matter, and not a categorical adjustment factor calculated from consistency measurements. Additionally, calculating an adjustment factor (as done by Le Jambre *et al.* 2007) assumes that the ordinal categories (consistency values 1-3) are continuous variables and that the distance between 1 and 2 is the same as between 2 and 3, an assumption we chose not to make.

Fecal egg counts and percent water content

The percent water content of feces was significantly related to fecal egg counts for springbok and zebra when tested alone, with fecal egg counts increasing as water content increased, although the slopes were very close to zero (Table 1). There was no significant influence of the measured fecal water content on egg counts once the ecological and demographic variables were included in analyses (Table 1). As a statistical parameter the effect of fecal water content was swamped out by the much great

differences in fecal egg counts by season and age (Table 2). For sex, however, the percent differences between males and females due to fecal water content and parasite egg counts are of similar magnitude, and the directionality (+/-) of the difference is opposite for parasite counts and water content for each species (Table 2). These two factors together make sex the variable with the greatest potential for water variation to confound significance estimates.

For strongly significant variables, such as season and age, water variation had little effect on the slope or significance of the egg count estimates. For variables which were borderline significant, like sex, water variation may still confound estimates, despite the non-significance of percent water as a model parameter. To demonstrate this point we performed the GLMs using estimated fecal egg counts (FEC) rescaled by the amount of dry matter per gram of fecal matter: $FEC_{dry\text{-}weight}=(FEC_{wet\text{-}weight})/(1-m/100)$, where m is the percentage of water in the sample.

Sex appeared nearly significant using count estimates based on wet weight (springbok: p=0.052; zebra: p=0.055; Table 3), but when analyses were performed with estimated dry-weight counts, host sex became non-significant for both species (springbok: p=0.234; zebra: p=0.268; Table 3).

Discussion

This study examined whether or not variation in fecal water content significantly biased estimates of parasite intensity recorded for two species of wild ungulates. We found evidence that water variation could lead to a spurious conclusion that parasitism was significantly different between males and females of each species. Although we

recorded significant differences in fecal water content among the categories of season, age and sex, we found no statistical relationship indicating an effect of fecal water content on model estimates of egg counts by season or age. The seasonal and age-related differences in fecal water content were obscured by much greater differences in strongyle egg counts for these same variables.

Patterns of variation in the percent water content of fecal samples in relation to the ecological and demographic variables were not consistent for both species studied. Springbok showed the greatest variation seasonally, but also had significant differences in fecal water content by age and sex. Zebra had the greatest variation in fecal water content by age, and little variation by season or sex. These differences between zebra and springbok may be due to species differences in feeding ecology, drinking behavior, mating system and digestive physiology.

Springbok are ruminants, have a mixed diet composed of grasses and shrubs, and seasonal changes in their preferred food sources (Skinner & Louw 1996). Springbok will drink when water is abundant, but do not require drinking water to achieve water balance in dry periods or regions (Nagy & Knight 1994), an ecological adaptation that may result in greater seasonal variation in fecal water content. In contrast to springbok, zebra are non-ruminant grazers and a much more water-dependent species (Skinner & Chimimba 2005); their relative homogeneity of diet type and water intake may reduce the seasonal variation in zebra fecal water content.

The drier feces observed for juveniles of both species may be due to dietary differences between juveniles and older animals. The digestive systems of juveniles must adjust from a primarily milk-based diet to a primarily plant-based diet during their first

year. Springbok lambs move to a plant-based diet far sooner than do zebra foals, which may explain why the age-related pattern in fecal water content was much less pronounced in springbok than in zebra. Springbok are weaned around four months of age while zebra are only weaned after 11 months (Skinner *et al.* 2002). The differences in fecal water content by sex for springbok and not zebra may relate to mating system; male zebra remain with female groups whereas territorial male springbok segregate from female groups (Skinner & Chimimba 2005). Sexual segregation in springbok may lead to sex differences in fecal water content through variation in diet, drinking frequency or spatial use between the sexes, however more research would be needed to explain this pattern.

Given observed differences in fecal water content, various correction factors have been proposed to account for water variation in fecal egg counts based on visually assessed categorical scales (Levine & Clark 1956; Gordon 1967; Le Jambre *et al.* 2007). Depending on the species studied, a categorical scale may or may not be able to adequately describe this variation. Our categorical fecal consistency scale described 32% of the variation in percent fecal water observed in springbok but only 3% of the variation for zebra, or 13% when juveniles were excluded from the analysis. There was far less variation in the percent water content or consistency of zebra feces than of springbok feces, making it difficult to capture differences on a categorical scale. The low success in describing water variation with a categorical scale implies that an adjustment factor may not be an adequate means of correcting for fecal water variation, and that the fit can vary substantially by species. We recommend evaluating the ability of a categorical scale to describe the water variation observed in a species of study before using it with correction factors developed for another species (*e.g.*, sheep: Gordon, 1967; Le Jambre *et al.* 2007).

We found a positive relationship between fecal water content and egg counts, although once we included the ecological and demographic variables in the model, fecal water content was no longer significant. A slight positive relationship found between fecal water content and egg counts indicates that water content was not reducing egg counts in this study. Le Jambre and colleagues (2007) found a significant negative relationship between a simulated fecal egg count and fecal water content in sheep given parasite burdens sufficient to induce diarrhea. If increasing fecal water content is negatively related to parasite count, then it could indeed be biasing estimates of parasite intensity. However, the severity of diarrhea produced in their experiment was rarely observed in springbok or zebra; nearly all feces encountered during our study were on the drier half of their six-point scale. It may be that diarrhea must be present in order for an effect of fecal water content on egg counts to be strongly apparent.

Variation in fecal water content may have a limited potential to confound estimates of parasite intensity from fecal egg counts in free-ranging wildlife systems. Heavily parasitized individuals are those most likely to exhibit signs of disease (*i.e.,* increased fecal water content from diarrhea). Many wildlife populations are subject to predation and predators may selectively remove heavily parasitized individuals in comparison to non-parasitized or minimally parasitized individuals (Ives & Murray 1997). Additionally, free-ranging animals can modulate their foraging to select food items which improve parasite resistance or contain antiparasitic compounds (Lozano 1991). These factors may help explain why, when contrasting patterns of parasite intensity in two wildlife species by season and age, we did not see a significant influence of fecal water content.

In conclusion, we found that fecal water content varied with each of the variables commonly examined in ecological studies (season, age and sex), and had a slightly positive relationship with increasing fecal egg counts. The differences in parasite counts between seasons and among age classes of hosts were so great that fecal water variation was trivial in comparison and did not alter fecal egg count conclusions. For sex, however, fecal water variation did change the model outputs and conclusions regarding parasite loads. In cases for which significance levels are borderline, or in which water content is increasing as egg counts are decreasing, we caution that analyses may be confounded by fecal water variation. We recommend assessing how variation in water content relates to the variables of interest to control for possible confounding effects when performing quantitative estimates of parasite intensity using fecal indices. Although we did not address individual variation here, we expect that controlling for water content of feces may improve the repeatability of successive parasite estimates collected from individuals.

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Table 1. Negative binomial GLMs of strongyle fecal egg counts by percent fecal water alone and percent fecal water with the ecological variables (age, sex, season) for springbok and zebra.

Species	Model	Parameter	Point estimate	S.E.	*Z*	*p*
springbok	water	fecal % water	0.019	0.01	2.6	0.008
	all variables	season[wet]	1.160	0.23	5.0	<0.001
		age[juvenile]	-1.972	0.37	-5.3	<0.001
		age[yearling]	0.375	0.23	1.6	0.103
		sex [male]	-0.386	0.19	-2.1	0.039
		fecal % water	-0.001	0.01	-0.1	0.884
zebra	water	fecal % water	0.014	<0.01	2.9	0.004
	all variables	season[wet]	0.542	0.10	5.3	<0.001
		age[juvenile]	-0.833	0.32	-2.6	0.009
		age[yearling]	-0.117	0.14	-0.8	0.405
		sex [male]	0.183	0.10	1.9	0.060
		fecal % water	-0.005	0.01	-0.6	0.564

Table 2. The percent change in median fecal egg count and mean percent water from dry to wet seasons, from juvenile to adult age classes and from females to males for zebra and springbok. Here, adults are all animals greater than one year, as there was no significant difference between animals 1-2 and 2+ years of age for fecal egg count or percent fecal water.

Species	Variable	% Difference between means or medians		
		Dry-Wet Seasons	Juveniles-Adults	Females-Males
zebra	egg count	125	316	27
	% water	2	94	-2
springbok	egg count	1300	1000	-18
	% water	28	25	13

Table 3. Negative binomial GLMs of strongyle fecal egg counts (eggs/g) based on wet-weights or dry-weights of feces by age, sex and season for springbok and zebra.

Species	Count method	Parameter	Point estimate	S.E.	*Z*	*p*	
springbok	wet	season[wet]	1.201	0.20	6.0	<0.001	***
		age[juvenile]	-2.023	0.34	-5.9	<0.001	***
		age[yearling]	0.385	0.23	1.7	0.094	*
		sex [male]	-0.357	0.18	-1.9	0.052	*
	dry	season[wet]	1.496	0.21	7.1	<0.001	***
		age[juvenile]	-2.159	0.36	-6.0	<0.001	***
		age[yearling]	0.455	0.24	1.9	0.062	*
		sex [male]	-0.232	0.19	-1.2	0.234	ns
zebra	wet	season[wet]	0.527	0.10	5.4	<0.001	***
		age[juvenile]	-0.665	0.18	-3.8	<0.001	***
		age[yearling]	-0.107	0.14	-0.8	0.442	ns
		sex [male]	0.187	0.10	1.9	0.055	*
	dry	season[wet]	0.622	0.10	6.2	<0.001	***
		age[juvenile]	-1.379	0.18	-7.7	<0.001	***
		age[yearling]	-0.157	0.14	-1.1	0.268	ns
		sex [male]	0.158	0.10	1.6	0.110	ns

***=p <0.001, **=0.001<p< 0.05, *=0.05<p <0.1, ns=p >0.1

Figure Legend

Figure 1. The mean±SE percent fecal water content for zebra and springbok by a) season, b) age and c) sex. Only adult individuals were used in the comparison of water content by sex.

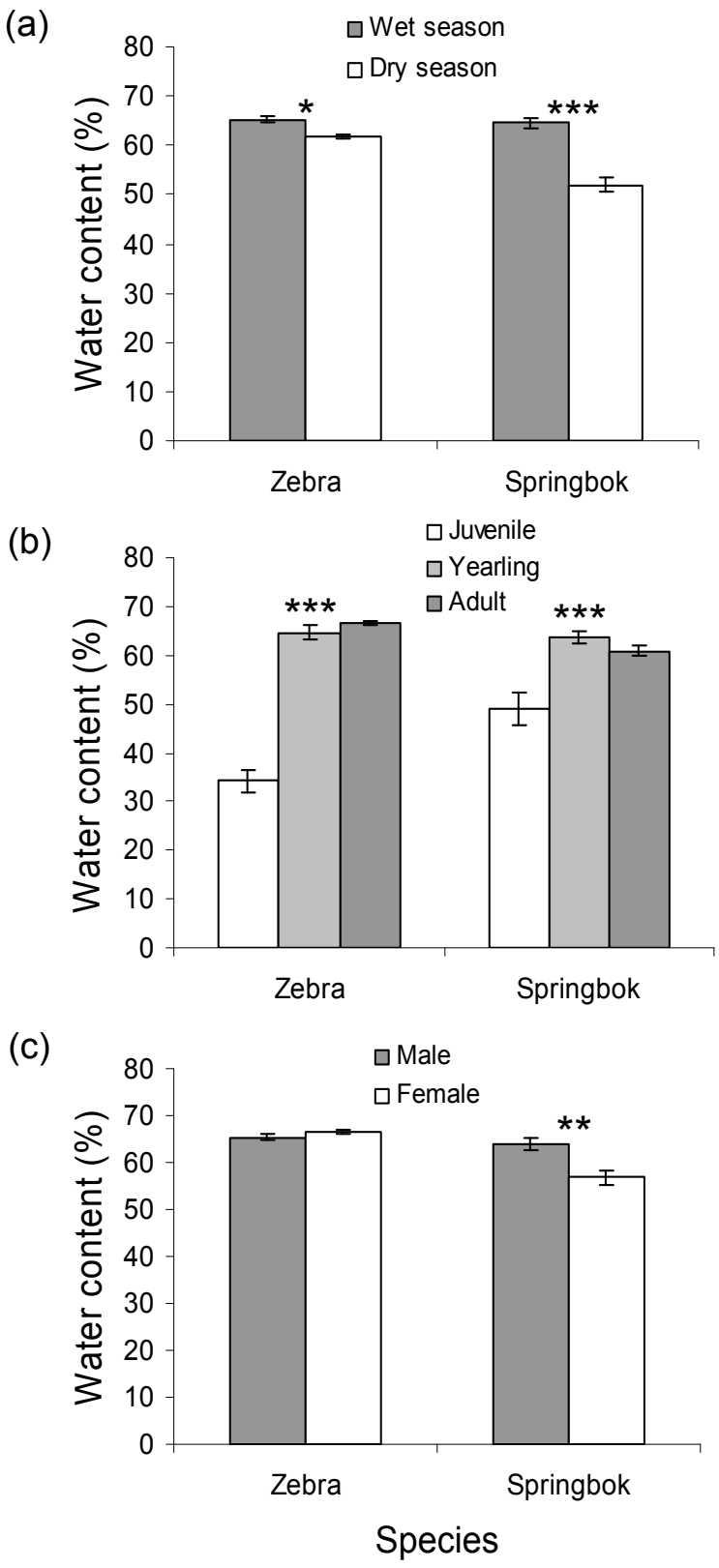
(a)
Wet season
Dry season
Water content (%)
80
70
60
50
40
30
20
10
0
*
***
Zebra
Springbok
(b)
Juvenile
Yearling
Adult
Water content (%)
***
***
Zebra
Springbok
(c)
Male
Female
Water content (%)
**
Zebra
Springbok
Species

Fig. 1

Chapter 4: The influence of seasonality and host demography on gastrointestinal parasitism of plains ungulates

Introduction

Seasonal changes in rainfall, temperature and resource availability may have a strong influence on disease dynamics (Altizer *et al.* 2006). Characterizing disease dynamics also requires an understanding of how the host age structure and immune response affects the host-parasite system (Cattadori *et al.* 2005; Cornell *et al.* 2008). These factors are not mutually exclusive, however, for host immunity may vary seasonally in relation to changes in reproduction, stress, nutrition, and photo period (Martin *et al.* 2008). Also, synchrony in host parturition concentrates young at times of resource abundance (Sinclair *et al.* 2000) adding a pulse of susceptible, immunologically naïve hosts to the population (Altizer *et al.* 2006). Finally, seasonal environmental changes can influence transmission rates of gastrointestinal parasites by affecting the intensity of parasite propagules produced, the development and survival of those propagules in the environment, and host contact with infectious free-living parasites (Stromberg 1997; Altizer *et al.* 2006).

Identifying how seasonality may affect host-parasite relationships first requires an understanding of the parasite life cycle and the host immune response to parasite infection. In this study we focus on two groups of directly transmitted, orally ingested parasites, the strongyle nematodes (Nematoda, Strongylida) and *Eimeria* spp. coccidia (Protozoa, Eimeriidae). Both of these parasite groups are generally pathogenic and responsible for widespread production losses in livestock (Bowman 2003). Strongyles are fairly generalist with respect to host species whereas *Eimeria* generally specialize on a single host species. Hosts can acquire protective immunity that is specific to a particular *Eimeria* species (Yun *et al.* 2000). The immune response to *Eimeria* is

complex, involving aspects of the T helper type 1 (Th1) intracellular and type 2 (Th2) humoral immune response systems, dominated by the Th1 response (Hong *et al.* 2006). In contrast, nematodes are multicellular parasites which do not invade host cells, and the immune response against gastrointestinal nematodes is mainly through the Th2 response (Urban *et al.* 1992). Once established, these relatively long-lived parasites are able to successfully evade the host's immune system by modulating and regulating the immune response in their favor (Hayes *et al.* 2004; Maizels *et al.* 2004). Host immune responses against nematodes are more effective in preventing establishment of invading larval stages, a process called concomitant immunity, than in clearing adult nematode burdens.

The strongyle nematode eggs excreted with feces undergo early-stage larval development in the fecal pellets, after which the infectious third-stage larvae migrate onto vegetation, seeking foraging hosts. Development to the third-stage larva is dependent on temperature and humidity, with increasing temperature and humidity generally associated with faster development and more eggs developing, but with upper and lower thresholds for both factors (O'Connor *et al.* 2006). It generally takes between five days to a few weeks for third-stage larvae to appear on vegetation following egg deposition, depending on the parasite species (O'Connor *et al.* 2006; Nielsen *et al.* 2007). First- and second-stage larvae are quite susceptible to desiccation (Nielsen *et al.* 2007). The motile third-stage larvae have a cuticle to protect against desiccation, but their movement requires a continuous film of moisture on the vegetation (O'Connor *et al.* 2006). Third-stage larval survival is greatly reduced in tropical conditions where larvae are exposed to high temperatures or prolonged dry periods (Banks *et al.* 1990; O'Connor *et al.* 2006; Nielsen *et al.* 2007). After ingestion, third stage strongyle larvae encyst in the mucosal wall of

the gut, develop into fourth stage larvae, and then excyst into the lumen or mucosal epithelium and develop into adult nematodes. Larvae may remain encysted and delay final development for months to years in times of environmental adversity, a process called hypobiosis (Gibbs 1982). Hypobiosis is a common phenomenon associated with seasonal changes, high nematode density in the host or development of host resistance (Balic *et al.* 2000). Despite similarities in the development and transmission of these parasites, strongyle species vary in larval niches in the gut and the feeding mode of adult nematodes (Balic *et al.* 2000).

The lifecycle of *Eimeria*, as an intracellular parasite, differs from that of the strongyle nematodes. When sporulated *Eimeria* oocysts are ingested by a host, the sporozoites excyst and enter epithelial cells of the small intestine initiating an asexual reproduction cycle, infecting new host cells in each cycle (Fayer 1980). This destruction of epithelial cells can result in diarrhea, anemia and in severe cases, death. A sexual phase also occurs in the epithelial cells producing the unsporulated oocyst which leaves the cell and is excreted in fecal matter. Unsporulated *Eimeria* oocysts require a period of development to become infectious, sporulated oocysts. In the environment, sporulation of oocysts is enhanced by warm temperatures and humidity, and moisture is the key limiting factor in the survival of sporulated oocysts in the environment (Fayer 1980).

