

Soil ingestion, nutrition and the seasonality of anthrax in herbivores of Etosha National Park

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Abstract. Anthrax, caused by the bacterium *Bacillus anthracis*, is a seasonally occurring infectious disease affecting primarily herbivorous wildlife and livestock. The seasonality of anthrax outbreaks varies among locations, making it difficult to develop a single consistent ecological description of this disease. Over 44 years of mortality surveillance, most anthrax cases in Etosha National Park, Namibia are observed in the wet season, although elephants have an anthrax mortality peak in the dry season. Focusing on three host species (plains zebra, *Equus quagga*; African elephant, *Loxodonta africana*; and springbok, *Antidorcas marsupialis*) occupying the endemic anthrax area of Etosha National Park, Namibia, we tested two commonly posited causes of anthrax seasonality in herbivores: increased pathogen exposure due to greater soil contact, and increased host susceptibility due to seasonal nutritional stress. These hypotheses were assessed using fecal sampling and measurement of the percentage of fecal silicates as an index of soil ingestion and fecal nitrogen, phosphorus and crude fiber as nutritional indices. Nutritional quality for all three species was higher in wet than dry seasons. Comparing among wet seasons, nutritional indices showed either a decline in nutrition with increasing rainfall or no significant pattern. All three species had greater soil ingestion in the wet season than the dry season. Higher soil contact during the anthrax peak suggests that anthrax seasonality may in part be due to heightened exposure to *B. anthracis* in wet seasons, for zebra and springbok. Elephant anthrax deaths do not correspond with the season of increased soil ingestion or grazing, suggesting that other behavioral mechanisms may overshadow foraging-based risk factors for this species. Nutritional stress is unlikely the primary causative factor in wet season anthrax systems, although nutritional stress sufficient to reduce resistance is difficult to assess non-invasively in wild herbivores. In contrast, increased soil ingestion may be an important predisposing factor for wet season anthrax outbreaks. Ultimately, the amount of soil ingested and its importance in the transmission of soil-borne pathogens will vary based on foraging behaviors, intake rates, grassland structure and on the likelihood that foraging areas intersect with pathogen aggregations in the environment.

Key words: anthrax; *Antidorcas marsupialis*; *Bacillus anthracis*; *Equus quagga*; Etosha National Park, Namibia; fecal nitrogen; fecal phosphorus; *Loxodonta africana*; nutrition; seasonality; soil ingestion.

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INTRODUCTION

Anthrax outbreaks occur world-wide and are of concern for animal conservation (Leendertz et al. 2004, Muoria et al. 2007), wildlife management (Clegg et al. 2007, Wafula et al. 2008), agriculture (Mongoh et al. 2008, Durrheim et al. 2009, Lewerin et al. 2010) and public health (Siamudaala et al. 2006). Anthrax is caused by the bacterium *Bacillus anthracis* which can infect a wide range of animal species, but mortalities are most commonly observed in herbivorous mammals. Anthrax can occur as different forms, depending on the route of infection: cutaneous, pulmonary or gastrointestinal. Airborne movement of anthrax spores is unlikely to expose animals to inhalational anthrax (Turnbull et al. 1998) and cutaneous lesions would be apparent on visual inspection. Because of obvious health and safety concerns, not many anthrax carcasses are necropsied to determine the route of infection. When animals have been necropsied, however, they have shown signs suggestive of gastrointestinal anthrax (Ebedes 1976, Wafula et al. 2008). For the purposes of this study, we assume that transmission occurs via the oral route and that herbivores are contracting gastrointestinal anthrax.

Although anthrax can be highly pathogenic in herbivores, with deaths estimated to occur a few days to two weeks following a lethal dose (WHO 2008), numerous surveys have failed to find concentrations of *B. anthracis* in the environment equivalent to experimental lethal doses. The anthrax bacterium has two life forms, vegetative cells and spores, and spores are the form required to induce an oral infection. Ingested lethal doses (LD₅₀) for herbivores (bovids or equids) are estimated to be 10⁷ to 10⁸ spores administered as a single dose, however quantities of spores per gram measured in the environment (in contaminated soil, water or scavenger feces) are many orders of magnitude lower (WHO 2008). It is unknown how well these studies on laboratory animals relate to conditions in the natural environment, though it is likely that with various environmental stressors, the LD₅₀s may be significantly lower under natural conditions. Herbivores can develop antibodies against *B. anthracis* (Fasanella et al. 2007, Lembo et al. 2011), providing evidence that

herbivores in endemic areas are exposed to sub-lethal doses of *B. anthracis*. Together, these results lead to numerous suppositions behind the timing of anthrax outbreaks in endemic areas, pertaining to whether outbreaks are facilitated by (1) an increase in host exposure, through changes in animal behavior or to the environment, (2) an increase in host susceptibility, as a result of various environmental, social or reproductive stressors or (3) variation in concentrations of *B. anthracis* in the environment, as a result of a bacterial lifecycle outside the host. There has been growing evidence of the activity of *B. anthracis* outside of hosts (Saile and Koehler 2006, Schuch and Fischetti 2009), however a definitive study has yet to demonstrate that the pathogen can multiply to the concentrations needed for a lethal dose. Clearly, during outbreaks lethal doses of *B. anthracis* are being contracted from the environment. Until further evidence emerges on the activity of *B. anthracis* in the environment, the search for mechanisms behind anthrax outbreaks centers on those factors affecting host exposure and susceptibility.

Anthrax outbreaks can be distinguished by those affecting primarily grazing or browsing herbivores. For browsing species in southern Africa (e.g., greater kudu, *Tragelaphus strepsiceros*) a link has been described between high densities of blowflies and anthrax epidemics (Braack and de Vos 1990, Clegg et al. 2007). Blowflies, after feeding on an infected carcass, rest on nearby trees and shrubs, defecating and regurgitating *B. anthracis* onto the vegetation that serves as a food source for browsing animals (Braack and de Vos 1990). This blowfly epidemic cycle has been suggested as the main transmission route for browsing species such as lesser kudu (*Tragelaphus imberbis*) in Mago National Park, Ethiopia (Shiferaw et al. 2002) impala (*Aepyceros melampus*) in Serengeti National Park, Tanzania (Hampson et al. 2011) and white-tailed deer (*Odocoileus virginianus*) in Texas (Blackburn et al. 2010). Browser outbreaks can occur at different times of the year (e.g., dry season in southern Africa (Pienaar 1960, 1961, de Vos 1989, Clegg et al. 2007); or after heavy rains in east Africa (Hampson et al. 2011)), but different species of blowflies also have peaks in population abundance at different times of the year (Braack and de Vos 1987). A blowfly-browser cycle explains

the large epidemics seen in these species, but not the source of infection for the index case of these outbreaks. Biting flies have also been implicated in some anthrax outbreaks, for example in atypical presentation of subcutaneous edema in horses in Italy (Palazzo et al. 2012), but will not be discussed further here (the role of insect vectors is reviewed by Hugh-Jones and Blackburn 2009).

For anthrax outbreaks of primarily grazing herbivores, no consensus exists on the general causes of outbreaks. This is in part due to the different environmental and ecological conditions under which outbreaks have occurred. The timing of anthrax outbreaks within a particular locality tend to have a seasonal signal, although the season during which most cases occur varies from locality to locality. Outbreaks consisting primarily of grazing wildlife or livestock occur in seasonal or weather conditions along a spectrum from drought (Fox et al. 1977, Shiferaw et al. 2002, Muoria et al. 2007, Wafula et al. 2008, Hampson et al. 2011), to hot dry seasons often following years of unusually high rainfall (Rees et al. 1977, Gates et al. 1995, Dragon et al. 1999, Turner et al. 1999, Himsworth and Argue 2008, Chikerema et al. 2012), to transition periods between dry-wet or wet-dry conditions (Fox et al. 1973, Parkinson et al. 2003, Siamudaala et al. 2006, Mongoh et al. 2008), to wet season outbreaks (Lindeque and Turnbull 1994, Durheim et al. 2009, Chikerema et al. 2012). Some authors do not specify the time scale in which the described weather patterns occur in relation to the outbreak. This makes it difficult to know, for example, if markedly wet or dry conditions were on the scale of days, weeks or months prior to case detection. Weather extremes are suggested as predictors of possible outbreaks in known anthrax areas (Hampson et al. 2011). The mechanisms behind these outbreaks, however, remain largely unknown. This is, in part, due to their often sporadic nature, the different conditions in which outbreaks occur, and the diversity of species affected, within and among ecosystems.

