

Context-dependent survival, fecundity and predicted population-level consequences of brucellosis in African buffalo

Erin E. Gorsich^{1*}, Vanessa O. Ezenwa², Paul C. Cross³, Roy G. Bengis⁴ and Anna E. Jolles⁵

¹Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA; ²Odum School of Ecology and Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA; ³U.S. Geological Survey, Northern Rocky Mountain Science Center, Bozeman, MT 59715, USA; ⁴Department of Agriculture, Forestry and Fisheries, Directorate of Animal Health, State Veterinary Office, Skukuza 1350, South Africa; and ⁵Department of Biomedical Sciences, Oregon State University, Corvallis, OR 97331, USA

Summary

1. Chronic infections may have negative impacts on wildlife populations, yet their effects are difficult to detect in the absence of long-term population monitoring. *Brucella abortus*, the bacteria responsible for bovine brucellosis, causes chronic infections and abortions in wild and domestic ungulates, but its impact on population dynamics is not well understood.

2. We report infection patterns and fitness correlates of bovine brucellosis in African buffalo based on (1) 7 years of cross-sectional disease surveys and (2) a 4-year longitudinal study in Kruger National Park (KNP), South Africa. We then used a matrix population model to translate these observed patterns into predicted population-level effects.

3. Annual brucellosis seroprevalence ranged from 8.7% (95% CI = 1.8–15.6) to 47.6% (95% CI = 35.1–60.1) increased with age until adulthood (>6) and varied by location within KNP. Animals were on average in worse condition after testing positive for brucellosis ($F = -5.074$, $P < 0.0001$), and infection was associated with a 2.0 (95% CI = 1.1–3.7) fold increase in mortality ($\chi^2 = 2.039$, $P = 0.036$). Buffalo in low body condition were associated with lower reproductive success ($F = 2.683$, $P = 0.034$), but there was no association between brucellosis and pregnancy or being observed with a calf.

4. For the range of body condition scores observed in the population, the model-predicted growth rate was $\lambda = 1.11$ (95% CI = 1.02–1.21) in herds without brucellosis and $\lambda = 1.00$ (95% CI = 0.85–1.16) when brucellosis seroprevalence was 30%.

5. Our results suggest that brucellosis infection can potentially result in reduced population growth rates, but because these effects varied with demographic and environmental conditions, they may remain unseen without intensive, longitudinal monitoring.

Key-words: African buffalo, bacteria, *Brucella abortus*, chronic disease, conservation biology, disease ecology, population growth

Introduction

Infectious diseases may have negative consequences for individual animals by reducing host survival or fecundity (Alexander & Appel 1994; Craig, Pilkington & Pemberton 2006). Accumulating evidence suggests that diseases may also have impacts at the population and ecosystem level

(Tompkins *et al.* 2011). However, examples of this remain largely limited to epidemics of virulent or emerging infections in endangered populations (Daszak, Cunningham & Hyatt 2003; McCallum *et al.* 2009; but see Smith *et al.* 2008). Experimental parasite removal in a few systems (Hudson, Newborn & Dobson 1992; Gulland *et al.* 1993; Albon *et al.* 2002) shows that endemic parasites may also influence populations. Theoretical models further suggest that regulation can occur in the absence of overt mortality (McCallum 1994). Despite concern for the impacts of

*Correspondence author. E-mail: eringorsich@gmail.com

infectious diseases on wildlife conservation, empirical support for endemic and persistent infections regulating host populations remains limited, especially for microparasitic infections, which tend to be more difficult to monitor over extended time periods in wildlife, because diagnosis typically requires invasive sampling (Cross *et al.* 2009; Honer *et al.* 2012).

Quantifying the effects of endemic pathogens in wildlife is further complicated by the fact that the survival and fecundity costs of persistent infections are likely to vary with resource availability or host body condition (Beldomenico & Begon 2010). Recent research suggests that this heterogeneity may be critical to transmission (Hoye, Fouchier & Klaassen 2012), disease severity and the population-level impacts of pathogens (Burthe *et al.* 2008). For example, the synergy between condition and disease at the population level was experimentally demonstrated in a study simultaneously supplementing food and removing intestinal parasites in a wild mice population (*Peromyscus leucopus* and *Peromyscus maniculatus*). The effects of parasites were exacerbated in the absence of food supplementation (Pedersen & Greives 2008), suggesting that the extent of host population regulation by parasites may be dependent on individual host condition. The association between host condition or resources and individual-level disease susceptibility and severity is numerous (Turner *et al.* 2012), as are associations among disease and host population dynamics (Cattadori, Haydon & Hudson 2005). Further, the effect of pathogens may vary with demographic and environmental conditions such as age, sex and geographic location that modify access to resources, competition and social status (Honer *et al.* 2012). Therefore, an understanding of the environmental and demographic variation in individual-level survival and fecundity costs of disease is crucial to predicting the population and landscape-level patterns of infection and impacts of disease (Blaustein *et al.* 2011).