In this study we examine seasonal, age- and sex-related variation in gastrointestinal (GI) parasite prevalence and propagule-shedding intensity in an assemblage of four herbivorous mammals utilizing a shared habitat in Etosha National Park, Namibia. We define prevalence as the proportion of individuals examined that are shedding parasite propagules in feces and intensity as the estimated number of parasite

propagules shed per gram of feces in infected individuals. Prevalence is an estimate of how common a parasite is in a host population at a particular period of time and intensity is an estimate of an individual's ability to control infection given that parasites are present and actively reproducing. Both prevalence and average intensity measures are needed to estimate the intensity of propagule inputs into the environment, an essential step towards understanding seasonal and demographic variation in host-parasite contact and transmission rates.

We expected to see a strong, positive relationship between parasite presence or intensity and monthly rainfall. In a sub-tropical, semi-arid environment, humidity may be the limiting factor for *Eimeria* or strongyle transmission, as dry conditions restrict development and reduce survival of propagules in the environment, and prevent larval movement (Fayer 1980; Banks *et al.* 1990; O'Connor *et al.* 2006; Nielsen *et al.* 2007). If acquired immunity limits parasitism in hosts, we expected to see a reduction in parasite intensity with host age, as immunity modulates infection in the most highly parasitized individuals and reduces susceptibility of older hosts (Woolhouse 1992). As an acquired immune response against *Eimeria* spp. is observed in other host species (Yun *et al.* 2000), we predicted that juvenile hosts would have greater *Eimeria* spp. presence and oocyst shedding intensity than adult hosts. In contrast, host acquired immunity is less effective at controlling strongyle nematode infections (Hayes *et al.* 2004; Maizels *et al.* 2004) and we therefore we predicted that strongyle presence and intensity would increase with host age. If differences in parasite prevalence or intensity occur by host sex, we expected to see greater differences in species that segregate sexually versus those that do not.

Methods

Study site

Etosha National Park is a 22,915 km^2 reserve in northern Namibia between 18˚30'-19˚30'S and 14˚15'-17˚10'E (Figure 1). Etosha contains a 4,760 km^2 salt pan, a dominant geological feature which is the remnant of a palaeolake (Hipondoka *et al.* 2006). The only perennial water comes from boreholes and artesian or contact springs (Auer 1997). The mean annual rainfall (± standard deviation) at Okaukuejo station in the center of the park was 384±137mm from 1934-2008. Rainfall is strongly seasonal and unimodal, with most rain falling between November and April, and peak rainfall in January and February (Figure 2). Although the rainfall season can begin as early as September, rainfall sufficient to facilitate significant grass growth generally occurs in January or February (du Plessis 2001). Beyond rainfall, precipitation in the form of dew does occur in immeasurable amounts (<0.1mm) for up to four weeks after cessation of the rains (Berry 1980). The mean monthly temperatures range from lows of 25°C maximum and 6°C minimum in June and July to highs of 34-35°C maximum in October-December and minimums of around 18°C November-February (Figure 2).

The vegetation in Etosha is classified as arid savanna (Huntley 1982). The focal area for this study was the Okaukuejo plains (Figure 1) which surround the Etosha pan and are comprised of extensively grazed short grassland and dwarf shrub savanna. The dominant grasses in this habitat are *Enneapogon desvauxii, Aristida adscensionis* and *Eragrostis nindensis*, and the dominant dwarf shrubs are *Leucosphaera bainesii, Cyathula hereroensis, Monechma tonsum, M. genistifolium* and *Petalidium englerianum*

(le Roux *et al.* 1988). This extensive grassland habitat is flanked by mopane (*Colophospermum mopane*) treeveld, but includes stands of woody plants including *Acacia nebrownii, A. tortilis, A. mellifera, A. reficiens, Catophractes alexandri, Aloe littoralis* and *Albizia anthelmintica* (le Roux *et al.* 1988). The soils in this area are relatively shallow with a limestone bedrock, moderate fertility, and high salinity and alkalinity (Beugler-Bell & Buch 1997).

Host species

The study focused on four species of herbivore that commonly utilize the grassland and dwarf shrub savanna habitats of Etosha, the "plains ungulates." These species are springbok (*Antidorcas marsupialis*), plains zebra (*Equus quagga*), gemsbok (*Oryx gazella*) and blue wildebeest (*Connochaetes taurinus*). Springbok, wildebeest and gemsbok are in the family Bovidae whereas zebra are in the family Equidae. Park-wide population estimates (with 95% confidence intervals all rounded to the nearest 100) for these species in 2005 were 15,600 (13,200-17,900) for springbok, 13,000 (10,900-15,000) for zebra, 5,700 (5000-6400) for gemsbok and 4,200 (3000-5500) for wildebeest (Namibian Ministry of the Environment and Tourism unpublished aerial survey data). Zebra and wildebeest are mainly grazers with a diet composed respectively of 92% and 90% C4 plants (Sponheimer *et al.* 2003; Codron *et al.* 2007). Springbok and gemsbok are considered mixed feeders although the diet of gemsbok has far more grass than the diet of springbok, with 81% vs 23% C4 plants in the diet, respectively (Sponheimer *et al.* 2003; Codron *et al.* 2007). The timing of parturition in Etosha is strongly seasonal in wildebeest and springbok (birth peaks occurring in January), somewhat seasonal in zebra

(births mostly occurring between December and April), and largely aseasonal in gemsbok where births occur throughout the year with peak activity between April-December (Gasaway *et al.* 1996).

Sample collection

Gastrointestinal parasitism was evaluated from examination of fecal pellets collected between July 2005 and April 2008, for a total of 1714 fecal samples for parasitological analysis from the four ungulate species. Collecting periods for parasitism were July-August 2005, February-October 2006, February-June 2007 and January-April 2008. We collected samples from zebra (*N*=742) and springbok (*N*=731) across all sampling periods, and wildebeest (*N*=133) and gemsbok (*N*=102) only during the first two sampling periods. Wildebeest and gemsbok had relatively lower population sizes than zebra and springbok, and consequently their grouping behaviors and seasonal movements made it difficult to collect sufficient fecal samples from these species in order to assess monthly or age variation in parasitism. For example, gemsbok juveniles hide for the first month of life and thereafter juveniles and yearlings tend to be congregated in nursery herds (Skinner & Chimimba 2005). As a result, many groups observed in this study contained only adult animals. Also, despite being present in the study area in the dry season of 2005, wildebeest were mostly absent from the study area in the dry season of 2006 (personal observation). Because of these sampling difficulties, we ceased sampling of gemsbok and wildebeest in July of 2006.

Fecal samples were acquired from the study area within the first week of each month, when possible, between 7:00-13:00. Each month we collected samples along all

roads and at each waterhole within the study area in order to collect a representative sample of hosts from the study area and to avoid resampling of individuals. We aimed for 40 samples per species per month, with attention to the age/sex distribution of those samples. During sample collection we used binoculars to watch individuals defecate, recorded the locations of feces, and then collected a homogenized sub-sample of the feces within 10 minutes of deposition. For each fecal sample collected we recorded the date, time, species, sex and age of the defecator. Age was assessed in three categories: juveniles were <1 year, yearlings 1-2 years, and adults 2+years old. Age and sex were determined via horn growth and morphology and genitalia for springbok (Rautenbach 1971), wildebeest (Atwell 1980) and gemsbok (Dieckmann 1980). Zebra age and sex were assessed based on relative size, pelage and genitalia (Smuts 1975).

Parasitological analysis

Fecal samples were evaluated for GI parasites within 48 hours of collection using a modification of the McMaster method for fecal egg counts (FAO 2005), a commonly used non-invasive method for quantifying parasitism (Bowman 2003). In brief, this method requires the combination of 4g of homogenized fresh fecal matter with 56ml of a saturated salt (NaCl) solution (specific gravity 1.2), removal of large plant debris via a tea-strainer, and filling of each chamber on a McMaster slide with a separate homogenized aliquot of the filtrate. The number of eggs or oocysts observed in each chamber using a compound microscope was added together and multiplied by 50 to get the number of eggs or oocysts per gram of feces. Parasite prevalences were calculated from presence/absence data acquired using this technique.

Fecal egg counts provide a non-lethal means for estimating relative nematode burdens among hosts (Stear *et al.* 1995; Seivwright *et al.* 2004), although the relationship between nematode burden and fecal egg count is generally of unknown sensitivity and specificity (Wilson *et al.* 2001). Fecal egg or oocyst counts do provide an accurate measurement of how the input of parasite propagules into the environment varies in relation to factors of interest. Stratifying the sampling time of fecal collection, as we did in this study, helps reduce bias introduced through daily variation in egg or oocyst output (Ezenwa 2003; Villanúa *et al.* 2006). We also assessed in Chapter 3 the potential biases introduced through variation in the water content of fecal samples.

Flotation techniques are best for recovery of nematode and cestode eggs and protozoan cysts from feces, but fail to recover trematode cysts or nematode larvae (Bowman 2003). The parasite groups observed in this study included nematodes in the order Strongylida (hereafter "strongyle nematodes") and the genus *Strongyloides*, coccidia in the genus *Eimeria*, and cestodes in the family Anoplocephalidae. The strongyle nematodes, *Eimeria* spp. and cestodes are orally ingested, with direct transmission for the nematodes and *Eimeria* spp. and via accidental ingestion of the intermediate arthropod host for the cestodes (Bowman 2003). *Strongyloides* spp. are facultative parasites that are transmitted via milk or penetration of host skin (Bowman 2003). The strongyle nematodes comprise superfamilies Strongyloidea, Trichostrongyloidea and Ancylostomatoidea, which are similar in egg morphology, epidemiology, and veterinary importance (Bowman 2003). Although little is known about the effects these parasites have on wild hosts, *Strongyloides* spp. and Anoplocephalid infestations are often asymptomatic in domestic animals. The strongyle

nematodes and coccidia are pathogenic and responsible for widespread production losses in livestock (Bowman 2003). Of the parasites observed, we focus on the strongyle nematodes and *Eimeria* spp. as parasites that are directly transmitted via the environment (*i.e.*, without intermediate hosts) and that are generally pathogenic to hosts.

Data analysis

We quantified parasitism based on the presence/absence of parasites in individual hosts and the intensity of parasite propagule counts. Our definitions for prevalence—the proportion of individuals examined who were infected—and intensity—the estimated number of parasite propagules from infected individuals only—are as defined by Margolis et al. (1982).

Evaluation of relationships between monthly rainfall and parasite presence/absence or intensity was done for zebra and springbok only. Temporal patterns of parasite occurrence in gemsbok and wildebeest were assessed only at the seasonal scale. We defined the seasons based on peak periods in parasitism and not rainfall, therefore the wet season was January-May and the dry season was June-October. Additionally, due to low numbers of juvenile or yearling wildebeest and gemsbok sampled, we combined these two categories and evaluated age patterns with a two-class distinction.

Statistical analyses of parasite presence were done using multivariate logistic regression with season, age and sex as independent variables. Analyses of parasite intensity were done using generalized linear models (GLMs) with a negative binomial link function and season, age and sex as independent variables. For the host species with

lower sample sizes, we analyzed parasite presence and not intensity, as the number of infected individuals was deemed too low for statistical examination of the counts in positive individuals. Where parasite prevalences were reported, we included 95% binomial confidence intervals based on the sample sizes examined. Statistical analyses were done using R 2.7.0 (R Development Core Team 2008).

Results

Overall patterns in parasite prevalence

All four parasite types were observed in springbok and wildebeest, but we recorded no *Eimeria* spp. in zebra and no Anoplocephalid cestodes in gemsbok (Figure 3). Springbok had high prevalence (>60% of individuals infected) of strongyles, *Eimeria* spp. and *Strongyloides* spp. The prevalence of Anoplocephalid tapeworms was low for all host species (<5% of individuals infected) so these parasites were not considered further for statistical analyses. Due to low prevalence, we also did not consider further *Strongyloides* spp. in zebra or wildebeest or *Eimeria* spp. in zebra or gemsbok. Zebra had very high prevalence (98% infected) of strongyle and very low prevalence (<3% infected) for the other three parasite types.

Parasitism and monthly rainfall

The presence and intensity of all three parasite types in springbok was significantly related to rainfall one and two months prior to collection, with the strongest relationship to rainfall one month prior (Table 1). The intensity of strongyle egg counts in zebra was also significantly related to rainfall one and two months prior, with the

strongest statistical relationship to rainfall one month prior (Table 1). Increased rainfall was positively correlated with parasite intensity in both host species and with prevalence in springbok hosts (Figure 4). In zebra, the monthly prevalence of strongyles never dipped below 92% (Figure 4), and in a total sample size of 742 individuals, only 14 were negative for strongyles using our identification methods, 13 of which were young juveniles. These juveniles had likely not yet acquired burdens of reproductively active strongyles.

Seasonality, host demography and parasitism

The presence and intensity of parasites was strongly seasonal in most cases, with more infected hosts observed in wet seasons than dry seasons (Table 2). Exceptions to this included *Eimeria* spp. in gemsbok and wildebeest, and strongyles in zebra, which exhibited no statistically significant seasonal patterns in parasite presence (Figure 5).

Eimeria spp. occurred significantly more in younger animals than adults for gemsbok and wildebeest, and although the presence of *Eimeria* spp. in springbok did not vary significantly between age classes, the intensity of infections was higher in juveniles than yearlings or adults (Table 2). Strongyle nematodes showed the opposite pattern to *Eimeria* spp., with strongyle presence and intensity significantly higher in adults and yearlings than juveniles for springbok and zebra (Table 2). *Strongyloides* spp. presence was not significantly related to age for gemsbok or springbok, but the intensity of *Strongyloides* spp. egg counts in springbok was significantly higher in juveniles than yearlings or adults.

Strongyle presence was significantly higher in male than female springbok (strongyle prevalence: males=83.6%, females=78.9%; Table 2), but when infected, females had significantly higher strongyle intensities than males (mean strongyle intensity: females=1184 eggs per gram (epg), males=853epg; Table 2). In contrast, strongyle intensity in zebra was significantly higher in male than female hosts (mean strongyle intensity: females=2252epg; males=2608epg; Table 2) and there were no significant differences in strongyle presence between males and females (strongyle prevalence: females=98.4%, males=98.2%; Table 2). *Strongyloides* spp. was significantly more prevalent in male than female gemsbok (*Strongyloides* prevalence: males=32.1%, females=14.5%; Table 2). There were no significant patterns with sex observed for *Eimeria* spp. in the host species or for any parasite types in wildebeest.

Discussion

Disease dynamics can be strongly affected by seasonality and age-related host immune responses (Cattadori *et al.* 2005; Altizer *et al.* 2006; Cornell *et al.* 2008). We found evidence of strong protective immunity against *Eimeria* spp., and a weaker immune response against strongyle nematodes. The seasonal patterns further demonstrated that the long dry season may limit survival of parasite stages in the environment and parasite transmission.

We observed strong seasonal differences in parasite prevalence and intensity of propagule shedding for most parasite types in the four host species, with both measures higher in wet seasons than dry seasons. The strong seasonal relationship was further related to monthly rainfall, with peaks in parasite prevalence and propagule shedding

intensity occurring 1-2 months after peak rainfall. As this time frame is sufficient for both *Eimeria* and strongyles to complete their life cycles, the marked increase in parasitism may be due to a resumption of parasite transmission, or parasite activity, in the case of arrested strongyle larvae. Rainfall may be the main constraining factor affecting parasite dynamics in semi-arid systems by severely limiting transmission (Chiejina *et al.* 1989). In a semi-arid area of Mauritania, Jacquiet et al (1995) found that young goats born during the dry season were free from GI nematode infections until the following rainy season, indicating a lack of transmission. At our study site, we attempted to recover larvae from grasses utilized by grazing herbivores in April 2007, at the end of the wet season, but the grasses were dry and we were unable to recover nematode larvae from the samples (W.C. Turner unpublished data). The rainfall patterns in Etosha may restrict parasite transmission for up to 6 months of the year.

A long dry season may also limit the diversity of parasites in hosts and their prevalence, even in comparison to other semi-arid areas. The parasite diversity we observed in Etosha seems fairly depauperate compared with Ezenwa's (2004a) study of parasitism in bovids at the Mpala Research Center in Kenya, an area also classified as semi-arid. These studies used similar methods for parasite identification and Ezenwa distinguished eight different types of gastrointestinal parasites in bovid hosts (Ezenwa 2004a), whereas we observed only four different types. Additionally, the prevalences of strongyle nematodes in bovids recorded in our study are lower than those observed in Mpala. Possible reasons for these differences could be that Mpala is much closer to the equator than Etosha, and latitudinal gradients have been observed in parasitism of other host taxa, with increased parasite species richness in hosts at lower latitudes (Lindenfors

et al. 2007). However, Etosha has a lower annual rainfall (384mm) which falls in one season compared to Mpala which has greater annual rainfall (400-500mm) and bimodal rainfall peaks. Ultimately, the temporal pattern of rainfall may be more important for distinguishing spatial patterns of parasitism than differences in annual rainfall.

Male springbok were significantly more likely to have strongyle nematodes than were female springbok, but there was no sex difference in the occurrence of the other parasite types. The observed sex difference in strongyle prevalence could be due to the mating system of the host species. Springbok males are territorial, and males of territorial species may have higher contact rates with infectious larvae within the boundaries of their territories than individuals in the more widely roaming female groups (Ezenwa 2004a). Wildebeest are also a territorial species and although we did not observe sex differences in strongyle prevalence, this could be due to a relatively lower sample size. There were sex differences in springbok and zebra strongyle intensity, but these patterns may be due to sex differences in fecal water content biasing the results of the counting method (see Chapter 3). We did not see sex differences in the occurrence or intensity of *Eimeria* infections, but this could be due to the strong age-related patterns where *Eimeria* infections are concentrated in juvenile hosts, the age class least likely to show variation between the sexes.