The different sets of environmental conditions associated with anthrax outbreaks in locations around the world have been posited as indicative of the underlying mechanisms causing anthrax in grazing animals. These mechanisms can be

differentiated by those thought to be more prominent during outbreaks in dry or wet conditions. Hypotheses under wet conditions tend to focus on the role of water transport, which could unearth buried spores and/or move spores from soil to vegetation or on arthropod vectors (Hugh-Jones and Blackburn 2009, Hampson et al. 2011). Hypotheses under dry conditions have included: (1) reduced resource availability causes increased animal density in localized areas; (2) animals feeding on dwindling or wilted vegetation increase their exposure to spores in the soil or dust; (3) water movement concentrates spores into low-lying areas on the landscape, and animals utilizing these areas (for food, water, mud or dust bathing) increase their exposure to spores; (4) animals consuming dry or prickly vegetation increase their rates of GI tract lesions, thereby increasing the likelihood that ingested spores enter the host's body and cause infection; and (5) various stressors (e.g., poor nutrition, competition, heat, parasitism, reproductive activities) lead to reduced host resistance (Van Ness 1971, Gainer and Saunders 1989, Dragon and Rennie 1995, Hugh-Jones and de Vos 2002, WHO 2008). All these hypotheses are ecosystem and host specific and do not directly address factors that may affect the sporulation, persistence, germination or possible multiplication of *B. anthracis* outside a host.

In this study, we assess factors behind the seasonality of anthrax in Etosha National Park, Namibia. Anthrax is widespread in northern and central Namibia (Beyer et al. 2012) and was first documented in the country on farms in 1879 and in Etosha in 1964 (Ebedes 1976). Throughout nearly 50 years of anthrax surveillance in Etosha, deaths in the plains ungulates (plains zebra, *Equus quagga*; springbok, *Antidorcas marsupialis*; and blue wildebeest, *Connochaetes taurinus*) sharply peak in the late wet season (March–April) whereas African elephant (*Loxodonta africana*) mortalities broadly peak in the late dry season (October–November), although cases occur throughout the year (Ebedes 1976, Lindeque and Turnbull 1994, Beyer et al. 2012). Anthrax mortalities in the plains ungulates occur annually, while elephant anthrax mortalities tend to be sporadic; most elephant anthrax cases recorded in the park were during droughts in the 1980s (Lindeque 1991). Anthrax control was conducted

in Etosha from 1968–1981, through methods such as burning or burying of carcasses, disinfection of watering points or closing of water points to restrict animal movements. Control efforts ceased after a large elephant anthrax outbreak in 1981 which overwhelmed carcass disposal efforts (Lindeque 1991). Since then, anthrax has been considered part of the natural ecosystem of Etosha and is not actively managed. Anthrax transmission in Etosha was originally thought to occur as a result of drinking at contaminated water holes (Ebedes 1976). Further study of the presence of *B. anthracis* in water or sediments of waterholes found few positive samples and low concentrations of the pathogen (Lindeque and Turnbull 1994), which lends support to the hypothesis that *B. anthracis* transmission occurs primarily during foraging.

Increased soil contact is often suggested as an important factor in anthrax outbreaks in grazing species (WHO 2008), but to our knowledge this has yet to be studied in any anthrax system. Soil ingestion is thought to be important in dry season anthrax outbreaks, when grass biomass is depleted or wilted and soils are dry and dusty. However, many herbivores are mixed-feeding species that consume a variety of plant species, depending on what is readily available and most nutritious at the time. These herbivores eat grasses when they are green and highly digestible, and switch to the leaves of shrubs and trees as grass quality declines. If herbivores are more likely to feed close to the soil (on grasses) during wet seasons, then soil ingestion should be higher in wet seasons. Herbivores are also known to intentionally consume soil either for trace minerals or to buffer the pH of the digestive system when grasses are highly digestible (Skipworth 1974, Ayotte et al. 2006). We therefore hypothesized that soil ingestion may be higher in wet seasons than dry seasons, for both grazing and mixed-feeding herbivores. This study explores the idea that the more soil an herbivore ingests, the greater its chance of contracting a lethal dose of *B. anthracis* from soil.

In addition, animal nutrition serves as a useful proxy for many hypothesized anthrax risk factors, such as reduced forage quality and availability, high animal density and heightened resource competition. Poor nutrition has also been suggested as one of the main stressors

affecting the susceptibility of animals to anthrax infections (Hugh-Jones and de Vos 2002). Grass nutritional quality will of course be better during the vegetation growing season than during the dormant season, but more rainfall during the growing season does not necessarily mean better forage quality. Higher rainfall leads to higher grass biomass, which may be more fibrous and hence less digestible than grasses grown during more moderate growing seasons. If rainfall extremes correlate with anthrax outbreaks, we hypothesized that animals may experience poorer nutrition not just during droughts, as expected, but also during periods of unusually high rainfall. Animal nutrition was assessed using estimates of fecal nitrogen, phosphorus and crude fiber. Fecal nitrogen and phosphorus are good indicators of forage quality in southern Africa (Grant et al. 2000).

Here we assess two commonly posited causes of anthrax seasonality in herbivores, that hosts have (1) greater pathogen exposure due to soil contact and (2) greater susceptibility due to nutritional stress. We test these with herbivore species in Etosha National Park that differ in the seasonal timing of anthrax occurrence. Although nutritional stress and soil contact have been suggested as important correlates with anthrax outbreaks in dry rather than wet seasons, we hypothesized that these factors could be general to any anthrax system where grazing species are the primary hosts, regardless of season. We describe patterns of anthrax occurrence in Etosha National Park from 44 years of disease surveillance. Finally, we discuss the relative roles of soil ingestion and nutrition in anthrax seasonality in Etosha and how these factors may apply in other grazing anthrax systems.

METHODS

Study site and species

The study area was the central Okaukuejo management section of Etosha National Park, which surrounds the western edge of the Etosha salt pan. The soils in the anthrax endemic area are high in calcium carbonate and of moderate to high alkalinity, salinity and drainage (Beugler-Bell and Buch 1997). Although much of the park is covered by mopane (*Colophospermum mopane*) scrubland or woodland, the plains around

Okaukuejo are primarily short grasslands and dwarf shrub savannas (le Roux et al. 1988). Three seasons are recognized in semi-arid Etosha, the cool dry season (May–August), the hot dry season (September–December) and the hot wet season (January–April). Temperatures range from an average monthly minimum of 6°C in July, to an average monthly maximum of 35°C in October–December (Fig. 1A). Mean annual rainfall at Okaukuejo was 390 mm from 1934–2011. Rainfall years are recorded from July to June, because the rainy season straddles the transition between calendar years (Fig. 1B). These rainfall years are labeled by the year of the second six months (January–June) because peak rainfall occurs in January/February. The years of study had either average rainfall (2005, 2007, 2008 and 2010; range 350–390 mm) or high rainfall (2006, 2009 and 2011; range 542–705 mm).