Bovine brucellosis, caused by the bacteria, *Brucella abortus*, is a globally distributed, zoonotic infection known to infect a broad range of mammalian hosts, including wild and domestic ungulates (Godfroid *et al.* 2011). Infection causes abortions and reduced milk production in cattle (Xavier *et al.* 2009; Neta *et al.* 2010). Reductions in fecundity from brucellosis have also been demonstrated in wildlife (Thorne *et al.* 1978) although no significant main effects on survival have been found (Joly & Messier 2005; Fuller *et al.* 2007). Brucellosis is transmitted by exposure to *Brucella*-infected birth products, including aborted fetuses or vaginal exudes (Stewart & Penzhorn 2004). Vertical transmission from mother to calf may also occur at birth or when bacteria are shed in milk (Lapraik *et al.* 1975; Nicoletti 1981), although serological data suggest that this route is less common (Rhyan *et al.* 2009). Wildlife is often implicated as maintenance reservoirs for transmission of brucellosis to livestock (Michel & Bengis 2012), fuelling conflicts between conservation and agricultural interests at wildlife-livestock interfaces

(Kilpatrick, Gillin & Daszak 2009; Gomo *et al.* 2012) and highlighting the need to understand the drivers and population level consequences of infection in wildlife (Godfroid *et al.* 2011).

We study the effects of environmental variation on disease in a free-living African buffalo population at Kruger National Park (KNP), South Africa. We combine a geographically extensive, 7-year cross-sectional seroprevalence data set compiled as part of the park's brucellosis surveillance effort with data from a longitudinal study where brucellosis infection, survival and reproduction were monitored for 4 years in 146 individual buffalo. In KNP, geological substrate and a latitudinal gradient in rainfall and temperature are strong drivers of savanna heterogeneity (Venter, Scholes & Eckhardt 2003), influencing plant biomass, forage quality (Ryan *et al.* 2012), buffalo behaviour (Winnie, Cross & Getz 2008) and body condition (Caron, Cross & Du Toit 2003). Thus, these variables can be used to define regions of differing buffalo densities and habitat quality. We expected this variation to influence the spatial distribution of brucellosis infection and test for independent, condition-dependent and region-dependent effects of brucellosis on host survival, fecundity and population growth. We test these hypotheses by analysing demographic, spatial and temporal patterns of infection using KNP's brucellosis surveillance data, and by testing for fitness costs of disease in our longitudinal study. We use a simple matrix population model to explore how condition-driven variability in vital rates and disease effects among buffalo herds may translate to contrasting outcomes of brucellosis infection for buffalo population growth.

Materials and methods

STUDY AREA AND POPULATION

Field data were collected in KNP (Mpumalanga province, South Africa, between 22°31' and 25°31' S, 30°45' and 32°00' E). The park covers approximately 20 000 km², represents a range of savanna habitats, and during the course of this study, supported buffalo population sizes ranging from approximately 23 000 to 30 000 individuals (Seydack *et al.* 2012). Both geology and climatic patterns structure plant and animal communities within KNP (Gertenbach 1983). The two main geological substrates are granite in the western section of KNP and basalt in the eastern section (Fig. 1). Granite soils are sandier and generally produce less biomass with lower nutrient concentrations (Mutanga *et al.* 2004). Rainfall, temperature and herbivore population density all follow a north-south gradient, with the highest values in the south-west (Venter, Scholes & Eckhardt 2003). Buffalo in southern KNP were also found to have a lower average body condition than in central and northern KNP (Caron, Cross & Du Toit 2003). We test whether buffalo in areas with lower resource availability (granite) or lower body condition (south) are associated with increased brucellosis seroprevalence by dividing the park into three latitudinal sections separated at the park's major rivers and two longitudinal sections based on geographic substrates (Fig. 1).