Eimeria spp. oocyst-shedding intensity or prevalence was significantly greater in young hosts than in adult hosts for the three Bovid species. *Strongyloides* spp. egg-shedding intensity in springbok also decreased as host age increased. Although a decline in levels of infection with age may be related to acquired immunity and/or age-dependent variation in host exposure (Woolhouse 1992), infection with *Eimeria* generally induces a

long-lasting protective immunity in hosts (Yun *et al.* 2000). In our system acquisition of immunity is likely and there may or may not be age-related differences in *Eimeria* exposure.

Age-related infection patterns for strongyle nematodes showed a significant increase in prevalence with increasing host age. This may indicate that immune protection is less successful against strongyle nematodes than the other parasite types. Adult nematodes are relatively long-lived. Immune protection in the host is most effective against establishment of incoming larvae or constraining fecundity of adult nematodes rather than expulsion of the adult population (Balic *et al.* 2000). Once an adult strongyle population or community is established, the host may not easily clear the worm burden. This may explain why strongyle prevalence was near 100% in zebra with the exception of a few young juveniles. Strongyle prevalence in springbok did change seasonally, but still showed a pattern of increase with host age.

Factors that may reduce egg-shedding rates include density-dependent effects within the nematode population reducing individual nematode fecundity or host immune responses. Immune responses against adult nematodes can include constraints on adult nematode size, which reduces fecundity (Rowe *et al.* 2008), loss of the vulvar flap in female nematodes, or expulsion of adult nematodes (Balic *et al.* 2000). We observed little evidence of strong protective immunity against strongyles, as the intensity of strongyle egg counts increased with host age in zebra and springbok.

In conclusion, there is evidence from this free-ranging wildlife system of strong protective immunity against *Eimeria* spp., and a weaker immune response against strongyle parasitism. The seasonal patterns further demonstrate the potential effect a

long dry season may have on limiting the survival or transmission of parasites in the environment. Future work on these issues would be strengthened by study of temporal variation in the abundance of infectious parasite stages in the environment and by addition of a finer taxonomic resolution in parasite identifications.

Table 1. The relationship between parasite presence/absence or intensity and rainfall one, two or three months prior to sample collection, for springbok and zebra. For parasite presence/absence, the results presented are from logistic regressions, for parasite intensity, the results are from GLMs.

			rain 1 month prior		rain 2 months prior		rain 3 months prior	
host species	parasite data type	dependent variable	statistic	*p*	statistic	*p*	statistic	*p*
springbok	presence	strongyles	24.1	<0.0001	22.2	<0.0001	0.9	0.3453
		Strongyloides	70.0	<0.0001	48.2	<0.0001	1.3	0.2574
		Eimeria	60.6	<0.0001	35.9	<0.0001	2.1	0.1497
	intensity	strongyles	6.0	<0.0001	3.5	<0.0001	-2.7	0.0080
		Strongyloides	7.9	<0.0001	3.2	<0.0001	-0.4	0.7052
		Eimeria	7.6	<0.0001	7.0	<0.0001	1.2	0.2480
zebra	intensity	strongyles	5.0	<0.0001	2.9	0.00425	-0.3	0.7644

Table 2. Relationships between parasite presence or intensity and season, host age and sex. Intensity is based on counts from positive hosts only. Because of low sample size of positive individuals, we did not assess intensity for gemsbok or wildebeest and we grouped juveniles and yearlings into one age class for assessing presence relationships. Directionality of relationships is represented as: + for season means parasite values (presence or intensity) were higher in the wet season than the dry season, + for age means parasite values increased with host age, + for sex means parasite values were higher for males than for females. Significance levels are presented as ***=$p<0.001$, **=$p<0.01$, *=$p<0.05$, ns=non-significant.

host species	data type	*N*	parasite	season	age	sex
springbok	presence	723	strongyles	+***	+*	+*
	intensity	591	strongyles	+***	+*	-***
	presence	723	Strongyloides	+***	ns	ns
	intensity	453	Strongyloides	+***	-***	ns
	presence	723	Eimeria	+***	ns	ns
	intensity	494	Eimeria	+***	-***	ns
zebra	presence	733	strongyles	ns	+***	ns
	intensity	728	strongyles	+***	+***	+**
wildebeest	presence	90	strongyles	+**	ns	ns
	presence	90	Eimeria	ns	-**	ns
gemsbok	presence	90	strongyles	+***	ns	ns
	presence	90	Strongyloides	+**	ns	+*
	presence	90	Eimeria	ns	-**	ns

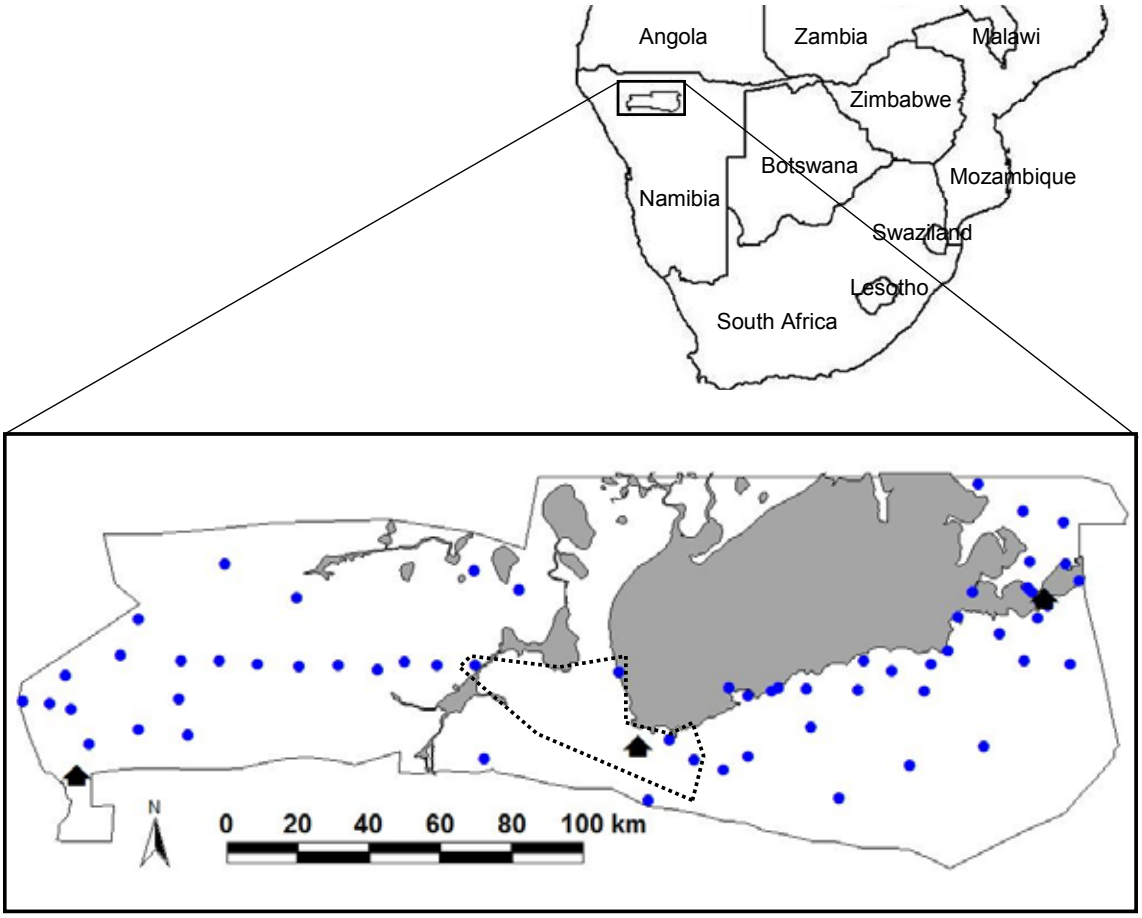

Figure 1. Etosha National Park in northern Namibia. Perennial watering points (springs or boreholes) are shown with blue circles, the focal study area is outlined with a dashed black line.

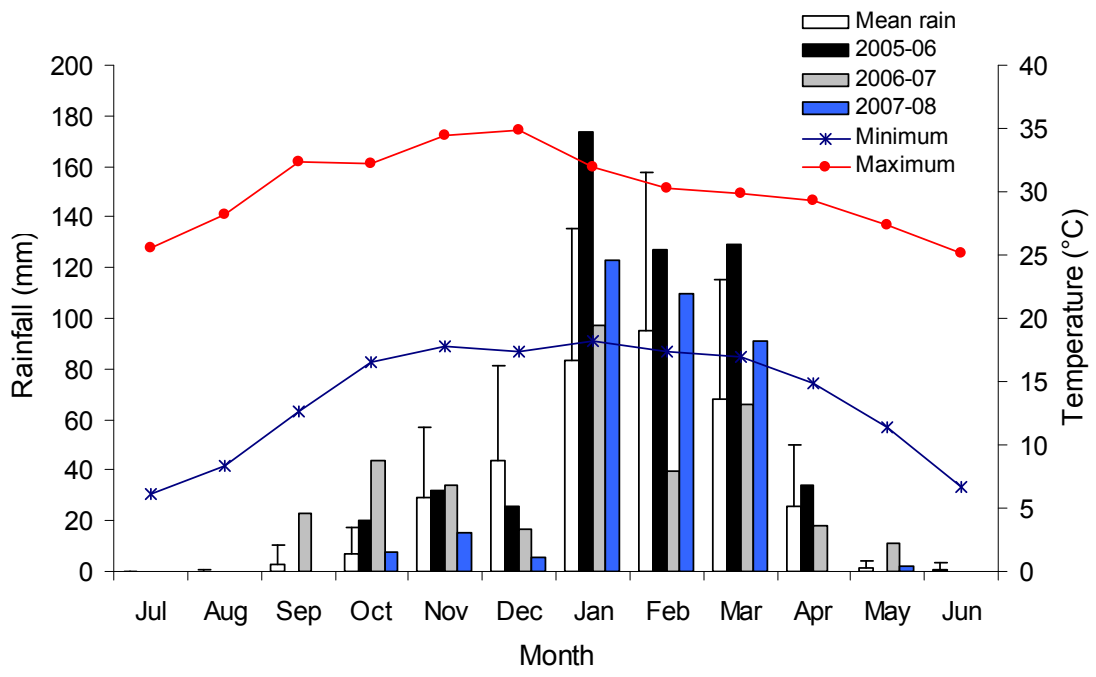

Figure 2. Monthly rainfall and monthly mean temperature minimums and maximums from Okaukuejo station in central Etosha National Park. Values presented include the mean and standard deviation of monthly rainfall records from July 1953- June 2008, and the monthly values recorded for each of the rainfall years encompassing this study, July 2005-June 2006, July 2006-June 2007, and July 2007-June 2008. Temperature data are averages of daily minimum and maximum recordings from 1974-1978 from Berry (1980).

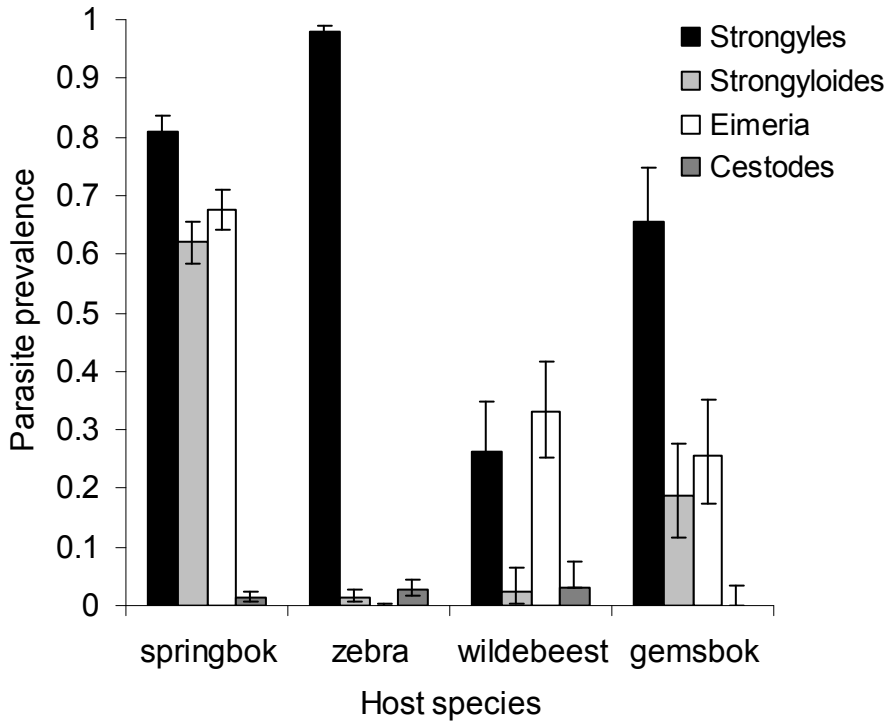

Figure 3. The prevalence and 95% binomial confidence intervals for strongyle nematodes, *Strongyloides* spp., *Eimeria* spp., and Anoplocephalid cestodes recorded in the four host species. Sample sizes were 731 for springbok, 742 for zebra, 133 for wildebeest and 102 for gemsbok.

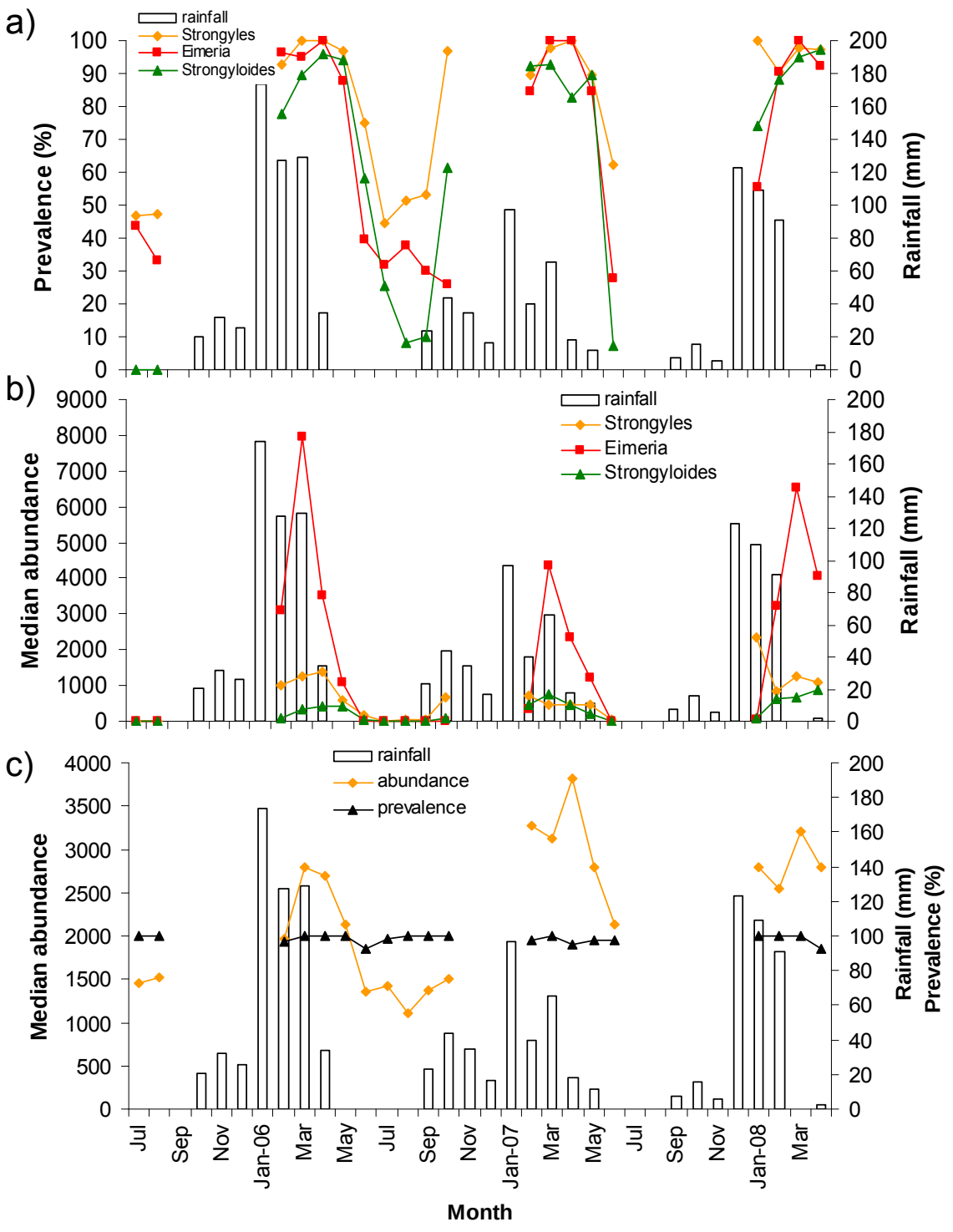

Figure 4. Variation with rainfall of a) springbok parasite prevalence, b) median springbok parasite abundance (the counts including zero values) and c) zebra strongyle prevalence and median abundance.