The three study species, plains zebra, springbok and African elephant, cover a range of body sizes, diet types and digestive physiologies. All three species graze when grasses are nutritious, but springbok and elephant are mixed-feeders, primarily browsing when grazing conditions are less favorable. Mean adult body masses (lower estimate from females, upper from males) from various populations for springbok adults range from 28–42 kg, zebra adults from 220–250 kg and elephant adults from 2800–5000 kg (Owen-Smith 1988). Springbok are ruminants, zebras are hind-gut fermenters and elephants have a simple digestive system. Population estimates (with 95% confidence intervals, rounded to the nearest 100) in 2005 were 15,600 (13,200–17,900) for springbok, 13,000 (10,900–15,000) for zebras, and 2,600 (1,900–3,300) for elephants (Namibian Ministry of the Environment and Tourism unpublished aerial survey data), although the springbok estimate is thought to be an undercount due to their small body size, aggregation and coloration.

Anthrax surveillance and diagnostics

The Etosha Ecological Institute has a database of mortality records from 1968 to the present. Anthrax surveillance in Etosha is opportunistic and park staff and researchers document mortalities observed for medium and large-bodied mammals, recording the date, species, location, suspected cause of death and the age and sex

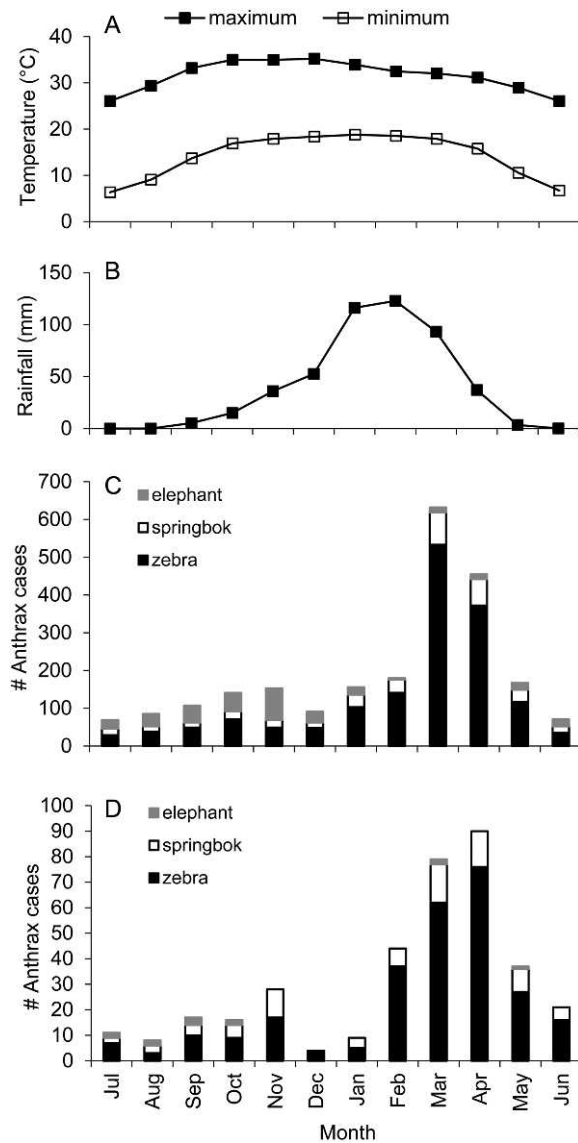


Fig. 1. Mean monthly temperature, rainfall and the number of confirmed anthrax cases by month. (A) The mean monthly minimum and maximum temperatures recorded at Okaukuejo station in central Etosha from January 1975–January 2004, the data currently available for this area, (B) mean monthly rainfall during the 2005–2011 rainfall seasons recorded at Okaukuejo, (C) monthly confirmed anthrax cases recorded 1968–2011 throughout Etosha for the study species, and (D) monthly confirmed anthrax cases for the study species recorded during the study period (2005–2011) in central Etosha.

when possible. Because anthrax is commonly observed in the park, staff and researchers have collected samples from observed carcasses for anthrax diagnostics whether or not anthrax is suspected as the cause of death. Opportunistic road-based surveillance will only recover a fraction of the total anthrax cases (Bellan et al., *in press*) but we do not expect seasonal biases in effort that would alter the seasonal patterns observed.

Prior to 1987, an anthrax diagnosis was determined primarily from examination of blood smears or visual signs at the carcass, with some samples sent to the Central Veterinary Laboratory in Windhoek for confirmation (Lindeque 1991). From 1987 until present, all sampled carcasses were tested for *B. anthracis* through bacterial culture and confirmation using standard protocols (WHO 2008). The samples collected were mostly swabs of blood or carcass exudates; occasionally soil samples were collected from beneath older carcasses for culture. The numbers of anthrax cases presented in this study are either those carcasses that were considered “confirmed *B. anthracis*” or “suspected *B. anthracis*.” Those listed as suspected cases had signs suggestive of anthrax, but were unconfirmed for various reasons.

Fecal sample collection

Animal nutrition and soil ingestion were evaluated from fecal measures, using samples collected from 2005–2011 (Table 1). All fecal samples were collected opportunistically along the road network and at waterholes. Every effort was made to reduce the possibility of resampling individuals, with collection conducted at different locations in the study area for each sampling day within the time period of interest.

For zebra and springbok, the wet season sampling occurred February–March and the dry season sampling July–August. These represent the wet season months prior to and including the monthly peak in anthrax occurrence and the dry season months with the lowest recorded anthrax occurrence (Fig. 1C, D). Zebra and springbok fecal samples from seven seasons (four wet, three dry; 2005–2009) were used for nutritional analyses. Additional fecal samples to assess soil ingestion from zebra and springbok (2008–2009) were carefully collected to ensure the samples

were not contaminated with soil. Collecting soil-free feces was challenging for springbok because they have small fecal piles and often defecate while walking; many potential samples were rejected as soil-contaminated. Fecal sampling to assess soil ingestion of zebra and springbok occurred in three seasons, the early-wet season, the wet season and the dry season.

Fecal samples from elephants were more difficult to collect than from the abundant populations of zebra or springbok, particularly during the wet season when elephants disperse into inaccessible woodland areas. Therefore the wet and dry season collection periods were longer and more opportunistic for elephants than for zebra and springbok (Table 1). For elephants, fecal samples were collected in the dry season from July–October and in the wet season from January–May during the period from August 2009 to April 2011. Fresh elephant samples were sought by visiting waterholes in early morning hours or from piles located along the road network. Fecal piles were examined to determine how recently they were defecated, and only moist boluses were sampled. Boluses were carefully broken apart and fecal matter was collected from the inner core to avoid soil contamination.

Fecal samples from juveniles were excluded. The zebra and springbok samples were collected from individuals observed defecating, and only samples from individuals one year and older were used in this study (aging criteria described in Turner and Getz 2010). For elephants, age was estimated by measuring the largest intact bolus, and boluses of diameter 14 cm or greater were considered to be from adults (Morrison et al. 2005).