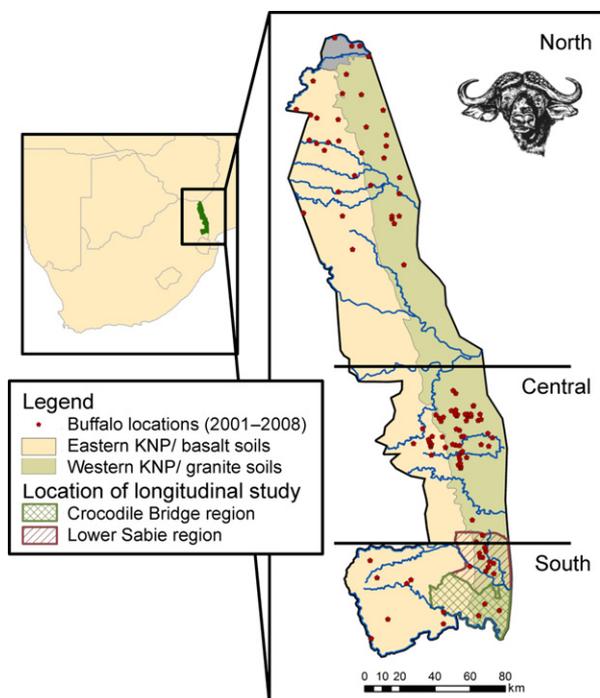


Fig. 1. Map of Kruger National Park (KNP) in southern Africa. Buffalo sampling locations for the cross-sectional disease survey and longitudinal study are shown within the geographically based sections of the park.

BRUCELLOSIS INFECTION

We collected information on brucellosis infection on two spatio-temporal scales. First, to determine the demographic and spatio-temporal patterns of infection, we conducted a cross-sectional, serological survey of 983 buffalo from 2001 to 2007 throughout KNP. Buffalo were captured as part of a study assessing the impacts of bovine tuberculosis (Cross *et al.* 2009) and as part of serological surveys conducted by South African National Parks and the Department of Agriculture, Forestry and Fisheries, Directorate Animal Health's State Veterinary Office. Age, sex, capture date and geographic location were recorded for each buffalo. Capture locations were targeted for the parkwide serological survey, but the animals captured each day were randomly selected (see Cross *et al.* 2009 for a detailed description). Age was estimated based on emergence of incisor teeth for animals younger than 5 years old and horn size for buffalo older than 5 (Grimsdell 1973; Sinclair 1977). Brucellosis testing from the cross-sectional survey was based on a panel of serological diagnostic tests. The Rose Bengal test (RBT) was used as a screening test and the complement fixation test (CFT) and the slow agglutination test (SAT) were used as confirmatory tests; we define a buffalo as seropositive for brucellosis if the buffalo was positive on the RBT and either the CFT or SAT based on their accuracy in cattle (Nielsen 2002). These tests have been validated in cattle (98.1%, 96.0% and 81.7% sensitivity and 99.8, 99.8 and 98.7 specificity for RBT, CFT and SAT, respectively; Grenier, Verloo & de Massis 2009) and have acceptable accuracy for diagnosis in African buffalo (98.6% and 37.4% sensitivity and 99.2% and 99.5% specificity; Gorsich *et al.* 2015a,b). All tests were performed by the Onderstepoort Veterinary Institute's diagnostic laboratory.

Secondly, to estimate the individual-level survival and fecundity costs of infection, we conducted a longitudinal study of 146 female buffalo. An initial 105 female buffalo were captured between June 2008 and October 2008. Buffalo were captured at two locations in the south-eastern section of KNP. Fifty-three buffalo were captured in the Lower Sabie region; 52 were captured in the Crocodile Bridge region (Fig. 1). Buffalo were radio-collared for re-identification and captured biannually at approximately 6 month intervals from their initial capture in 2008 until August of 2012. As natural mortalities occurred throughout the study period, new buffalo were captured and monitored, so that 41 buffalo were added throughout the course of the study.

This sampling design allowed us to test for independent, condition-dependent, and region-dependent costs of infection on female buffalo. During each capture period, we recorded brucellosis status, body condition, age, pregnancy status and whether there was a calf associated with each buffalo. Buffalo calves (defined as animals <1 year old) often associate with their mother until the next breeding season and were distinguishable from other calves by being within a few metres from a female (Sinclair 1977). Pregnancy status was determined by rectal palpation of the uterus (which has a nearly 100% sensitivity rate after 51 days of gestation in Egyptian buffalo; Aly *et al.* 2011). The gestation period for African buffalo is approximately 11 months (Sinclair 1977). Age determination was calculated by tooth eruption in younger buffalo and by incisor wear in older buffalo (Jolles 2007). Body condition was assessed on a scale of 1–5 by palpation of four main areas where fat is stored on buffalo: ribs, spine, hips and the base of the tail (see Ezenwa, Jolles & O'Brien 2009 for details and validation). Brucellosis testing was conducted using an ELISA test (Brucellosis Serum Ab ELISA test, IDEXX #P04130), with an estimated sensitivity of 93% and specificity of 87% (Gorsich *et al.* 2015a). Our analyses do not compare between data sets with different diagnostic tests to avoid potentially confounding effects of differences in diagnostic test accuracy. We estimated the time of mortality as midway between the last capture period the animal was observed and the subsequent capture period (6 months later) when it was recovered unless the exact mortality date was known. This assumption is supported by our sampling design that recovered most animals quickly after death. Of the 43 mortality events reported here, all but six were retrieved before the next capture period.