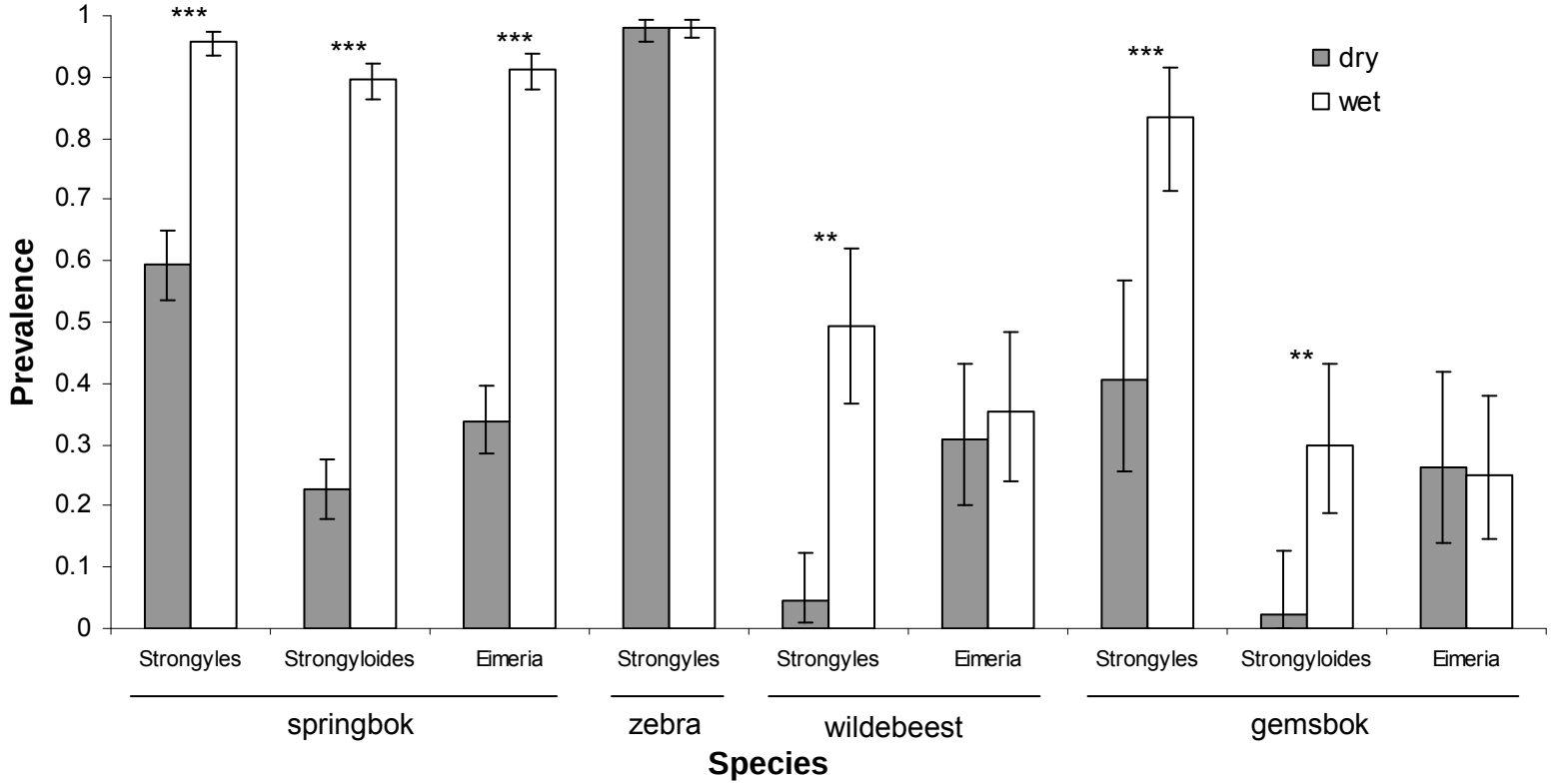

Figure 5. Seasonal differences in parasite prevalence in the four host species. Prevalences are shown with 95% binomial confidence intervals based on sample sizes. Statistical significance between seasons is presented as ***=$p<0.001$, **=$p<0.01$.

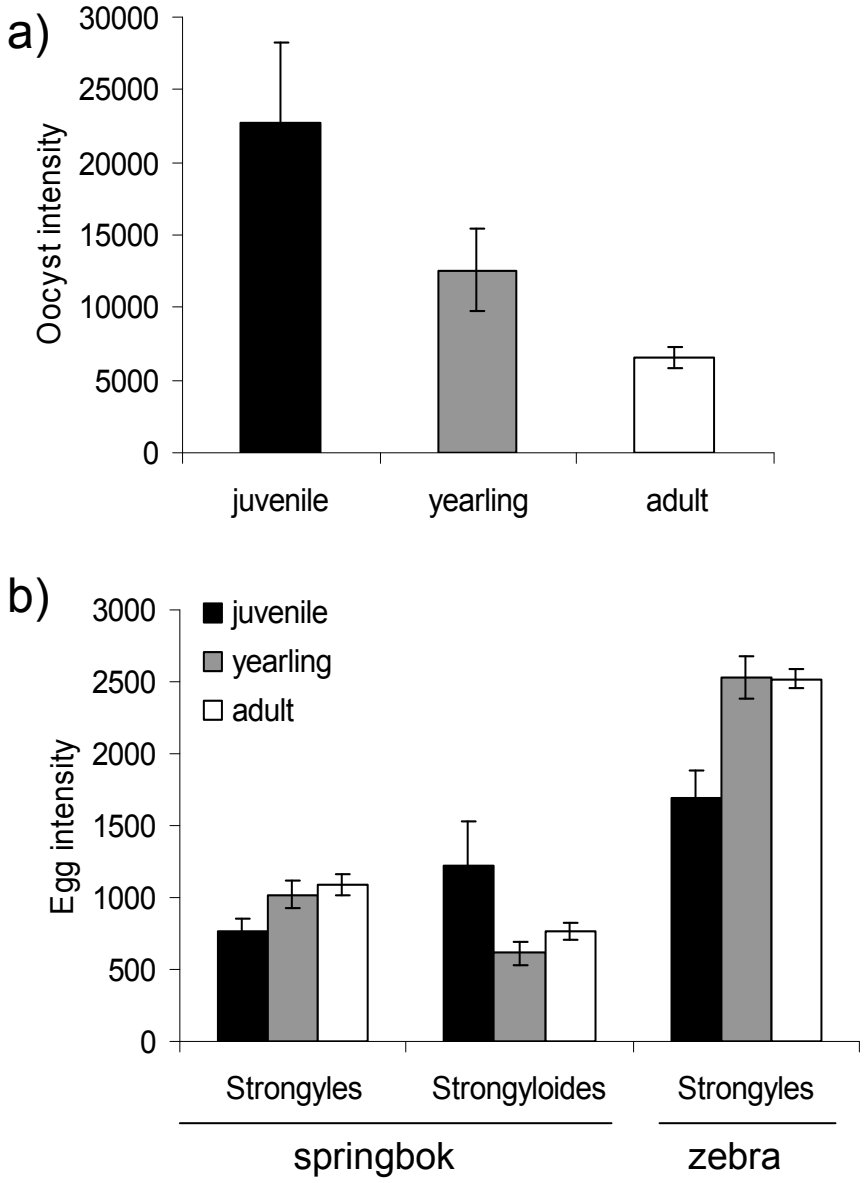

Figure 6. The mean (±SE) propagule shedding intensity by age class for a) *Eimeria* spp. in springbok and b) nematodes in springbok and zebra.

Chapter 5: A survey of *Eimeria* spp. in ungulates of Etosha National Park, Namibia and description of three new *Eimeria* species from springbok (*Antidorcas marsupialis*)

Introduction

Despite a significant body of research into the diversity, pathogenicity, transmission and host specificity of *Eimeria* (Apicomplexa: Eimeriidae) species in livestock and laboratory animals, very little is known about these parasites in wildlife. Only 4% of eimeriid coccidia are estimated have been described phenotypically (Tenter *et al.* 2002). This study focuses on describing three new species of *Eimeria* observed in springbok (*Antidorcas marsupialis*) and the seasonal, age- and sex-related patterns of infection. Springbok are a medium-sized, mixed-feeding herbivore and a commercially important game species located in the more arid regions of southern Africa (Skinner & Chimimba 2005).

In general, *Eimeria* species have a high degree of host specificity (Fayer 1980). To our knowledge, no descriptions of *Eimeria* species in springbok have been published. A published abstract attributed the mortality of several springbok on a game farm in South Africa to coccidiosis caused by an unidentified *Eimeria* (Lopez-Rebollar *et al.* 1997). These authors isolated oocysts from the feces of one carcass and found lesions in the intestine which were consistent with pathology caused by *Eimeria* spp. No other information was provided to detail the size or shape of the oocysts.

We did a survey of *Eimeria* spp. prevalences in ungulates of Etosha National Park, Namibia, and in this work observed distinct differences in the morphology of *Eimeria* oocysts recovered from springbok feces. We then characterized the diversity of *Eimeria* spp. in springbok based on phenotypic descriptions of the sporulated oocysts, and during that work discovered a third, smaller species of *Eimeria*. Here we describe the results of the prevalence survey, provide a morphological description of the three

species of *Eimeria*, and show seasonal and demographic patterns of infection for two of these new species.

Methods

Study area

Etosha National Park is a 22,915 km^2 reserve in northern Namibia between 18˚30'-19˚30'S and 14˚15'-17˚10'E. Etosha contains a 4,760 km^2 salt pan, the dominant geological feature which is the remnant of a palaeolake (Hipondoka *et al.* 2006). The vegetation is classified as arid savanna (Huntley 1982) with a single wet and a single dry season each year. Rainfall is strongly seasonal, mainly falling between November and April, with the greatest monthly rainfall occurring in January and February (Engert 1997). The only perennial water comes from boreholes and artesian or contact springs (Auer 1997).

Sample collection and parasitological analysis

We carried out a survey of *Eimeria* spp. prevalence in an ungulate assemblage of Etosha. Sample collection for this work took place between July-September of 2005 and 2006, obtaining 767 fecal samples from 13 host species. Ungulate species surveyed included red hartebeest (*Alcelaphus buselaphus*), springbok, black-faced impala (*Aepyceros melampus petersi*), blue wildebeest (*Connochaetes taurinus*), black rhino (*Diceros bicornis*), plains zebra (*Equus quagga*), mountain zebra (*Equus zebra*), giraffe (*Giraffa camelopardalis*), elephant (*Loxodonta africana*), gemsbok (*Oryx gazella*), common warthog (*Phacochoerus africanus*), eland (*Taurotragus oryx*) and greater kudu

(*Tragelaphus strepsiceros*). Rare or furtive ungulate species for which adequate samples sizes were not easily acquired were excluded from consideration.

In addition to the ungulate survey, we carried out a detailed study of parasites in springbok from July 2005 to April 2008, collecting 726 fecal samples from springbok in the Okaukuejo section of Etosha. The collecting periods were July-August 2005, February-October 2006, February-June 2007 and January-April 2008. Monthly sample sizes ranged from 27-48 individuals, with a mean of 37 samples per month. We classified the wet season as January-May, and the dry season as June-October. We used these samples as the basis for estimating prevalence and seasonal and demographic patterns of infection for the new *Eimeria* species.

For both studies we stratified the time of sample collection for each species to avoid daily variation in oocyst output (Ezenwa 2003; Villanúa *et al.* 2006). We chose the sampling times based on when a species tended to congregate around point water sources, to maximize sample sizes per unit effort for each species. Therefore samples from most species were collected in the morning (7:00-13:00) excepting giraffe, which drink in the late afternoons, and elephant and black rhino, which drink at night. In the survey we collected samples that we either saw deposited or fresh fecal piles from areas recently utilized by observed groups. For elephant and rhino, we searched near waterholes at dawn, collecting any fresh samples deposited during the night.

For springbok hosts, we used binoculars to watch individuals defecate, recorded the age and sex of the defecator and the fecal locations, and then collected a homogenized sub-sample of the feces within 10 minutes of deposition. Springbok age was distinguished into juvenile (<1 year), yearling (1-2 years) and adult (2+ years) age classes

(Rautenbach 1971). When collecting feces, every effort was made to sample along a particular route or waterhole only once per month, to avoid resampling of individuals within a month. However, if all roads were driven before reaching the target of 40 samples per month, we would return to high density areas to collect additional samples.

Fecal samples were evaluated for *Eimeria* oocysts within 48 hours of collection using a modification of the McMaster method for fecal egg counts (FAO 2005), a commonly used non-invasive method for quantifying parasitism (Bowman 2003). In brief, this method requires the combination of 4g of homogenized fresh fecal matter with 56ml of a saturated salt (NaCl) solution (specific gravity 1.2), removal of large plant debris via a tea-strainer, and filling of each chamber on a McMaster slide with a separate homogenized aliquot of the filtrate. The number of oocysts observed in each chamber using a compound microscope was added together and multiplied by 50 to calculate the number of oocysts per gram of feces (OPG). In rare cases where the number of oocysts on the slide was too high to accurately count, we diluted the sample with additional salt solution and adjusted the counts accordingly. These counts were done with unsporulated oocysts as this work was part of a larger study evaluating variation in springbok gastrointestinal parasite intensity using the McMaster quantitative technique. Two of the *Eimeria* species were easily distinguished in an unsporulated form at the magnification required for the McMaster technique (10x), however, the smallest species was not distinguishable from plant material due to its shape and size. Therefore the third species was not discovered until examinations were done under higher magnification on sporulated oocysts for morphological description of the larger two species.

We performed a Web of Science search using the key words "Eimeria" and the genus and species names for each of the bovids surveyed to see if any *Eimeria* species from these hosts had been described that were not included in Levine and Ivens' (1986) reference book. We found no published species descriptions for our study species that were not included in this book, so we used this reference to assess the possible occurrence of known *Eimeria* species from our survey (Figure 2). Of the bovids surveyed, species of *Eimeria* have been previously described in impala (*Aepyceros melampus*, though not the subspecies *A. melampus petersi*), wildebeest and eland, but not hartebeest, kudu, springbok or gemsbok (Levine & Ivens 1986).

Oocyst descriptions in springbok

In April 2007 and February 2008, we collected pooled samples of springbok feces to concentrate large numbers of oocysts for morphological descriptions. We transported these samples to the University of Pretoria, South Africa, for morphological examination and photographing using a more powerful microscope than was available at the field site. Oocysts were isolated from fecal matter via straining to remove larger debris, a series of washes and centrifugation with double distilled water until the supernatant was clear, suspension in saturated salt solution, recovery of the surface solution containing the oocysts and a final wash. Oocysts were then stored in a 2% potassium dichromate ($K_2Cr_2O_7$) solution at room temperature to allow sporulation.

Descriptions of the sporulated oocysts followed the guidelines of Duszynski and Wilber (1997) and used their abbreviations. These included oocyst shape, oocyst length (L) and width (W), length to width ratio (L/W), and presence/absence of the micropyle

(M) and micropyle cap (MC). Description of the sporocyst included shape, L, W, L/W ratio. Measurements presented are the mean and range of values and all units are in μm.

Data analysis

To compare factors affecting the presence or absence of the different *Eimeria* species in individuals, we performed multivariate logistic regressions of oocyst presence against the categorical variables season (wet or dry), age (juvenile, yearling or adult) or sex (male or female). We evaluated variation in oocyst intensity—the oocyst counts from infected individuals only—in comparison to season and host age and sex using multiple regression of log(count+1) transformed counts. We used the oocyst counts from the McMaster slides in statistical analysis rather than the more usually reported derived number of oocysts per gram of feces (=observed count*50) to avoid large discontinuities at low densities when the data are reported in 50-increment bins before transformation. Statistical analyses were performed in JMP (SAS Institute 2001).

Results

Survey of Eimeria *spp. prevalence in ungulates*

During the survey for *Eimeria* across the ungulate assemblage, *Eimeria* spp. were observed to occur only in hosts from the family Bovidae and not in warthog, giraffe, the two zebra species, black rhino or elephant. Of the host species surveyed, springbok had the highest prevalence of *Eimeria* spp. at 36.0% (*N*=100) of individuals infected (Figure 1). Prevalence of *Eimeria* spp. in the other bovids was 32.1% in blue wildebeest (*N*=84),

26.5% in black-faced impala (*N*=49), 25.9% in gemsbok (*N*=38), 21.1% in red hartebeest (*N*=19), 6.8% in greater kudu (*N*=59) and 0% in eland (*N*=27; Figure 1).

In host *A. melampus*, two species have been described, *E. impalae* and *E. neitzi* (Levine & Ivens 1986). The oocyst we observed in the subspecies *A. melampus petersi* was smaller than either described species (observed, 24 x 18 µm, Figure 2B; *E. impalae*, 33 x 22 (30-36 x 20-24) µm; *E. neitzi*, 32 x 30 (29-34 x 28-33) µm). In *C. taurinus*, two *Eimeria* have been described, *E. connochaetei* and *E. gorgonis* (Levine & Ivens 1986). We observed three potentially different oocyst types from *C. taurinus*, all of which were larger than these two described species (Figure 2C-E; observed oocyst sizes: 30 x 20 µm, 45 x 35 µm and 30 x 22.5 µm; *E. connochaetei*, 22 x 14 (20-20 x 13-15); *E. gorgonis*, 23 x 16.5 (20-26 x 15-18)). These results suggest the oocysts observed in this study are new to science.

Species descriptions of Eimeria *from springbok*

Morphological examination of sporulated oocysts showed evidence of three different species of *Eimeria* from springbok hosts based on size, general shape and the presence or absence of a micropyle and micropyle cap:

***Eimeria antidorcasi* n. sp.**

Type-host: *Antidorcas marsupialis* (Zimmermann, 1780)

Type-locality: Okaukuejo, Etosha National Park, Namibia; 19˚10'S and 15˚55'E

Prevalence: 61.0% averaged across seasons from 726 fecal specimens examined

Site: Unknown. Oocysts collected from fecal specimens

Etymology: The specific epithet *antidorcasi* is derived from the genus of the type host, because this was the most commonly observed oocyst from the host species.

Description (Figure 3A-D)

Sporulated oocyst. Oocyst shape: tear-shaped, with a prominent micropyle at the pointed end; wall characteristics: smooth; L x W: (n=91) 28.7 x 20.3 (22.1-32.9 x 15.9-26.5); L/W: 1.4 (0.6-1.7); M: present and prominent at pointed end of oocyst; MC: absent.

Sporocyst. Sporocyst shape: ovoid and somewhat pointed at the tips; L x W: (n=70) 12.1 x 8.0 (9.1-14.2 x 6.1-9.8); L/W: 1.5 (1.2-2.0).

***Eimeria lammekia* n. sp.**

Type-host: *Antidorcas marsupialis* (Zimmermann, 1780)

Type-locality: Okaukuejo, Etosha National Park, Namibia; 19˚10'S and 15˚55'E

Prevalence: 16.5% averaged across seasons from 726 fecal specimens examined

Site: Unknown. Oocysts collected from fecal specimens

Etymology: The specific epithet *lammekia* is derived from the Afrikaans word, "lammetjie" meaning "little lamb" to reflect the greater prevalence and intensity of infections observed from juvenile springbok.

Description (Figure 4A-D)

Sporulated oocyst. Oocyst shape: ellipsoidal; wall characteristics: smooth, thickest on end with M; L x W: (n=14) 38.7 x 28.4 (33.8-44.4 x 25.0-31.0); L/W: 1.37 (1.3-1.4); M: present; MC: present, prominent and clear, can be torn off during processing.