Springbok and zebra samples were each pooled for laboratory analyses, whereas elephant fecal samples were analyzed individually (Table 1). Pooling was implemented because the quantity of fecal material available was of insufficient mass for individual-level analyses. For zebra and springbok, five individual samples were pooled into one, based on the sequential dates of collection. Ten pooled samples per season were analyzed for nutrient content, for a total of 70 pooled samples from zebra and from springbok (i.e., from a total of 350 individual samples for each species). For soil ingestion analysis, five

Table 1. Fecal collection periods by species for the nutrition analyses (fecal nitrogen, crude fiber and phosphorus) and silicate analysis.

| Year and season | Sampling months | Sample type | N/species | Sample source |
|-------------------------|-----------------|-------------|-----------|-----------------|
| Springbok and zebra | | | | |
| Nutrition | | | | |
| 2005 dry | July–August | pooled | 10 | Turner 2009 |
| 2006 wet | February–March | pooled | 10 | Turner 2009 |
| 2006 dry | July–August | pooled | 10 | Turner 2009 |
| 2007 wet | February–March | pooled | 10 | Turner 2009 |
| 2008 wet | February–March | pooled | 10 | Turner 2009 |
| 2009 wet | February–March | pooled | 10 | Imologhome 2011 |
| 2009 dry | July–August | pooled | 10 | Imologhome 2011 |
| Silicates | | | | |
| 2008 early-wet | December | pooled | 5 | Imologhome 2011 |
| 2009 wet | February–March | pooled | 5 | Imologhome 2011 |
| 2009 dry | July–August | pooled | 5 | Imologhome 2011 |
| Elephant | | | | |
| Nutrition and silicates | | | | |
| 2009–2011 dry | July–October | individual | 26 | Havarua 2011 |
| 2009–2011 wet | January–May | individual | 26 | Havarua 2011 |

Note: For laboratory analysis the zebra and springbok samples are composites (pooled) of five individual fecal piles.

pooled samples per season were collected from zebra and springbok. For elephants 26 fecal samples were collected in each season. These samples were used for both nutrient and soil ingestion analysis.

Soil and nutrient analysis of feces

Nutritional quality was estimated using three common fecal indices of dietary quality: fecal nitrogen, phosphorus and crude fiber. Fecal nitrogen is a useful index for the dietary quality of forage (Leslie et al. 2008), although tannins (present in the leaves of some dicotyledonous plants) can increase the concentration of fecal nitrogen observed (Leslie and Starkey 1985, Wrench et al. 1997), so is a better nutritional proxy for grazers than browsers. Fecal phosphorus correlates with dietary phosphorus, and measurements are not affected by diet selection, so concentrations of this mineral are often included in estimates of dietary quality for mixed-feeding or browsing species (Wrench et al. 1997, Grant et al. 2000). Crude fiber provides an estimate of the digestibility of the forage consumed, where the higher the crude fiber, the lower the digestibility.

Nutrition estimates were conducted using standardized laboratory techniques (AOAC 1996). Nitrogen and phosphorus were measured using the Kjeldahl method. Fiber analysis was conducted with the crude fiber method, by digesting the sample in sulphuric acid and

sodium hydroxide. Fecal samples were oven-dried at 60°C for 48 hours and ground to fit through a 1 mm mesh. A 1–2 g portion of the ground sample was analyzed for the quantity of fecal nitrogen, phosphorus and crude fiber, and results are presented as percent of fecal dry matter.

The acid-insoluble ash (i.e., silicate) technique was used to determine the percentage of silicates in feces, a technique used in public health and wildlife management to estimate soil ingestion (Wong et al. 1988, Beyer et al. 1994). In this method 1 g dry weight of ground feces is incinerated and then dissolved in concentrated HCl, leaving a residue of acid-insoluble inert silicates. Wong et al. (1988) calculated the recovery efficiency of silica added to fecal samples at above 90% and the method was able to detect changes in the silica concentration representing 0.4% of fecal dry weight. This method was modified for elephants because of the large undigested plant pieces present in elephant feces. For elephants, 20 g of dry fecal matter (unground) was suspended in 1 liter of de-ionized water and stirred vigorously, coarse suspended plant matter was scooped from the surface and the remaining material was isolated by filtration then incinerated as per the original protocol. The percentage of silicates was calculated as a percentage of the original sample (1 g for zebra and springbok, 20 g for elephant).

Nutritional and fecal silicate analyses were

conducted at the Ministry of Agriculture, Water and Forestry Laboratory or the Analytical Laboratory Inc. in Windhoek, Namibia.

Data analysis

Correlations between annual rainfall and the number of observed anthrax cases were evaluated for the four main host species observed in Etosha over a 44 year period, 1968–2011. Although wildebeest were not a study species for the nutrition and soil ingestion components, we included them in this analysis as they are one of the main host species. Only cases recorded from the central Okaukuejo section of Etosha were used since surveillance effort in other sections of the park has been less consistent over time. Numbers of anthrax cases and accumulated rainfall were calculated for rainfall years (June–July) rather than calendar years, and rainfall data from Okaukuejo station were used. No mortality data were available for 1975, so this year was excluded from analysis.

Fecal nitrogen variation among species, a result of digestive capabilities, is considerable, and hence, does not necessarily reflect differences in dietary quality (Leslie et al. 2008). Since the three study species have very different digestive morphologies and efficiencies, we did not attempt to make comparisons among species in the nutrition parameters. Therefore, the effect of season (wet or dry) on the nutrition measurements was analyzed separately for each species using two-tailed *t* tests. The concentrations of fecal nitrogen, phosphorus and crude fiber are non-binomial proportions, and we used a logit transform ($\log(y/[1 - y])$) prior to statistical analyses (Warton and Hui 2011) to normalize the data distributions.

Linear regressions were used to examine inter-annual relationships between nutrition parameters and rainfall variation for springbok and zebra. For these analyses we used only the wet season samples, to examine annual variation in nutrition patterns prior to the peak in anthrax occurrence for these species. Since wet season samples were collected in the middle of the rainy season (February–March), the sum of rainfall for the months prior to sample collection (January–February) was used to analyze rainfall effects on nutrition parameters.

Seasonal differences in the fecal silicate data

(wet vs dry or early-wet vs wet) were compared using two-tailed Wilcoxon signed-rank tests for each species. Silicates are indigestible and are excreted in feces in proportion to the amount ingested (Beyer et al. 1994), however, the amount of undigested plant matter in feces will vary among species based on digestive capabilities, affecting the proportion of fecal silicates observed. As a result, we did not compare the percentage of fecal silicates among species. Instead, we calculated estimates of soil ingested (kg/day) for each species in each season using the equations of Mayland et al. (1975), to account for species differences, to make the species data more comparable and to evaluate how much contact animals could have with *B. anthracis* spores in soil. Since silicates in herbivore feces can be of soil or dietary origin—grasses (not browse) contain silica for structural support—we therefore modified the equations of Mayland et al. to include corrections for the contribution of dietary silica and also made an adjustment for the amount of organic matter in the soil.

If i_p is the dry matter intake of plant material (kg/day) and d is the digestible proportion of the forage, then the fecal mass of plant origin (kg/day), f_p , is

$$f_p = (1 - d)i_p. \quad (1)$$

Further, if s_f is the proportion of silicates in feces, y is the proportion of dietary silica from grasses, and g is the proportion of grass in the diet, then $s_f - (y \times g)$ is the proportion of silicates of soil origin in feces and $1 - (s_f - (y \times g))$ is the fraction of feces that is of plant origin. Hence, the total fecal output each day is given by

$$f_t = f_p / \left(1 - \left(s_f - (y \times g)\right)\right). \quad (2)$$

Finally, if x is the proportion of organic matter in the soil, then the amount of soil ingested (kg/day), i_s , is

$$i_s = (f_t - f_p) / (1 - x). \quad (3)$$

These equations do not take into consideration the contribution of gut microbes, parasites or soil organic matter to the total fecal mass, all of which we assume are negligible. The parameter values and data sources used for these calculations are shown in Table 2. Most literature sources presented data as means without an estimate of

Table 2. Parameters used to calculate soil ingestion for each species and season.

| Species | Parameter | Definition | Wet season estimate | Dry season estimate | Units | Source |
|-----------|-----------|----------------------|---------------------|---------------------|------------|--------------------------------|
| All | s_f | silicates in feces | Fig. 5 | Fig. 5 | proportion | this study |
| | x | soil organic matter | 0.025 | 0.025 | proportion | Turner <i>unpublished data</i> |
| | y | dietary silica† | 0.02 | 0.02 | proportion | Bezeau et al. 1966 |
| Springbok | g | grass in diet | 0.82 | 0.14 | proportion | Nagy and Knight 1994 |
| | d | forage digestibility | 0.62 | 0.62 | proportion | Van Hoven et al. 1984‡ |
| | i_p | intake rate | 2.5 | 1.3 | kg/day | Nagy and Knight 1994 |
| Zebra | g | grass in diet | 0.95 | 0.95 | proportion | Codron et al. 2005 |
| | d | forage digestibility | 0.54 | 0.37 | proportion | Bodenstein et al. 2000 |
| | i_p | intake rate | 6.1 | 6.2 | kg/day | Bodenstein et al. 2000 |
| Elephant | g | grass in diet | 0.5 | 0.4 | proportion | Codron et al. 2006 |
| | d | forage digestibility | 0.21 | 0.18 | proportion | Ruggiero 1992§ |
| | i_p | intake rate | 55.3 | 45.8 | kg/day | Ruggiero 1992 |

† The proportion of s_f estimated to be of dietary origin.