Sporocyst. Sporocyst shape: elongate-ovoid; L x W: (n=8) 19.2 x 9.6 (15.1-21.8 x 7.5-11.2); L/W: 2.0 (1.8-2.4).

Remarks. These large oocysts rupture easily under the pressure of the 100x magnification oil immersion objective, making it difficult to acquire measurements (Figure 5).

***Eimeria gasawayi* n. sp.**

Type-host: *Antidorcas marsupialis* (Zimmermann, 1780)

Type-locality: Okaukuejo, Etosha National Park, Namibia; 19˚10'S and 15˚55'E

Prevalence: unknown, described from pooled fecal samples

Site: Unknown. Oocysts collected from fecal specimens

Etymology: The specific epithet *gasawayi* is named after the late William C. Gasaway, who studied springbok in Etosha National Park.

Description (Figure 6A-D)

Sporulated oocyst. Oocyst shape: round; wall characteristics: smooth; L x W: (n=27) 15.8 x 14.1 (10.1-20.6 x 7.7-20.3); L/W: 1.2 (1.0-1.3); M: absent; MC: absent.

Sporocyst. Sporocyst shape: ovoid; L x W: (n=15) 8.8 x 5.2 (6.2-12.7 x 4.1-8.5); L/W: 1.7 (1.3-2.2).

Eimeria antidorcasi *and* E. lammekia *infection patterns*

Of infected individuals, the oocysts shed per gram of feces (OPG) were higher for *E. antidorcasi* than *E. lammekia* (*E. antidorcasi*: average=9,283 OPG, median=1,700 OPG, maximum=373,050 OPG; *E. lammekia*: average=4,036, median=450, maximum=120,050). The monthly prevalence of *E. antidorcasi* and *E. lammekia* exhibited very different seasonal infection profiles (Figure 7). The prevalence of *E.*

antidorcasi was above 80% for much of the wet season and dropped to below 30% in the dry season, whereas the prevalence of *E. lammekia* was always below 30%.

The number of hosts excreting oocysts of *E. antidorcasi* varied significantly by season but not by host age or sex (logistic regression: Wald statistic[season]=246.9, $p<0.0001$; Wald statistic[age]=1.7, $p=0.4283$; Wald statistic[sex]=0.1, $p=0.7543$). *Eimeria antidorcasi* was more prevalent in wet seasons than dry seasons (87.7% vs 16.7%, respectively). For *E. lammekia*, the number of individuals infected varied significantly by age and season but was not significantly related to host sex (logistic regression: Wald statistic[age]=46.3, $p<0.0001$; Wald statistic[season]=11.12 $p=0.0008$; Wald statistic[sex]=0.1, $p=0.6990$). *Eimeria lammekia* occurred most often in juveniles and presence decreased with increasing host age (prevalence of juveniles=35.2%, $N=145$; yearlings=14.2%, $N=134$; adults=11.2%, $N=446$) and was somewhat more prevalent in wet than dry seasons (19.5% vs 12.1%, respectively).

The intensity of *E. antidorcasi* was significantly related to season and host age but not sex (multiple regression: season, $F=59.0$, $p<0.0001$; age, $F=9.8$, $p<0.0001$; sex, $F=0.2$, $p=0.6424$). *Eimeria antidorcasi* counts were significantly higher in wet seasons than dry seasons (mean and standard error (SE) of log-transformed counts: wet season, 1.73±0.04, $N=377$; dry season, 0.97±0.07, $N=66$) and increased significantly between each age class from juveniles to adults (mean and SE of log-transformed counts: juveniles, 1.87±0.11, $N=74$; yearlings, 1.74±0.08, $N=92$; adults 1.51±0.04, $N=276$).

The intensity of *E. lammekia* excreted by hosts was significantly related to host age and season, but not sex (multiple regression: age, $F=9.4$, $p=0.0002$; season, $F=4.3$, $p=0.0394$; sex, $F=0.3$, $p=0.6130$). The intensity of *E. lammekia* excreted was highest in

juveniles and decreased as host age increased (mean and SE of log-transformed counts: juveniles, 1.45±0.11, *N*=51; yearlings, 1.12±0.15, *N*=19; adults 0.86±0.08, *N*=50) and was also higher in wet than dry seasons (mean and SE of log-transformed counts: wet season, 1.25±0.08, *N*=84; dry season, 0.92±0.11, *N*=36), although the difference was more pronounced for *E. antidorcasi* than for *E. lammekia*.

Discussion

Springbok showed the greatest *Eimeria* prevalence of the thirteen host species surveyed in Etosha National Park over the course of two dry seasons. The relatively high prevalence may be because springbok are the most abundant herbivore species in Etosha (Ministry of the Environment and Tourism unpublished aerial survey reports). We recorded *Eimeria* spp. in six of seven species of Bovidae surveyed, and no *Eimeria* in the six species surveyed outside of Bovidae. Based on a basic examination of oocyst sizes, none of the oocysts observed in this study have yet been described.

Springbok are the only extant species in the genus *Antidorcas* and there have also been no *Eimeria* described from this species or the most closely related species, *Ammodorcas clarkei*, Clarke's gazelle from Ethiopia and Somalia). Within the Antilopini tribe, there have been *Eimeria* described in four different species: *Gazella gazella* (*E. chinkari*, *E. idmii*), *G. subgutturosa* (*E. abenoui*, *E. elegans*, and *E. gazella* and *E. rheemi*), *G. dorcas* (*E. dorcadis*), and *Litocranius walleri* (*E. walleri*) (Levine & Ivens 1986; Hussein & Mohammed 1992; Mohammed & Hussein 1992). None of these species shares a geographic distribution with springbok; they are located in east Africa (*L. walleri*), north Africa (*G. dorcas*), the middle east (*G. gazella*) and southern and

central Asia (*G. subgutturosa*). Therefore, we feel confident that the *Eimeria* observed in springbok were new to science.

Of the new species described in this study from springbok hosts, *E. antidorcasi* was more prevalent in hosts than was *E. lammekia*, and the prevalence of *E. gasawayi* is unknown. Both *E. antidorcasi* and *E. lammekia* were more prevalent in wet seasons than dry seasons, although this difference was far more pronounced for *E. antidorcasi* than for *E. lammekia*. In wet seasons increased temperatures and humidity may enhance *Eimeria* transmission. Additionally, springbok in Etosha National Park have a restricted parturition season in January (Gasaway *et al.* 1996) adding a pulse of immunologically naïve hosts to the population in the wet season. The prevalence and intensity of *E. lammekia* was greater in juveniles than older age classes. *Eimeria antidorcasi* had greater intensity in juveniles than other age classes, but the prevalence was not significantly different among age classes. From studies of *Eimeria* in other host-parasite systems (Yun *et al.* 2000), a decrease in the prevalence or intensity of infections with age is consistent with expectations of host acquired immunity. We found no evidence of sex differences in the prevalence or intensity of either *E. antidorcasi* or *E. lammekia.*

The three *Eimeria* species observed from springbok were distinctly different in the size, shape and phenotypic characteristics of the oocysts. Further research must be done in order to characterize all the details of the morphology of the sporocysts and the sporozoites and to create a composite line drawing of the oocysts (Duszynski & Wilber 1997). This process would be enhanced through the use of differential interference contrast microscopy instead of light microscopy, for increased contrast and detail in the photomicrographs. Additionally, a molecular description of these species should be done

to provide insight into the differences between these species and their relationships to other known eimeriids in the Bovidae.

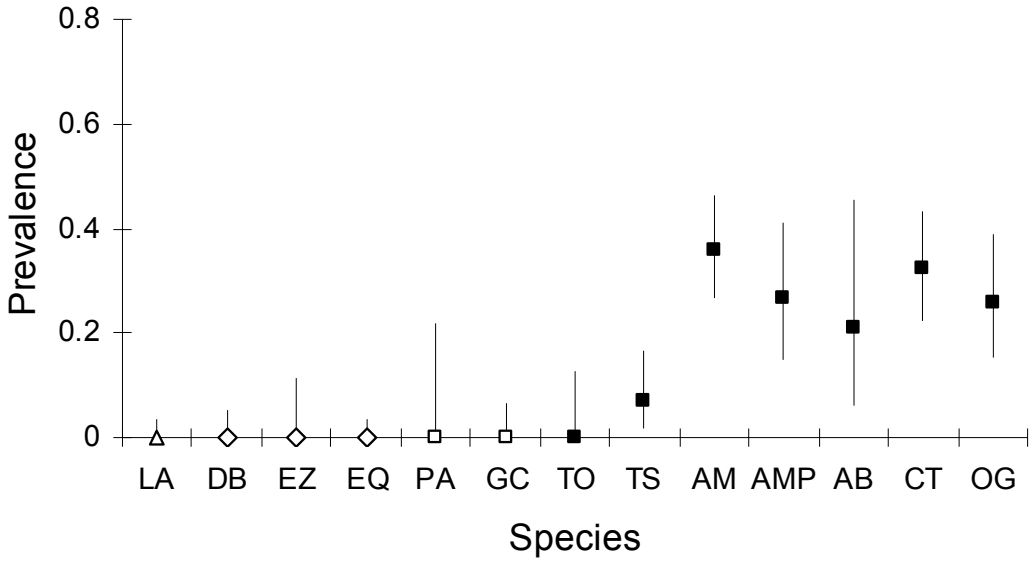

Figure 1. The prevalence of *Eimeria* spp. recorded in an assemblage of ungulates in Etosha National Park and the 95% binomial confidence intervals. Hosts in the order Perrisodactyla are represented with open diamonds and the order Proboscidea with an open triangle. Hosts in the order Artiodactyla are represented with squares: the family Bovidae with closed squares, non-Bovids with open squares. Host species include AB, *Alcelaphus buselaphus* (red hartebeest); AM, *Antidorcas marsupialis* (springbok); AMP, *Aepyceros melampus petersi* (black-faced impala); CT, *Connochaetes taurinus* (blue wildebeest); DB, *Diceros bicornis* (black rhino); EQ, *Equus quagga* (plains zebra); EZ, *Equus zebra* (mountain zebra); GC, *Giraffa camelopardalis* (giraffe); LA, *Loxodonta africana* (elephant); OG, *Oryx gazella* (gemsbok); PA, *Phacochoerus africanus* (common warthog); TO, *Taurotragus oryx* (eland); TS, *Tragelaphus strepsiceros* (greater kudu). The sample sizes range from 15 for PA to 100 for LA, EQ and AM.

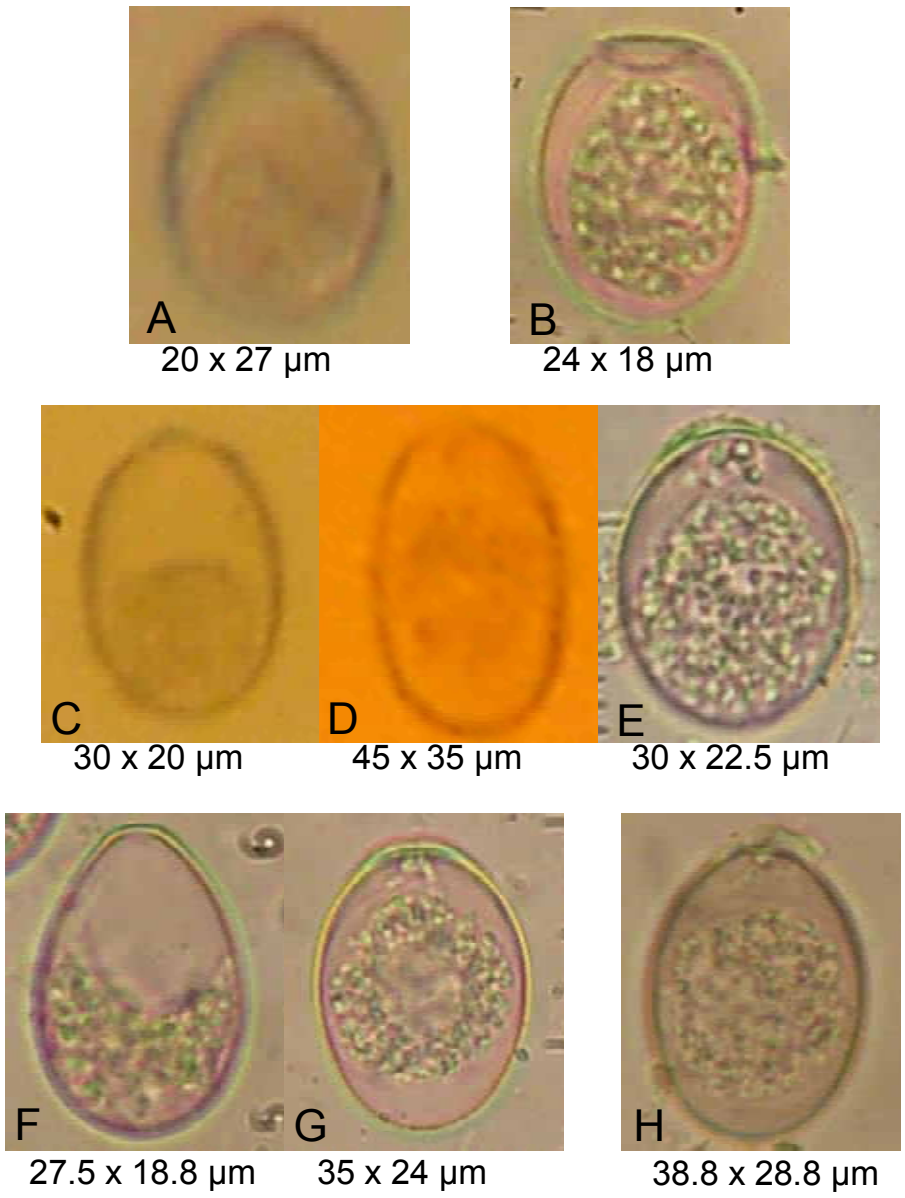

Figure 2. *Eimeria* spp. oocysts observed during the ungulate survey in A, *A. buselaphus* (red hartebeest); B, *A. melampus petersi* (black-faced impala); C-E, *C. taurinus* (blue wildebeest); F-G, *A. marsupialis* (springbok); H, *O. gazella* (gemsbok). The photos are not to scale and the measurements for each oocyst are presented below the photos. Photos A, C and D were taken at 10x magnification and B, E-H were taken at 40x magnification. All photos were taken holding a digital camera against the ocular. Although *Eimeria* oocysts were observed in kudu, no photos were taken.

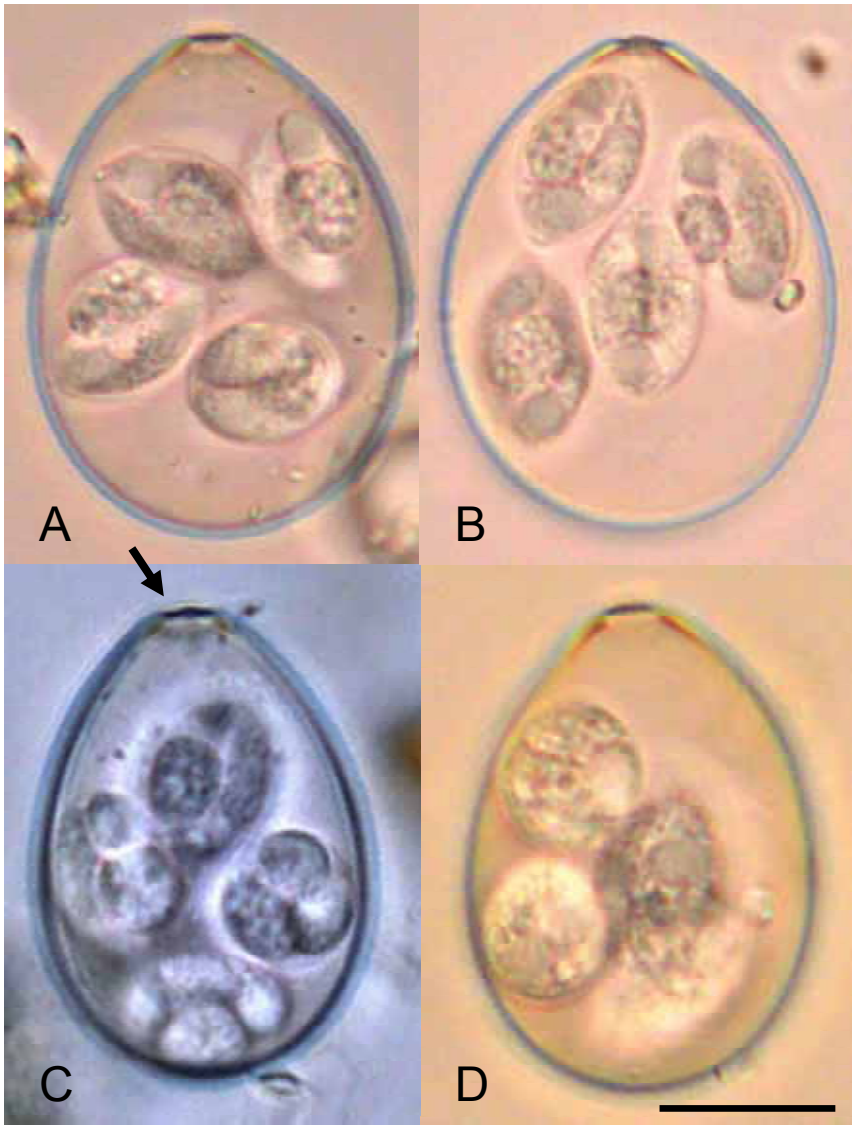

Figure 3. *Eimeria antidorcasi* n. sp. sporulated oocysts observed from springbok feces, photos A-D. The black arrow indicates the micropyle. The black bar is 10µm. Photos by WCT.

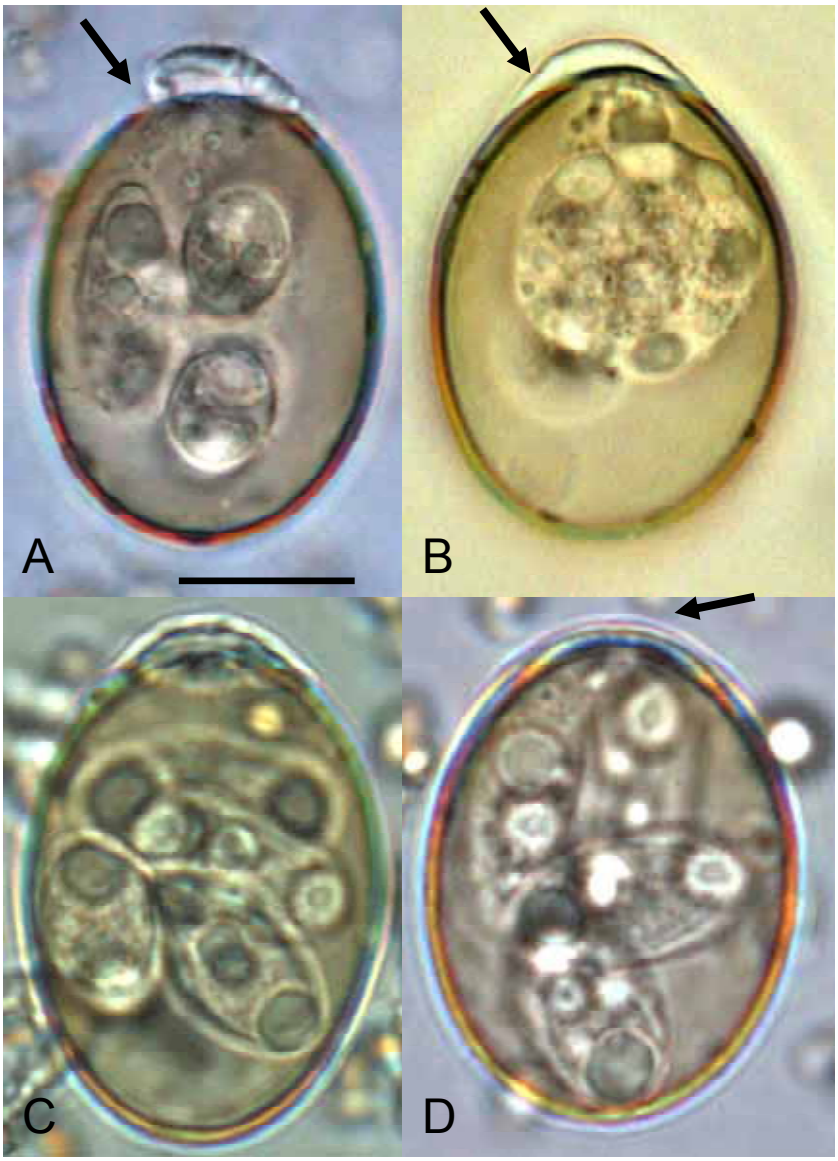

Figure 4. *Eimeria lammekia* n. sp. oocysts observed from springbok feces: A, the sporulated oocyst indicating the torn micropyle cap with a black arrow, B, an unsporulated oocyst with an undisturbed micropyle cap indicated with the black arrow, C, sporulated oocyst, D, sporulated oocyst with missing micropyle cap. The black bar is 15µm. Photos A, C, D by Rhulani Nkuna, B by WCT.

Figure 5. *Eimeria lammekia* unsporulated oocyst that ruptured under pressure of the 100x oil immersion objective. Photo by WCT.

Figure 6. *Eimeria gasawayi* n. sp. sporulated oocysts observed from springbok feces, photos A-D. The black bar is 10µm. Photo A by WCT, B-D by Rhulani Nkuna.

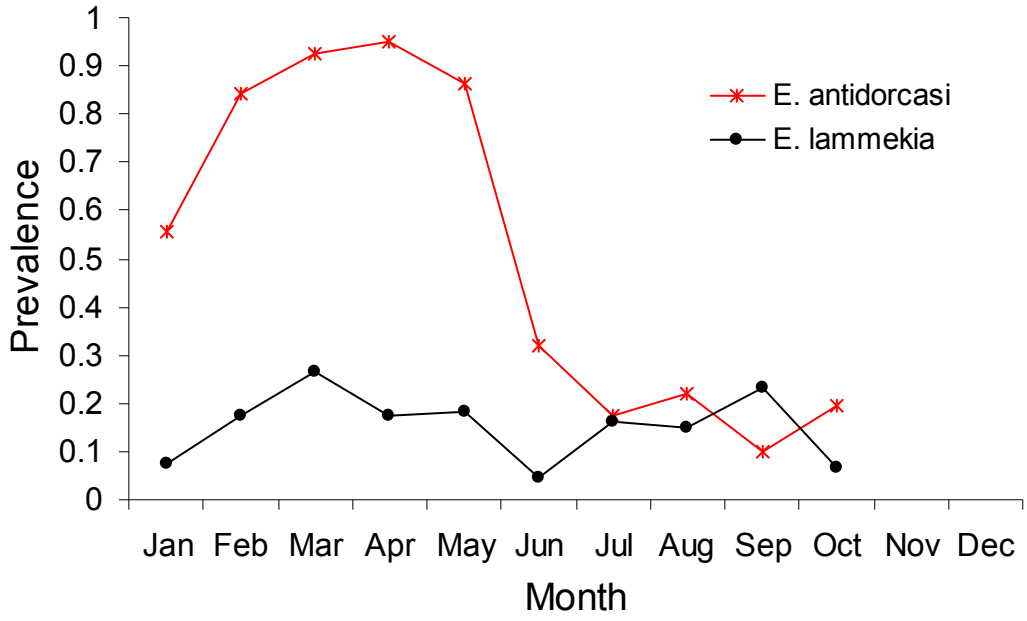

Figure 7. Monthly prevalence of *E. antidorcasi* and *E. lammekia* from Okaukuejo combining samples collected from July 2005-April 2008. Sample sizes per month range from 27 for January to 120 for March. No samples were collected in November and December.

Chapter 6: Effects of gastrointestinal parasites and parasite interactions on body condition of springbok (*Antidorcas marsupialis*) along a rainfall gradient

Introduction

Multiple parasite infections often co-occur in hosts. Heterospecific parasite interactions within hosts may be direct (e.g., competition for space) or indirect (e.g., mediated by host immune responses) and can be positive (e.g., synergistically engaging the host's immune system), negative (e.g., competing for host resources) or neutral (e.g., no correlative effects detectable) (Esch & Fernandez 1992). The type of interaction and its directionality tend to vary depending on the parasites involved. Indirect parasite interactions via host immunity can occur through cross-immunity between similar types of parasites (a negative interaction) or through suppression of the acquired immune response (a positive interaction) (Cox 2001). These interactions are by no means mutually exclusive, a community of parasites may experience both direct and indirect interactions (Lello *et al.* 2004).

Broadly, parasites can be divided into two groups, intracellular or microparasites (i.e., viruses, bacteria, protozoa or fungi) and extracellular or macroparasites (i.e., nematodes, cestodes or arthropods). The adaptive immune response produces lymphocytes (T helper or Th cells) which differentiate into Th1 or Th2 cells, two mutually exclusive pathways which defend against intracellular parasites (the Th1 pathway) or extracellular parasites (the Th2 pathway) (Abbas *et al.* 1996). Interactions between intracellular and intercellular parasites tend to result in enhanced host susceptibility to one type of parasite through decreased host immunity (Bentwich *et al.* 1999; Borkow *et al.* 2001; Cox 2001; Chen *et al.* 2005), or by increasing disease severity (Graham *et al.* 2005).

Gastrointestinal (GI) parasite infections are observed to suppress host appetite, change gastrointestinal function and protein metabolism, cause diarrhea, anemia and, in extreme cases, death in their ungulate hosts (Fox 1997; Bowman 2003). However, the physiological effects of parasitism in wildlife hosts are far less understood than in livestock. Studies from wildlife populations indicate that GI parasites are associated with reduced host physical condition or body mass (Stien *et al.* 2002; Holmstad *et al.* 2005; Lello *et al.* 2005; Newey *et al.* 2005; Craig *et al.* 2008). Parasite-induced reductions in body condition are further associated with reduced host fecundity (Stien *et al.* 2002). Hosts with high parasite burdens may also be subject to higher rates of predation than other hosts (Hudson *et al.* 1992a; Murray *et al.* 1997; Packer *et al.* 2003). By reducing host survival or fecundity, nematodes can affect the dynamics of wildlife host populations (Hudson 1986; Gulland 1992; Hudson *et al.* 1992b; Murray *et al.* 1997; Stien *et al.* 2002; Deter *et al.* 2007).

In this study we focus on two types of gastrointestinal (GI) parasite infections, in particular those of nematodes (extracellular parasites) and protozoa in *Eimeria* spp. (intracellular parasites) in a free-ranging population of springbok (*Antidorcas marsupialis*). We explore if there are positive or negative interactions between observed groups of GI parasites related to presence or intensity of parasitism within hosts. We then examine if parasite presence or intensity varies among three study areas situated along an environmental rainfall gradient. Finally, we examine parasite, host and environmental factors associated with changes in host physical condition.

Methods

Study area

Etosha National Park is a 22,915 km^2 reserve in northern Namibia between 18˚30'-19˚30'S and 14˚15'-17˚10'E (Figure 1). Etosha contains a 4,760 km^2 salt pan, the dominant geological feature which is the remnant of a palaeolake (Hipondoka *et al.* 2006). The vegetation is classified as arid savanna (Huntley 1982) with a single wet and a single dry season each year (Figure 2). Rainfall is strongly seasonal, mainly falling between November and April, with the greatest monthly rainfall occurring in January and February (Engert 1997). The only perennial water comes from boreholes and artesian or contact springs (Auer 1997).

The main study area for our research was around Okaukuejo station in the center of the park. In addition, we selected two other study areas in the far west and east of Etosha to evaluate how the east-west rainfall gradient evident across the park may influence parasitism. The three areas selected were Otjovasandu in the far west, Okaukuejo in the center, and Namutoni in the far east of Etosha (Figure 1). The mean annual rainfall from each of these study areas increases from west to east: 301mm in Otjovasandu, 349mm Okaukuejo, and 444mm in Namutoni from 1971-2008. During 2006-2007 when we sampled all three areas, the rainfall differences were larger than apparent in the annual means: 2006 was a wetter year, with annual rainfall of 450mm in Otjovasandu, 542mm in Okaukuejo and 676 in Namutoni, whereas 2007 was a drier year, with 212mm in Otjovasandu, 350mm in Okaukuejo and 510mm in Namutoni (Figure 2).

Study species

Springbok are an arid-adapted species of gazelle occurring in the more arid regions of southern Africa (Nagy & Knight 1994; Skinner & Chimimba 2005). This species is categorized as a selective, mixed feeding herbivore (Hofmann *et al.* 1995). The diet of springbok is composed mostly of browse, one estimate being 77% using stable isotope methods (Sponheimer *et al.* 2003). Diet composition changes seasonally and grazing tends to occur during the rains, when grasses are green and highly digestible (Bigalke & van Hensbergen 1990). Springbok are the most abundant herbivore in Etosha National Park, with a population estimate of 15,600 in 2005 (the 95% confidence interval rounded to the nearest 100 is 13,200-17,900; cf. Namibian Ministry of the Environment and Tourism unpublished aerial survey data).

The parasite groups measured include nematodes in the superfamily Trichostrongylidea (hereafter "strongyle nematodes") and the genus *Strongyloides*, cestodes in the genus *Moniezia*, and two species of coccidia in the genus *Eimeria* (*E. antidorcasi* and *E. lammekia*). The strongyle nematodes, *Eimeria* and *Moniezia* are orally ingested, with direct transmission for the nematodes and *Eimeria*, and via accidental ingestion of the intermediate arthropod host for *Moniezia* (Bowman 2003). *Strongyloides* spp. females are transmitted either via milk or larval penetration of host skin and migration to the gut where they mature and produce eggs through parthenogenesis (Bowman 2003; Streit 2008). Only female worms of *Strongyloides* spp. are parasitic; males are free-living and sexual reproduction only occurs in the free-living stages.

Although little is known about the effects these parasites have on wild hosts, *Strongyloides* spp. and *Moniezia* rarely cause detectable disease in domestic animals. In contrast, the strongyle nematodes and *Eimeria* are pathogenic and responsible for widespread production losses in livestock (Bowman 2003). The strongyle nematode genera which have been recorded in springbok include *Agriostomum*, *Cooperia*, *Cooperioides*, *Dictyocaulus*, *Haemonchus*, *Impalaia, Longistrongylus*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Paracooperia* and *Trichostrongylus* (Round 1968; Horak *et al.* 1982; De Villiers *et al.* 1985). Three species of *Eimeria* from springbok were described in this study (Chapter 5).

Sample collection and parasitological analysis

Gastrointestinal parasitism was evaluated using fecal estimates of propagule abundance from randomly selected individual springboks. Fecal specimens were collected from our main study area in Okaukuejo between July 2005 and April 2008, for a total of 731 fecal samples. Collecting periods in Okaukuejo were July-August 2005, February-October 2006, February-June 2007 and January-April 2008. Monthly sample sizes ranged from 27-48 individuals, with a mean of 37 samples per month. To examine spatial patterns in parasite infections, we additionally collected samples from the other two study areas in April-May 2006, July-August 2006 and March-April 2007. In Otjovasandu and Namutoni, we aimed for 30 fecal samples per area per month of sampling, collecting a total of 160 samples per area. For both data sets, the wet season was classified as January-May, and the dry season June-October.

In all study areas, we collected samples between 7:00-13:00. As the distances were far between study areas, we sampled each study area in rotation. Okaukuejo was always sampled in the first week of the month, Namutoni in the second week, and Otjovasandu in the third week. The laboratory for parasite analysis was based in Okaukuejo, so when working in other study areas, samples were collected over 2.5 days, stored in a refrigerator on the vehicle and then processed immediately on return to the laboratory. Samples from Okaukuejo were generally processed within one day of collection; the samples from other areas were processed at most 2.5 days from collection and often at shorter time intervals.

When collecting feces, every effort was made to sample along a particular route or waterhole within the study area only once per month, to avoid resampling of individuals. During collection, we used binoculars to watch individuals defecate, recorded the fecal locations, and then collected a homogenized sub-sample of the feces within 10 minutes of deposition. For each fecal sample collected, we recorded the date, time, age, sex,and body condition of the defecator, its group size and spatial location. Age and sex were determined via horn growth and morphology, and genitalia (Rautenbach 1971). Age was assessed in three categories: juveniles were <1 year, yearlings 1-2 years, and adults 2+years old. We adapted to springbok the ordinal visual scoring system for physical condition (1=very poor, 2=poor, 3=fair, 4=good, 5=excellent) developed by Berry and Louw (1982) for wildebeest. These categories were based on the shape of the hindquarters and the visibility of the ribs and pelvis.

Fecal samples were evaluated for GI parasite propagules using a modification of the McMaster method for fecal egg counts (FAO 2005), a commonly used non-invasive

method for quantifying parasitism (Bowman 2003). Flotation techniques are best for recovery of nematode and cestode eggs and protozoan cysts from feces, but fail to recover trematode cysts or nematode larvae (Bowman 2003). In brief, this method requires the combination of 4g of homogenized fresh fecal matter with 56ml of a saturated salt (NaCl) solution (specific gravity 1.2), removal of large plant debris via a tea-strainer, and filling of each chamber on a McMaster slide with a separate homogenized aliquot of the filtrate. The number of eggs or oocysts observed in each chamber using a compound microscope was added together and multiplied by 50 to get the number of eggs or oocysts per gram of feces. In rare cases where the number of oocysts on the slide was too high to accurately count, we diluted the sample with the salt solution and adjusted the counts accordingly.

Data analysis

Analyses of the presence or absence of parasites within individual hosts was done using logistic regression. To test factors related to the presence or absence of each parasite type, we analyzed each parasite type separately, with the categorical variables season (wet or dry), sex (male or female), age (an ordinal variable with three age classes representing juvenile, yearling and adult age classes), body condition score (ordinal variable 1-5) and the presence or absence of the other parasite types as independent variables. We examined these patterns from each of the three study areas independently, to evaluate the repeatability of results from different regions of the park. We then assessed if parasite presence varied significantly along the rainfall gradient. For this analysis (and all further analyses comparing study areas) we combined data from the

three study areas and reduced the Okaukuejo dataset to match the sampling periods for the other two study areas. These analyses incorporated as independent variables study area (an ordinal variable representing an increase in annual rainfall) and those factors which were significantly related to each parasite's occurrence based on results from the larger Okaukuejo dataset.

Since the dependent variable for intensity excludes any samples with a zero count, we considered the sample sizes of parasite intensity for Otjovasandu and Namutoni too small for independent analyses. Therefore we focused on the larger Okaukuejo dataset for evaluation of key factors related to parasite intensity. As independent factors we considered the categorical variables season, host age, sex, body condition, and the continuous variables of egg or oocyst counts for other parasites. These factors were tested for collinearity and were found to have variance inflation factors (VIF) below 1.7; VIFs below 10 are considered acceptable (Quinn & Keough 2002). We then tested for differences in parasite intensity between the study areas along the rainfall gradient using variable selection as for parasite presence between study areas. Intensity analyses were performed using multiple regression after transforming all parasite counts by log(count+1) to reduce the otherwise extremely long-tailed distribution associated with these counts. We used the observed egg or oocyst counts from the McMaster slides in statistical analysis rather than the more usually reported derived number of propagules per gram of feces (=count*50) to avoid large discontinuities at low densities when the data are reported in 50-increment bins before transformation.

We used ordinal logistic regression models to assess factors related to variation in host body condition. We evaluated the influence of a number of environmental, host and

parasite variables using Akaike's information criteria (AIC) to compare alternative models (Burnham & Anderson 2002). The variables examined include rainfall one, two, or three month's prior to sample collection, host age and sex, and the log(count+1) intensity for each parasite type. For these models we include all samples, including zero values for parasite intensities. Backward and forward selection techniques converged on the same model, and we present the AIC values for each stepwise model. Finally, we tested if host body condition varied between the study areas using ordinal logistic regression. This model included the factors selected in the best fit model of body condition based on AIC values, as well as study area as an ordinal variable.

Multiple regressions were performed in JMP (SAS Institute 2001) and logistic regressions were performed in R 2.7.0 (R Core Development Team, 2008).