‡ Digestibility was not assessed for the same seasons as this study, therefore used the average for both.

§ Digestibility estimated as gross assimilation efficiency: $(i_p - f_i)/i_p$.

variation, and as a result we could not calculate confidence intervals around the estimates of soil ingestion.

RESULTS

Anthrax occurrence

Anthrax cases occur throughout the year, but a peak in cases is apparent towards the end of the rainfall season (Fig. 1). This peak is driven by the strong seasonality in zebra mortalities, since 52% of all confirmed cases were plains zebras (Table 3). The number of zebra and springbok anthrax cases were positively related to annual rainfall (zebra: $R^2 = 0.14$, $t = 2.6$, $p = 0.0131$, $N = 43$; springbok: $R^2 = 0.12$, $t = 2.3$, $p = 0.0260$, $N = 43$; Fig. 2) although annual rainfall describes only 12–14% of the variation in case numbers observed for these species. For elephant and wildebeest, there was no significant relationship between annual rainfall and anthrax cases (elephant: $R^2 = 0.01$, $t = -0.7$, $p = 0.4974$, $N = 43$; wildebeest: $R^2 = 0.03$, $t = 1.2$, $p = 0.2339$, $N = 43$).

Nutrition

Fecal nutrition indices all indicated that herbivores accessed higher quality forage in wet seasons. Fecal nitrogen was significantly higher in wet than dry seasons for all species (zebra: $t = 6.4$, $P < 0.0001$, $N = 70$; springbok: $t = 6.9$, $P < 0.0001$, $N = 70$; elephant: $t = 5.3$, $P < 0.0001$, $N = 52$; Fig. 3A). Fecal phosphorus content was also significantly higher in wet than dry seasons (zebra: $t = 8.0$, $P < 0.0001$, $N = 70$; springbok: t

$= 9.8$, $P < 0.0001$, $N = 70$; elephant: $t = 3.0$, $P = 0.0046$, $N = 52$; Fig. 3B). Fecal crude fiber content was significantly lower in wet than dry seasons (zebra: $t = -5.0$, $P < 0.0001$, $N = 70$; springbok: $t = -6.4$, $P < 0.0001$, $N = 70$; elephant: $t = -2.1$, $P = 0.0398$, $N = 52$; Fig. 3C).

Springbok fecal nitrogen was negatively related to rainfall ($F = 14.3$, $p = 0.0005$, $N = 40$; Fig. 4) and crude fiber was positively related to rainfall ($F = 12.2$, $p = 0.0012$, $N = 40$; Fig. 4). The fecal crude fiber of zebra in wet seasons also increased with increasing rainfall ($F = 19.5$, $p < 0.0001$, $N = 40$; Fig. 4), but zebra fecal nitrogen was not significantly related to rainfall ($F = 0.1$, $p = 0.8150$, $N = 40$; Fig. 4). There were no significant relationships between fecal phosphorus and rainfall for either species (Fig. 4).

Fecal silicates and estimates of soil ingestion

The fecal silicate content was significantly higher in the wet than dry season for all three species (zebra: $Z = 2.5$, $P = 0.0119$, $N = 10$; springbok: $Z = 2.5$, $P = 0.0090$, $N = 10$; elephant: $Z = 3.7$, $P = 0.0002$, $N = 52$; Fig. 5A). Comparing the early-wet season to the mid-wet season, zebra had significantly higher fecal silicates in the mid-wet season than the early-wet season, but springbok showed no significant difference in fecal silicates between the two wet season estimates (zebra: $Z = 2.1$, $P = 0.0361$, $N = 10$; springbok: $Z = 1.3$, $P = 0.2101$, $N = 10$; Fig. 5A).

Estimates of the quantity of soil ingested (in kg/day; Fig. 5B) for each species show that elephants have the greatest quantity ingested in the wet season of all species (1.4 kg/day) however

Table 3. The number of confirmed and suspected anthrax cases recorded in Etosha National Park from 1968–2011.

| Scientific name | Common name | Anthrax | Suspected | Total |
|-----------------------------------|---------------------|---------|-----------|-------|
| <i>Equus quagga</i> | plains zebra | 1586 | 346 | 1932 |
| <i>Connochaetes taurinus</i> | blue wildebeest | 660 | 224 | 884 |
| <i>Antidorcas marsupialis</i> | springbok | 384 | 70 | 454 |
| <i>Loxodonta africana</i> | African elephant | 297 | 225 | 522 |
| <i>Oryx gazella</i> | gemsbok | 38 | 9 | 47 |
| <i>Tragelaphus strepsiceros</i> | greater kudu | 14 | 8 | 22 |
| <i>Diceros bicornis</i> | black rhino | 12 | 12 | 24 |
| <i>Giraffa camelopardalis</i> | giraffe | 7 | 8 | 15 |
| <i>Struthio camelus</i> | ostrich | 6 | 3 | 9 |
| <i>Acinonyx jubatus</i> | cheetah | 10 | 0 | 10 |
| <i>Taurotragus oryx</i> | eland | 4 | 0 | 4 |
| <i>Equus zebra</i> | mountain zebra | 4 | 4 | 8 |
| <i>Alcelaphus buselaphus</i> | red hartebeest | 3 | 1 | 4 |
| <i>Panthera leo</i> | lion | 3 | 0 | 3 |
| <i>Raphicerus campestris</i> | steenbok | 1 | 1 | 2 |
| <i>Aepyceros melampus petersi</i> | black-faced impala | 1 | 0 | 1 |
| <i>Canis mesomelas</i> | black-backed jackal | 1 | 0 | 1 |
| <i>Panthera pardus</i> | leopard | 1 | 0 | 1 |
| <i>Mellivora capensis</i> | honey badger | 0 | 1 | 1 |
| Total | | 3032 | 912 | 3944 |

this becomes negligible in the dry season. Zebra soil ingestion estimates remain relatively high in both seasons (0.7 vs 0.6 kg/day in wet vs dry season) whereas springbok ingest relatively little soil, with estimates in both seasons of below 100 g/day. If body size is taken into account, zebra have the highest soil ingestion per unit body mass, followed by springbok and elephants (Fig. 5C).

DISCUSSION

Many infectious diseases have a seasonal component to their outbreaks, and for pathogens with an environmental life stage, a seasonal outbreak cycle can be caused by variation in the host, the pathogen or in environmental conditions. The relationships among these three components are often complex, making the search for causal mechanisms initiating disease outbreaks akin to searching for a needle in a haystack. This study evaluated two factors thought to influence the timing of anthrax outbreaks, enhanced host susceptibility due to nutritional stress and enhanced host exposure due to soil ingestion. The goals were two-fold, to determine if these factors were important in describing anthrax seasonality in Etosha National Park, a primarily wet-season anthrax system, and to evaluate how generally they may apply across grazing systems with different seasonal outbreak patterns.

Seasonality of nutrition

To understand the role of nutrition in reducing host resistance, we must know the range of nutritional values indicative of nutritional stress. However, nutritional requirements are not static; they vary seasonally and among individuals of a species depending on age, sex and reproductive status. Nutrient requirements are generally lower in dormant seasons as a result of physiological and behavioral mechanisms (Kuntz et al. 2006, Parker et al. 2009), such as altered intake rates, digesta passage rates, diet composition, habitat use, active times, and the timing of growth and reproduction. When resource quality or availability is insufficient, animals draw on body reserves, experiencing a decline in body fat and body protein (Parker et al. 2009). Poor nutrition further leads to a reduction in animal fecundity and survival (Gaillard et al. 2000).

Nutritional thresholds of around 1.9–2 g fecal phosphorus/kg (0.9–2%) and 13–16 g fecal nitrogen/kg (1.3–1.6%) have been suggested as levels below which herbivores are nutritionally stressed and cannot maintain body mass or rumen function (Wrench et al. 1997, Grant et al. 2000). The mean fecal nitrogen and phosphorus estimates for springbok were above these thresholds in both wet and dry seasons. The non-ruminant species, zebra and elephant, were below these nitrogen thresholds in both seasons and elephants were also below the phosphorus thresholds in both seasons. The studies present-

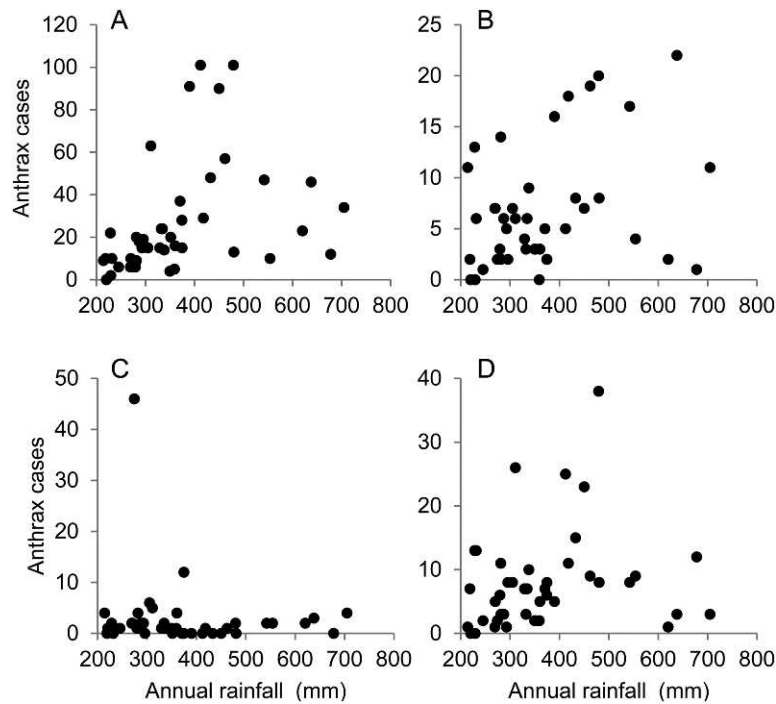


Fig. 2. The number of confirmed anthrax cases recorded annually in the Okaukuejo section of central Etosha, in relation to rainfall for plains zebra (A), springbok (B), African elephant (C) and blue wildebeest (D), from 1968–2011. Annual rainfall and mortality case records were calculated based on rainfall years (July–June) and not calendar years. All points are lower bounds for the actual number of cases; however, we assume the bias in underestimating actual numbers is proportionately similar each year.

ing nutrition thresholds, however, were conducted primarily on ruminants. Although Wrench et al. (1997) had zebra as a study species, most zebra samples were below their recommended thresholds, a result which was not discussed. Dietary thresholds of fecal nutrition indices determined for ruminant species are unlikely to apply to non-ruminant herbivores (e.g., elephants, Woolley et al. 2009), since they only take into account the proportion of nitrogen or phosphorus per unit feces. Ruminants have lower intake rates, greater digestive efficiency, and select higher quality forages than non-ruminant herbivores of similar body mass (Foose 1982, Duncan et al. 1990). Thus, these thresholds alone are unlikely to provide concrete evidence of whether Etosha's herbivores are in nutritional stress.

A comparison of fecal nutrition estimates among populations suggests that the nutritional concentration of forage ingested by herbivores in Etosha is similar to or better than what these

species access in other savanna areas. Springbok in Etosha had similar quantities of fecal nitrogen and phosphorus to springbok in the Northern Cape (Mbatha and Ward 2006) and similar fecal nitrogen but higher phosphorus to springbok in the Kalahari (Stapelberg et al. 2008). Fecal nitrogen and phosphorus levels recorded in elephants in Pilansberg, South Africa (Woolley et al. 2009) were similar to those observed in this study. Elephants in Parc National des Volcans, Rwanda, had a mean fecal nitrogen of 1.3% (across seasons) (Plumptre 1995), which is considerably higher than seen in Etosha's elephants (0.9% in wet season, 0.7% in dry season). The Rwandan area, however, is montane forest, and likely more productive than the semi-arid savanna of Etosha. Zebras in the Northern Province lowveld, South Africa, had lower fecal nitrogen than Etosha in both seasons (Bodenstein et al. 2000). This comparison among populations suggests that forage limitation is an unlikely stressor influencing anthrax seasonality in Eto-

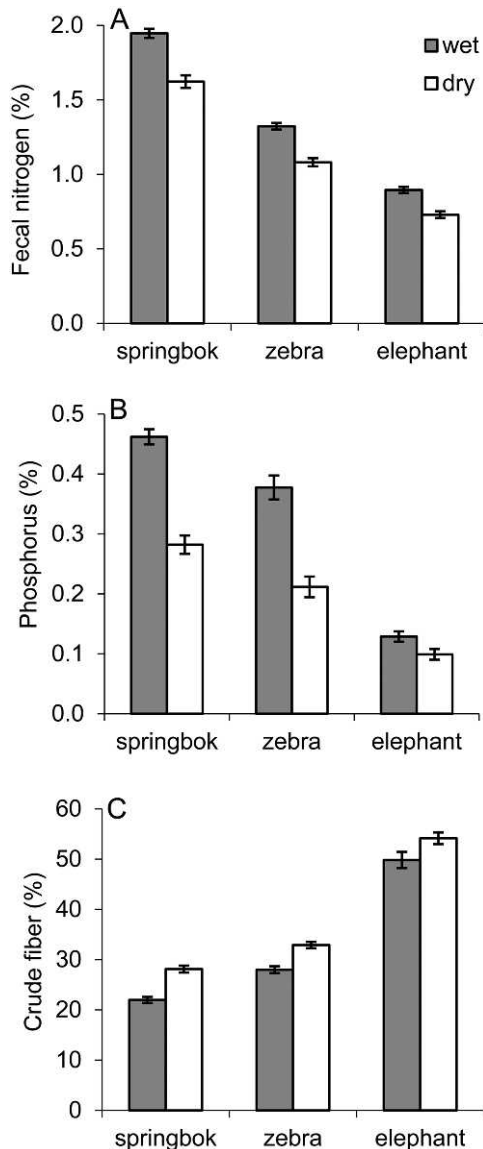


Fig. 3. The percentages of fecal nitrogen (A), phosphorus (B) and crude fiber (C) in fecal samples for zebra, springbok and elephant (mean \pm SE).

sha. This is supported by an earlier study showing that during drought conditions, rates of pregnancy, recruitment and mortality in Etosha's herbivores were not resource limited (Gasaway et al. 1996).