Results

Parasite presence and co-occurrences

Where significant relationships were found between the co-occurrences of different parasites, these relationships were positive, with the occurrence of one parasite related to the occurrence of a second parasite (Table 1). These positive relationships were observed between strongyles and *Strongyloides* spp. and between *Strongyloides* spp. and *E. antidorcasi*. Comparing strongyle and *Strongyloides* spp. co-occurrence in hosts from Okaukuejo, 428 individuals had both parasites, 25 had *Strongyloides* only, 163 had strongyles only, and 115 had neither parasite type. Comparing *E. antidorcasi* with *Strongyloides* spp., 374 hosts had both parasites, 79 had *Strongyloides* only, 69 had *E. antidorcasi* only, and 204 had neither parasite type. The models assessing parasite

occurrence and co-occurrence in the three study areas were fairly consistent in the directionality and significance of statistical patterns (Table 1). Most discrepancies involved patterns that were significant in the main study area were not in the other study areas, a difference perhaps due to the relatively lower sample sizes in these two areas. For all parasite types except *E. lammekia*, parasite prevalence was consistently higher in wet than dry seasons (Table 2). The only parasite with strong patterns of occurrence by host age was *E. lammekia*, with juvenile hosts parasitized significantly more than other age classes, across all study areas (Table 2).

Comparing parasite presence across the rainfall gradient, only *Strongyloides* spp. occurrence varied significantly among the study areas (Table 3). *Strongyloides* presence was significantly lower in Otjovasandu than the other two study areas, in both wet and dry seasons (Table 2). When we examined patterns of parasite co-occurrence among study areas, a significantly negative association emerged in the co-occurrence of *E. antidorcasi* and *E. lammekia* that was not evident in analyses from the main study area: 38 individuals had both parasites, 297 had *E. antidorcasi* only, 32 had *E. lammekia* only, and 155 had neither *Eimeria* species (Table 3).

Parasite propagule intensities

The only significant relationships among parasite intensities showed increased *Strongyloides* intensity was significantly related to increased strongyle intensity and *E. antidorcasi* intensity (Table 4). Higher strongyle intensities were associated with significantly poorer host condition (Table 4). The intensities of all parasites except *E. lammekia* were significantly higher in wet than dry seasons, and where age-related

patterns in intensity occurred, juveniles tended to have higher intensities than other age classes (Table 4).

There were significant differences among study areas in the intensity of strongyles, *Strongyloides* spp. and *E. antidorcasi* but not *E. lammekia* (Table 3). Significance tests tended to separate Otjovasandu from the other two study areas. Hosts in Otjovasandu had lower intensities of *Strongyloides* spp. and *E. antidorcasi* and higher intensities of strongyles than the other two study areas (Figure 3).

Host body condition

The body condition scores of hosts sampled in this study ranged from 2 (poor) to 5 (excellent). We observed individuals in class 1 (very poor) only twice, but neither defecated while observed. The best fit model (lowest AIC value) for host condition included the variables age, intensity of strongyles, *E. antidorcasi* and *Strongyloides* spp., rainfall two months prior and sex (ranked first in Table 5; model parameters in Table 6). The top three models all had $\Delta AIC<2$, indicating these three models are equally plausible given the data (Burnham & Anderson 2002). The difference between these three models was the inclusion or exclusion of the variables sex or *E. lammekia* intensity, indicating that these factors may add little towards describing the observed variation in body condition. The parameters that described most of the observed variation were strongyle intensity, rainfall two months prior and host age, although overall, the variance in condition explained was low (Table 6). Relationships between body condition and the intensity of the three parasite types from the best fit model are shown in Figure 4.

Host body condition varied significantly among study areas (Table 3). Hosts from Otjovasandu had higher body condition scores than hosts from Okaukuejo or Namutoni (mean and standard error of condition scores by area: Otjovasandu, 3.67±0.04; Okaukuejo, 3.49±0.04; Namutoni, 3.49±0.04).

Discussion

Parasite interactions

Significant positive associations in the intensities and co-occurrences of parasites were observed between strongyle nematodes, *E. antidorcasi* and *Strongyloides* spp. Although both *E. antidorcasi* and strongyles were related to *Strongyloides* spp., these two parasites were not directly related to each other. The association between *E. antidorcasi* and *Strongyloides* spp. may be driven by the increased intensity of each parasite in juveniles. Juvenile hosts may not be able to fight both infections simultaneously, as *Strongyloides* spp. generates a Th2 immune response (Bleay *et al.* 2007) and *Eimeria* primarily generates a Th1 immune response (Hong *et al.* 2006). There was a significant negative relationship in the co-occurrence of the two *Eimeria* species when examined across the three study areas. A negative association between closely related species could indicate cross-immunity, however the immunity towards *Eimeria* is generally quite species-specific (Hong *et al.* 2006).

It is much more difficult to understand mechanisms, either direct or indirect, driving the observed parasite interactions that do not occur via the immune system (cross-immunity or immunosuppression). Direct interactions between helminthic parasites may include competition for host resources (Holmes 1961), however, outside of experimental

systems, these mechanisms cannot be known. Behnke and colleagues (2005) found that interactions between helminthic parasites of wood mice were highly context dependent, and where positive associations occurred, these were generally weak patterns. The patterns of co-occurrence we observed between study areas were fairly consistent, although the levels of significance were variable.

Spatial patterns of parasitism

Strongyloides spp. prevalence and intensity varied strongly among study areas. Both measurements were lower in Otjovasandu, the study area receiving the least rainfall, and the intensity of *Strongyloides* spp. increased across the rainfall gradient. Similarly, *E. antidorcasi* intensity was lowest in Otjovasandu. The drier conditions in Otjovasandu may limit transmission of infective larvae or survival of free-living stages (Stromberg 1997) or oocysts. Rainfall differences are only one way in which these study areas differ; they also vary in habitat types and the abundance and diversity of host species, additional factors that may relate to rates of parasitism. However, in a semi-arid system, rainfall may be a key factor limiting parasite transmission (Chiejina *et al.* 1989; Jacquiet *et al.* 1995). Host body condition was significantly higher in Otjovasandu than the other study areas. A reduction in parasitism in this study area may relate to improved host body condition, although this should be viewed with caution, given the amount of variation in body condition unexplained by our model. Variation in vegetation type and quality and host density may further explain variation in host body condition among study areas.

Host body condition

We observed significant differences between host body condition and environmental and parasite factors, and body condition varied among host age classes. Population-level assessments of body condition show that body condition is lower at the conclusion of the dry season than the wet season (Gasaway *et al.* 1996). With the onset of the rains in January, grass cover and greenness on the Etosha plains swiftly increases within a 10-day window after an accumulated rainfall of around 30mm (du Plessis 2001). The improved quality and availability of vegetation in response to rainfall is likely behind the observed relationship between rainfall two months prior and improved springbok condition.

The onset of the rains also results in an increase in parasitism, with the presence and intensity of all parasite types from springbok significantly related to rainfall one month prior (Chapter 4). In both wet and dry seasons, individuals with higher strongyle nematode intensities had poorer body condition than individuals with lower strongyle intensities. Strongyle nematodes were most strongly related to host condition of the parasite groups examined. Relationships between *E. antidorcasi* and *Strongyloides* spp. and body condition were only observed when the parasite community was examined together in relation to host condition. When the parasites were examined independently, we only observed a significant relationship between host condition and strongyle intensity. These results indicate the importance of examining how the parasite community in concert may relate to host condition. Further research into the diversity of species within the strongyle nematodes could identify which parasite species have the largest impact on host body condition.

The relationship between parasites and host condition may indicate the symptoms of disease resulting in decreased condition, a reduced ability to control parasite infections in hosts that are in poor condition, or a synergism between these two processes (Beldomenico *et al.* 2008). Regardless of whether increased parasitism or high parasite propagule shedding rates is a result of poor host condition or simply a consequence of it, a reduction in host body condition for certain individuals may influence springbok population dynamics. The springbok population shows no signs of food limitation and yet is not increasing (Gasaway *et al.* 1996), indicating that predation or disease may be limiting population growth. It is possible that these parasites, in particular the strongyle nematodes, may be associated with reduced reproductive success or survival of individual hosts (Hoberg *et al.* 2001).

A reduction in reproductive success from parasitism would affect males and females differently. Reduced body condition from parasitism may limit male reproductive opportunities, as only males in excellent physical condition can hold quality territories (Ritter & Bednekoff 1995; Skinner *et al.* 1996). For females a reduction in body condition may decrease fecundity (Stien *et al.* 2002; Lello *et al.* 2005), offspring condition (Hakkarainen *et al.* 2007) or the sex ratio of embryos (Krüger *et al.* 2005), all factors that may affect population growth rates. Relatively little is known about population dynamics of springbok. Increases in kidney fat of female springbok are associated with an increased proportion of female embryos (Krüger *et al.* 2005). Rainfall effects on springbok body mass show that the amount of dry season (May-September) rainfall in the year before birth is more important for body mass than current or prior year rainfall (van Hensbergen *et al.* 1992). Inputs of rainfall during the dry season can

increase juvenile survival for other species (Owen-Smith *et al.* 2005), but Etosha is a strongly seasonal system and winter rainfall rarely occurs (Figure 2). The proportion of juveniles per adult female observed four months after birth is negatively related to rainfall the prior year (Turner unpublished data), a pattern which could be related to effects of vegetation quality, predators or parasites, but has yet to be explained.

Host morbidity resulting from parasite infection is often implicated in facilitating predation (Hudson et al. 1992, Murray et al. 1997, Packer et al. 2003). However, whether predators preferentially select prey in poorer condition depends on how easily individuals from a prey species can be captured. If a prey species is difficult to capture, then predators will preferentially select individuals in poorer condition (Temple 1987; Wirsing *et al.* 2002). In Etosha there are numerous predator species that can hunt adult springbok, including lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*), cheetah (*Acinonyx jubatus*), leopard (*P. pardus*) and black-backed jackal (*Canis mesomelas*) (Gasaway *et al.* 1991; Stander 1992; Hayward & Kerley 2008, Ministry of Environment and Tourism unpublished mortality records). The lion diet is primarily springbok in terms of numbers killed and biomass consumed, but the hunting success rate is low, with 13% success for springbok compared to other prey species which varied from 11-52% success (Stander 1992). Therefore, lions may be preferentially selecting springbok in poorer condition. The observed associations between GI parasites and host condition may alter the population dynamics of springbok.

Table 1. Logistic regression models of parasite presence by season, host age, sex and body condition, and the presence or absence of other parasite types, assessed separately for each of the three study areas. Directionality of relationships is represented as: + for season means parasite presence was greater in the wet season than the dry season, + for age means parasite presence increased with host age, + for sex means parasite presence was higher for males than for females, and – for condition means parasite presence was associated with reduced host body condition. Significance levels are presented as ***=$p<0.001$, **=$p<0.01$, *=$p<0.05$, ^=$p<0.1$; ns=non-significant. Sample sizes for the models from each study area were Otjovasandu 156, Okaukuejo 692, Namutoni 157.

	Independent variables								
dependent variable	season[wet]	age[juvenile]	sex[male]	condition	strongyles	Strongyloides	*E. marsupialis*	*E. lammekia*	area
E. antidorcasi	+***	-*	ns	ns	ns	ns	x	-^	Otjovasandu
E. antidorcasi	+***	ns	ns	ns	ns	+***	x	-^	Okaukuejo
E. antidorcasi	+***	ns	ns	ns	ns	+^	x	ns	Namutoni
E. lammekia	ns	-**	-*	ns	ns	ns	ns	x	Otjovasandu
E. lammekia	+***	-***	ns	ns	ns	ns	ns	x	Okaukuejo
E. lammekia	ns	-***	ns	ns	ns	ns	ns	x	Namutoni
strongyles	+**	ns	ns	ns	x	ns	ns	ns	Otjovasandu
strongyles	+***	+*	+*	ns	x	+***	ns	ns	Okaukuejo
strongyles	+***	-^	ns	ns	x	+^	ns	ns	Namutoni
Strongyloides	+***	ns	ns	ns	ns	x	ns	ns	Otjovasandu
Strongyloides	+***	ns	ns	-**	+***	x	+***	ns	Okaukuejo
Strongyloides	+**	ns	ns	ns	ns	x	+^	ns	Namutoni

Table 2. The prevalence recorded for each parasite type in the three study areas, separated by season or age.

		parasite prevalence					
		season		age			
parasite	area	dry	wet	juvenile	yearling	adult	total *N*
E. antidorcasi	Otjovasandu	0.10	0.84	0.41	0.73	0.61	156
E. antidorcasi	Okaukuejo	0.22	0.88	0.51	0.69	0.62	692
E. antidorcasi	Namutoni	0.16	0.90	0.67	0.74	0.60	157
E. lammekia	Otjovasandu	0.10	0.11	0.36	0.15	0.04	156
E. lammekia	Okaukuejo	0.12	0.20	0.35	0.14	0.11	692
E. lammekia	Namutoni	0.09	0.13	0.40	0.04	0.05	157
strongyles	Otjovasandu	0.50	0.95	0.73	0.91	0.80	156
strongyles	Okaukuejo	0.59	0.96	0.69	0.86	0.83	692
strongyles	Namutoni	0.52	0.99	0.90	0.87	0.79	157
Strongyloides	Otjovasandu	0.04	0.66	0.41	0.62	0.43	156
Strongyloides	Okaukuejo	0.23	0.90	0.54	0.69	0.63	692
Strongyloides	Namutoni	0.34	0.89	0.70	0.78	0.67	157

Table 3. Comparisons among study areas of a) parasite occurrence, b) parasite intensity and c) host body condition. Parasite intensity data are log(count+1) transformed; models of parasite intensity include samples with non-zero parasite counts, the model of host body condition includes all samples. The factors in each model are significant factors identified from the larger Okaukuejo dataset. Sample sizes from each area considered in these models were 160 for Otjovasandu, 204 for Okaukuejo and 160 for Namutoni.

a) Parasite occurrences. Model types: logistic regression

	Independent variables															
Parasite	area		season		age		condition		strongyles		*Strongyloides*		*E. antidorcasi*		*E. lammekia*	
occurrence	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*
Strongyloides	35.1	<0.0001	54.8	<0.0001	59.7	<0.0001	4.0	0.2635	3.4	0.0657			7.6	0.0058		
Strongyles	0.6	0.7242	52.2	<0.0001	1.4	0.5014					3.5	0.0630				
E. antidorcasi	4.7	0.0964	109.9	<0.0001	6.8	0.0330					7.5	0.0062			4.4	0.0367
E. lammekia	2.7	0.2575	0.1	0.7794	55.5	<0.0001										

b) Parasite intensities. Model types: multiple regression

	Independent variables															
Parasite	area		season		age		condition		strongyles		*Strongyloides*		*E. antidorcasi*		*E. lammekia*	
intensity	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Strongyloides	34.5	<0.0001	37.2	<0.0001					6.9	0.0092			1.9	0.1640		
Strongyles	4.6	0.0101	80.8	<0.0001			3.0	0.0300			36.9	<0.0001				
E. antidorcasi	5.9	0.0078	27.2	<0.0001	1.1	0.3279										
E. lammekia	1.3	0.2702			5.2	0.0081			2.7	0.1038						

c) Body condition. Model type: ordinal logistic regression

	Independent variables															
Dependent	area		season		age		condition		strongyles		*Strongyloides*		*E. antidorcasi*		*E. lammekia*	
variable	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*	*Wald*	*p*
condition	15.1	0.0005	0.2	0.6273	31.4	<0.0001			9.2	0.0025	3.1	0.0802	1.8	0.1785		

Table 4. Relationships among parasite intensity and season, host age, sex and body condition, and the counts (intensities including zero values) of other parasites. Directionality of relationships is represented as: + for season means parasite intensity was higher in the wet season than the dry season, + for age means parasite intensity increased with host age, + for sex means parasite intensity was higher for males than for females, and – for condition means parasite intensity was associated with reduced host body condition. Significance levels are presented as ***=p<0.001, **=p<0.01, *=p<0.05, ^=p<0.1; ns=non-significant. Data are from the Okaukuejo study area only.

		Independent variables							
dependent variable	season[wet]	age	sex[male]	condition	strongyles	Strongyloides	*E. antidorcasi*	*E. lammekia*	*N*
strongyles	+***	ns	ns	-***	x	+***	ns	ns	563
Strongyloides	+***	-^	+^	-^	+***	x	+*	ns	433
E. antidorcasi	+***	-**	ns	ns	ns	ns	x	ns	442
E. lammekia	ns	-***	ns	ns	+^	ns	ns	x	120

Table 5. Models of host body condition comparing the effects of various environmental, host and parasite factors. Models are ranked by ΔAIC, which indicates the difference between the AIC value of each model and the model with the lowest AIC value (rank 1). Models with ΔAIC<2 are equally plausible given the data (Burnham & Anderson 2002). Variable abbreviations are: str, strongyle nematodes; strd, *Strongyloides* spp.; Ea, *E. antidorcasi*; El, *E. lammekia*; rain1, rain2 and rain3 are the monthly rainfall one, two or three months prior to sample collection.

Rank	Model	Model χ^2 *P*	AIC	ΔAIC	R^2 (U)
1	age, sex, str, strd, Ea, rain2	<0.0001	1135.327	0.00	0.1125
2	age, sex, str, strd, Ea, El, rain2	<0.0001	1136.589	1.26	0.1132
3	age, str, strd, Ea, rain2	<0.0001	1136.62	1.29	0.1109
4	age, sex, str, strd, Ea, El, rain2, rain3	<0.0001	1138.086	2.76	0.1137
5	age, str, Ea, rain2	<0.0001	1139.461	4.13	0.1072
6	age, sex, str, strd, Ea, El, rain1, rain2, rain3	<0.0001	1139.888	4.56	0.1139
7	age, str, rain2	<0.0001	1153.096	17.77	0.1038
8	age, str	<0.0001	1172.389	37.06	0.0869
9	age	<0.0001	1201.335	66.01	0.0637

Table 6. Model coefficients for the best logistic regression of host body condition based on AIC values.