Seasonality of soil ingestion

Although increased soil exposure has been hypothesized as a cause of anthrax outbreaks in

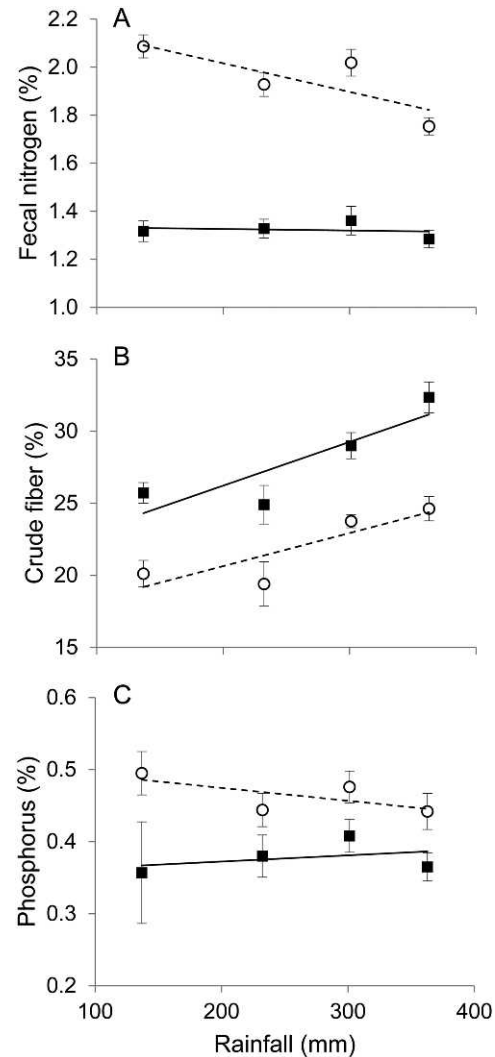


Fig. 4. Rainfall variation and fecal nitrogen (A), fecal crude fiber (B) and phosphorus (C) in springbok (open circles) and zebra (closed squares) during four wet seasons in Etosha National Park (2006–2009; mean \pm SE). Rainfall values are the sum of monthly rainfall from January and February, the months prior to the wet season fecal sample collection period (February and March) for each year.

dry conditions (WHO 2008), the higher rates of soil ingestion in wet seasons observed in this study (Fig. 5) correspond with seasonal patterns of soil ingestion observed elsewhere. In other soil ingestion studies, conducted primarily in North America, the highest rates of soil ingestion tend to occur in the spring (roughly equivalent to the

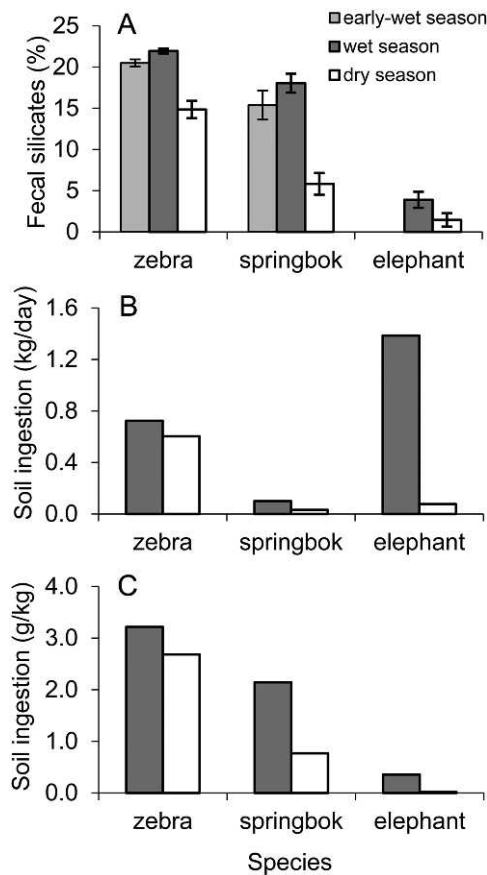


Fig. 5. (A) Percentage of fecal silicates (mean \pm SE), (B) estimates of soil ingestion ($\text{kg}\cdot\text{day}^{-1}$) and (C) soil ingestion per unit body mass ($\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) for zebra, springbok and elephant. No elephant fecal samples were collected during the early-wet season. Parameters used to estimate soil ingestion are presented in Table 2.

southern hemisphere wet season). Bighorn sheep (*Ovis canadensis*) in Alberta had a strong peak in fecal silicates during the late spring/early summer, with measured values up to 51% of dry matter, while during the rest of the year, fecal silicate measurements were $<5\%$ (Skipworth 1974). Mule deer (*Odocoileus hemionus*) in Colorado had the greatest soil ingestion in the spring (29.6 g/day), second highest in the winter (18.3 g/day) with lower values for summer/autumn (<10 g/day) (Arthur and Alldredge 1979). Pronghorn (*Antilocapra americana*) in Utah had a peak in soil ingestion during the spring and then a second peak in the autumn (Arthur and Gates 1988). Cattle grazing during a drought in summer/

autumn in Idaho did ingest more soil as forage availability decreased (Mayland et al. 1975); however, soil ingestion was not evaluated during the spring.

Ingested soil can come from a variety of sources and behaviors, either inadvertent through consumption of soil along with forage (Arthur and Alldredge 1979) or intentional for trace minerals or to buffer the digestive system (Kreulen 1985, Ayotte et al. 2006). The sources of mule deer soil ingestion varied throughout the year: in spring from soil attached to roots of low growing grasses and forbes, in winter from direct soil consumption, and in summer/autumn from soil particles attached to above ground plant parts (Arthur and Alldredge 1979). In cattle during drought conditions, soil attached to shallow-rooted plants was thought to be the main source of soil ingestion (Mayland et al. 1975).

The fecal silicate data from Etosha indicate that exposure, at least to soil, is significantly higher in wet than dry seasons for all three herbivore species (Fig. 5). If pathogens in the environment are spatially aggregated, such as *B. anthracis* at anthrax carcass sites, then it is essential to know how and when animals contact these local areas. The amount of soil ingestion from inadvertent versus intentional sources would alter pathogen exposure, depending on the probability that grazing or lick areas intersect with pathogen aggregations. Since carcass sites can be nutrient hot spots and alter the community composition or nutritional quality of grasses (Towne 2000, Bump et al. 2009), we speculate that these areas could be sources of attraction to herbivores. If increased soil exposure puts animals at a greater risk of ingesting a soil-borne pathogen, then herbivores should be at the highest risk during the vegetation growing season, when grasses are most palatable and soil moisture and low grass height supports the plucking of whole plants (and the associated soil attached to roots) while grazing. The grass community structure and species composition will further affect the likelihood of soil ingestion while grazing. In short grass habitats, such as Etosha's endemic plains, herbivores forage near ground level throughout the year. In areas with tall, high biomass grasses, grazers would feed closest to the soil when biomass is depleted or when grass growth begins

after rainfall. Also, deeper-rooted (perennial) grasses are less likely to be pulled from the soil while foraging than are shallow-rooted (annual) grasses. We propose that differences in grassland structure, coupled with variation in the diet selection of herbivores, may in part explain the differences in anthrax seasonality among grazing systems.

Nutrition, soil ingestion and anthrax in Etosha

Nutritional stress is unlikely to lead to increased host susceptibility during wet season outbreaks, because animals are ingesting higher quality forage in wet than dry seasons (Fig. 3). Comparing among years, higher wet season rainfall did not lead to improved nutrition for zebra or springbok. The years of study included average and high rainfall years, including 2009, which was the highest rainfall recorded since 1976, but no drought years. Years of high rainfall produce high biomass, less digestible forage, as seen from the relationship between fecal crude fiber and rainfall (Fig. 4). Wet-season forage quality and hence herbivore nutrition may peak during intermediate rainfall years, and decline at high and low rainfall levels. The number of zebra anthrax cases was positively associated with annual rainfall, however the greatest numbers of zebra anthrax cases were recovered in years of intermediate rainfall (Fig. 2), when forage quality may be at its highest. This weak relationship between anthrax occurrence and annual rainfall is surprising given that many anthrax outbreaks in other systems follow years of unusually high rainfall (Rees et al. 1977, Gates et al. 1995, Dragon et al. 1999). Whether examined seasonally or inter-annually, the timing of anthrax in Etosha is unlikely to be influenced by poor nutrition. Although certain individuals may have poorer nutrition when forage conditions are good, in these cases nutrition would be a secondary, not primary, factor behind an individual's declining resistance. The ultimate cause of decline for these individuals would be factors such as injury, infection, old age, or various social or reproductive stressors.