Parameter	d.f.	Coefficient	SE	χ^2	*P*
age[yearling-juvenile]	1	-1.110	0.291	14.72	0.0001
age[adult-yearling]	1	-0.758	0.209	13.20	0.0003
sex	1	-0.129	0.080	2.62	0.1057
strongyles	1	-1.114	0.178	38.99	<0.0001
E. antidorcasi	1	-0.282	0.103	7.49	0.0062
Strongyloides spp.	1	0.391	0.188	4.32	0.0377
rain 2 months prior	1	0.009	0.002	23.43	<0.0001

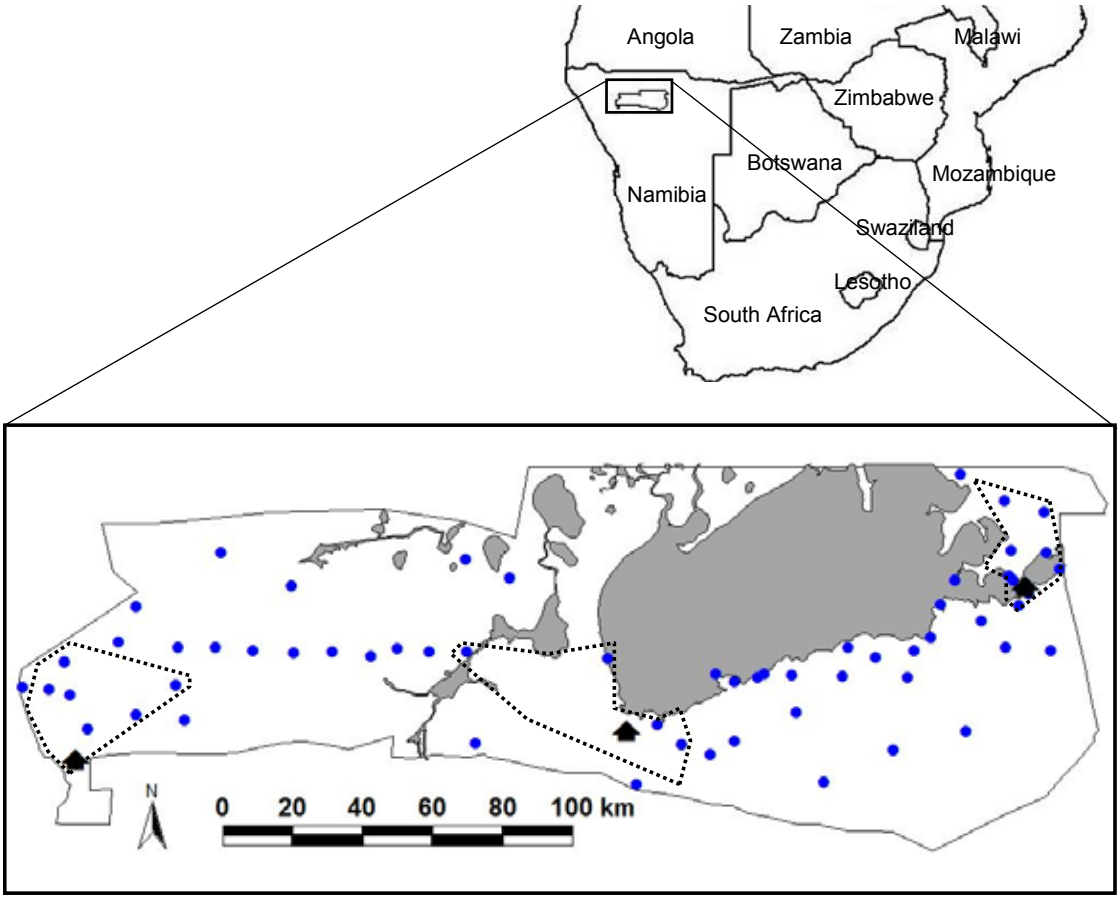

Figure 1. Etosha National Park in northern Namibia. Perennial watering points (springs or boreholes) are blue circles, the three study areas are indicated with dashed lines. From west to east, the study areas are Otjovasandu, Okaukuejo and Namutoni. The location of management stations for each area is indicated with a house symbol. Rainfall data were collected at these management stations.

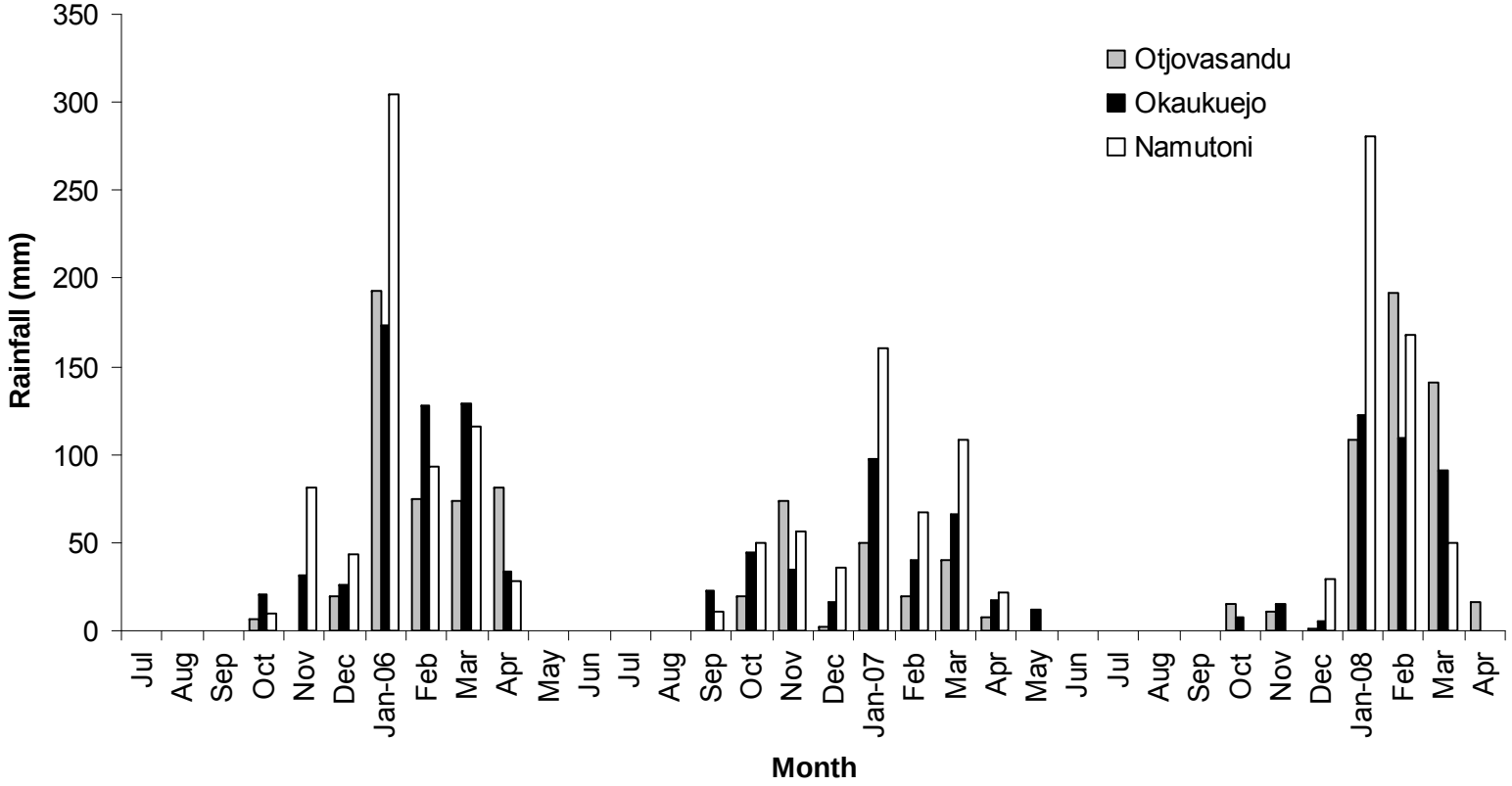

Figure 2. Monthly rainfall during the study period, July 2005-April 2008, in each of the three study areas.

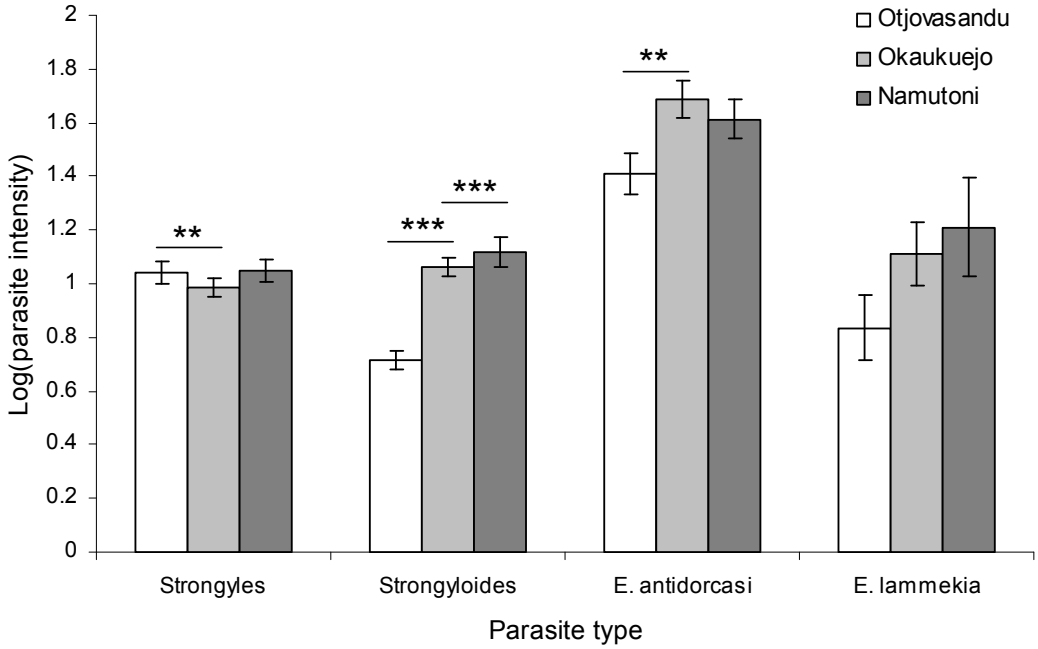

Figure 3. Mean and standard error of parasite intensity by parasite type and study area. Significant differences between study areas are indicated as ***=p<0.001, **=p<0.01. Annual rainfall in the study areas increases from Otjovasandu to Okaukuejo to Namutoni.

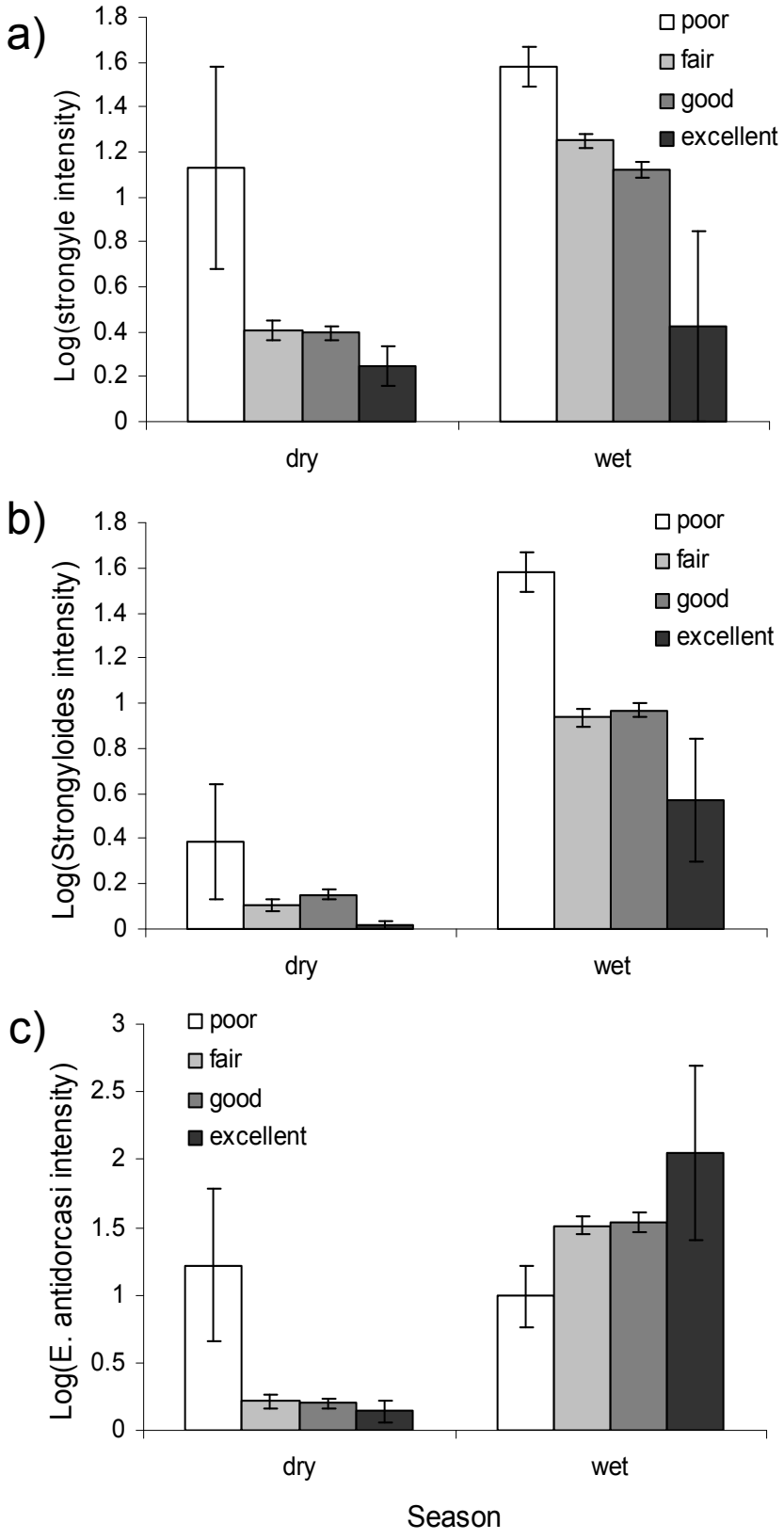
a)
Log(strongyle intensity)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
poor
fair
good
excellent
dry
wet
b)
Log(Strongyloides intensity)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
poor
fair
good
excellent
dry
wet
c)
Log(E. antidorcasi intensity)
3
2.5
2
1.5
1
0.5
0
poor
fair
good
excellent
dry
wet
Season

Figure 4.

Figure 4. Mean propagule-shedding intensities by host body condition score (poor-excellent) and seasons for a) strongyle nematodes, b) *Strongyloides* spp. and c) *E. antidorcasi.* Error bars show the standard error of the mean. The sample sizes for each condition class are variable: poor=23, fair=295, good=358, excellent=17. Here, intensity includes all samples, not just those positive for a particular parasite type.

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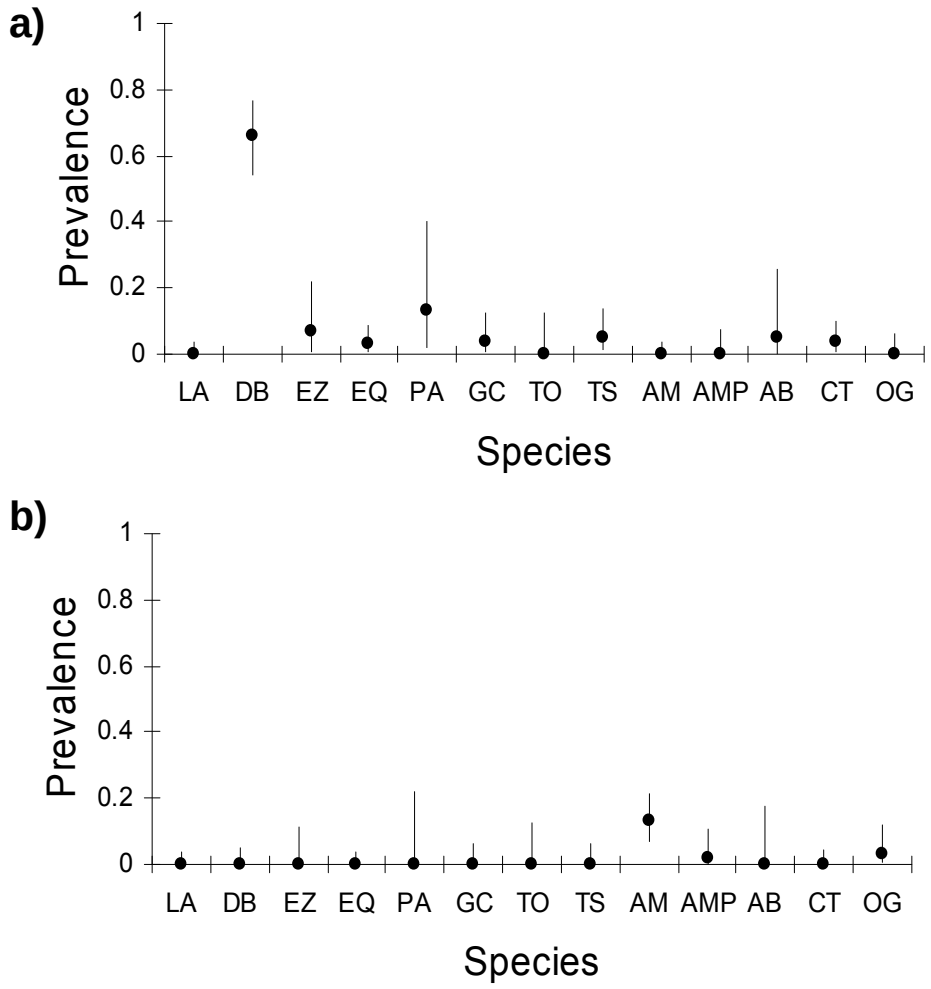
Appendix I



The prevalence and 95% binomial confidence intervals (based on sample size) of a) Anoplocephalid cestodes and b) *Strongyloides* spp. in ungulates (methods discussed in Chapter 2). AB, *Alcelaphus buselaphus*; AM, *Antidorcas marsupialis*; AMP, *Aepyceros melampus petersi*; CT, *Connochaetes taurinus*; DB, *Diceros bicornis*; EQ, *Equus quagga*; EZ, *Equus zebra*; GC, *Giraffa camelopardalis*; LA, *Loxodonta africana*; OG, *Oryx gazella*; PA, *Phacochoerus africanus*; TO, *Taurotragus oryx*; TS, *Tragelaphus strepsiceros*.