In contrast, increased soil ingestion may be an important predisposing factor for wet season anthrax outbreaks. However, the amount of soil ingested and its importance in disease transmis-

sion will vary considerably based on a species' foraging behavior and physiology, as is evident from estimates of soil ingestion in the study species (Fig. 5). Springbok soil ingestion was low overall, but higher in wet than dry seasons (100 vs 30 g/day, respectively), likely due to a seasonal dietary shift from grazing to browsing. When browsing on shrubs and trees, springbok feed above ground level, encountering less soil with their forage. Therefore springbok exposure to *B. anthracis* in the soil may be considerably higher in wet than dry seasons. Overall, if quantities of soil ingested are indicative of pathogen contact, then springbok may have much less exposure to *B. anthracis* than other herbivores utilizing the endemic area. Relatively few springbok anthrax carcasses are observed despite an abundant population, a fact previously attributed to their small body size and the speed with which scavengers can consume a carcass, decreasing the likelihood of carcass detection (Lindeque 1991). Our results offer an alternative explanation: that fewer springbok anthrax carcasses are found because they are less likely than other herbivores to be exposed to lethal doses of *B. anthracis*.

Although soil ingestion is an important risk factor for exposure to a soil-borne pathogen, it is unlikely to be the sole factor responsible for the seasonality of anthrax. Zebra in Etosha provide an example of this, because estimates of soil ingestion were relatively high in both seasons (720 vs 600 g/day in wet vs dry seasons, respectively) despite a strong seasonal peak in anthrax occurrence (57% of all zebra anthrax cases were recorded in March and April). Relatively high soil ingestion in all seasons is consistent with zebra digestive physiology (requiring higher intake rates per unit body mass than ruminants; Foose 1982) and diet selection, since they primarily graze throughout the year (Bodenstein et al. 2000, Codron et al. 2007, Havarua 2011). Zebra had the highest soil ingestion per unit body mass of the three species examined, and high rates of soil ingestion may in part explain why zebra are the main anthrax host species seen in Etosha. However, given the similarities in soil ingestion in both seasons, soil ingestion alone is unlikely to explain the strong seasonality observed in zebra anthrax cases (Fig. 1). Zebra and springbok may be more abundant

in the endemic area during the wet season than the dry season and a portion of these populations does migrate east along the southern edge of the Etosha pan in the dry season (Turner, *personal observation*). However, local density in the endemic area is quite variable in the wet season because herds move throughout the region following areas of recent rainfall and vegetation response. In the dry season animal movements are restricted by the few available perennial water sources in the endemic area and local densities remain high near water throughout the dry season. Further research is required to understand how other seasonal factors, such as animal movements and space use, parasite infections and reproductive cycles may modulate both the risk of exposure to *B. anthracis* from soil and the threshold for a lethal infection.

Seasonal peaks in soil ingestion and anthrax cases suggest that zebra and springbok wet-season outbreaks may in part occur due to heightened exposure. By the same measure, elephants should have greater exposure to *B. anthracis* during wet seasons (Fig. 5), but most elephant mortalities have been recorded in the late dry season (Fig. 1C). Elephant had the lowest fecal silicate values and the lowest soil ingestion per kg of body mass of the three species, but ingest large quantities of food each day. Therefore, in the wet season when elephants graze more, their estimated soil ingestion was 1.4 kg/day whereas in the dry season, soil ingestion was estimated at only 80 g/day. Elephants either (1) have a greater susceptibility to infection during the dry season despite reduced soil exposure from foraging, (2) are exposed via a different transmission route or (3) have other factors not considered in this study that alter their exposure or susceptibility to *B. anthracis*. If animals only seasonally utilize an anthrax endemic area, they can only be exposed to *B. anthracis* when in that area, regardless of seasonal variation in risk factors. Elephants primarily use the endemic area around Okaukuejo during dry seasons, dispersing into inaccessible woodland or shrubland areas during the wet season (Havarua, *personal observation*). Therefore, although foraging factors indicate exposure should be higher in wet seasons than dry seasons, elephant exposure to *B. anthracis* in the endemic area is seasonally restricted.

For elephants, behaviors other than foraging may be more important for the transmission of *B. anthracis*. The time elephants spend dust bathing increases as temperature increases (Rees 2002) and elephant deaths peak when conditions are hot, dry and dusty, indicating a possible risk of inhalational anthrax from dust bathing. Other elephant-specific behaviors could lead to increased contact with the pathogen. Mourning behaviors have been documented where individuals sniff, handle and taste the body or bones of dead elephants, whether or not they are closely related to the dead individual (e.g., Douglas-Hamilton et al. 2006). During our study period we did not see evidence of a strong seasonal pattern in elephant anthrax mortalities; they have been sporadic and timed throughout the year (compare Fig. 1C vs D). Many of the elephant anthrax mortalities in Etosha were recorded from two epidemics in dry seasons during drought conditions in the 1980s (40% of all elephant anthrax cases in Okaukuejo section from 1968–2011 were recorded in 1981 and 1989). Drought conditions could delay their seasonal dispersal, forcing elephants to remain near water in the endemic area for longer than usual. Ultimately, further research into behavioral mechanisms and movement patterns will be required to understand the seasonality of elephant anthrax in Etosha.

Conclusions

Beyond a system- and species-specific description of anthrax occurrence, our goal was to inform our understanding of the ecology and seasonality of anthrax. Although increased soil exposure is thought to be an important risk factor in anthrax outbreaks during dry seasons (WHO 2008), this study demonstrates the opposite, that herbivore soil exposure is greatest during wet seasons. Our results indicate that soil exposure is not simply a function of grass biomass, but affected by a variety of ecological and behavioral factors that must be considered when attempting to determine causal mechanisms for an anthrax outbreak. Nutrition, on the other hand, is difficult to address in the context of a wildlife disease, because of the limited insight into animal physiology possible from non-invasive nutritional indices and the uncertainty in what represents clinically poor nutrition for a particular species,

as opposed to normal seasonal fluctuations in nutrition.

The challenge in determining causal mechanisms for seasonally occurring infectious diseases is that many environmental, ecological and behavioral factors also vary seasonally. In order to facilitate a common understanding of environmental conditions associated with anthrax outbreaks and what impact those conditions may have on animal populations and individual behavior, we recommend that reports of anthrax outbreaks describe in greater detail the environmental conditions observed prior to and during outbreaks, environmental changes associated with the cessation of anthrax mortalities, and the time scale in which these conditions occur. For outbreaks in grazing species, it would also help to know the grazing conditions during outbreaks: if animals are feeding on dry, low grasses remaining from the prior growing season or if they are feeding on lush green growth from recent rainfall (e.g., Wafula et al. 2008). Disentangling the many correlated seasonal environmental factors will enable us to move beyond lists of associated conditions during an outbreak to an understanding of specific causal factors in the temporal occurrence of outbreaks among endemic systems, akin to the assessment of environmental risk factors behind the spatial occurrence of anthrax (Blackburn et al. 2007, Mongoh et al. 2008, Epp et al. 2010). A greater understanding of temporal triggers would enhance management efforts targeted to reduce the likelihood of outbreaks in endemic areas.

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