STATISTICAL ANALYSIS: DEMOGRAPHIC AND SPATIO-TEMPORAL PATTERNS OF INFECTION

We tested for age, sex and spatio-temporal patterns of infection with the parkwide, cross-sectional disease survey using logistic regression (logit link function and binomial errors). To determine age and sex patterns of infection, we fit models of brucellosis seroprevalence to the entire cross-sectional data set. Capture locations in this cross-sectional survey are displayed in Fig. 1; sample sizes and sources are summarized in Table 1. Age is represented as a categorical variable in all models from this data set and includes calves (0–1 years), juveniles (2–3.5 years), subadults (4–5.5 years), adults (6–8.5 years) and mature adult buffalo (9+ years). To determine age and sex patterns of infection, we used model selection to test for their additive or interactive influence on brucellosis seroprevalence after accounting categorically for annual variation in sampling locations with a term representing sampling year and park section.

Table 1. Annual prevalence (Prev) and sample sizes (*N*) observed in the south, central and northern sections of Kruger National Park

	South		Central		North		Total	
	<i>N</i>	Prev, %						
2001	19	26.32	73	12.33	0	–	92	15.22
2002	21	47.62	45	22.22	0	–	66	33.33
2003	44	25.00	74	17.57	0	–	118	20.34
2004	24	12.50	69	8.70	0	–	93	9.68
2005	228	26.75	72	19.44	0	–	300	25.0
2006	0	–	0	–	117	9.40	117	9.40
2007	0	–	0	–	197	18.27	197	18.27
Total	336	26.79	333	15.62	314	14.97	983	19.23

To examine the spatio-temporal patterns of infection, we analysed those areas with spatial and temporal overlap: 2001–2005 in southern and central KNP. We fit models including age, sex, year, season, park section (central, southern), geology (granite vs. basalt soils) and buffalo density. We considered all two-way interactions and all models account for age and sex because this reduced data set was highly age-structured ($N = 670$). Season was included in the models as a categorical variable based on rainfall and temperature (wet: October–March, dry: April–September). Buffalo density was also included in models because density is frequently a strong determinant of infection prevalence (Cross *et al.* 2010). Buffalo density was approximated as the density of buffalo counted around each capture location each year. Density calculations were based on counts during annual, parkwide, dry season herbivore helicopter surveys (KNP megaherbivore censuses 2007–2012) in circular areas representing the median (245 km²) and maximum (327 km²) home range sizes (Ryan, Knechtel & Getz 2006). Data on buffalo herd size were collected for a subset of the census data (2005–2007). Preliminary analyses found no association between seroprevalence and herd size, so we use density for all analyses. Model selection was based on Akaike Information Criteria (AIC, Burnham & Anderson 2002). We report the top eight models (Table S1, Supporting information), and because models within 2 units of the model with the lowest AIC value provide equally good fit to the data (Burnham & Anderson 2002), we report the most parsimonious model within this range. All statistical analyses were conducted in R version 2.15.2 (R core development team 2012 R Foundation for Statistical Computing, Vienna).

STATISTICAL ANALYSIS: CONDITION, SURVIVAL AND FECUNDITY CONSEQUENCES OF BRUCELLOSIS

We used general linear mixed models to determine whether host condition decreases after brucellosis infection. We consider the following independent variables during model selection: brucellosis infection status, capture location, age, season, buffalo density, and whether the buffalo was observed with a calf (Table S2, Supporting information). We test for heterogeneous effects of disease on condition by considering all two-way interactions between independent variables and account for repeated sampling by including animal ID as a random effect. Of the 140 buffalo observed in this study, this analysis includes 230 sample points from 33 buffalo that seroconverted from test negative to test positive during the study period. Therefore, this analysis tests for differences in host body condition before vs. after infection. Capture location was represented as a categorical variable, indicating whether animals were captured in the Lower Sabie or Crocodile

Bridge region of the park. Age was a continuous variable in years because the longitudinal sample design allowed more accurate estimations of buffalo age. We modelled the effect of age using either a linear or a linear and quadratic term.

Mortality in brucellosis-positive and brucellosis-negative buffalo was analysed using a Cox proportional hazards regression model (CPH) with brucellosis as a time-dependent variable (Table S2, Supporting information, Cox 1972). We modelled brucellosis as a lifelong infection (Nicoletti 1980), assuming that a buffalo that has tested positive on at least two capture periods remains positive for life (but only seven were positive for one capture period and assumed to be test errors). We represented age and initial capture location as time independent or fixed variables. Host body condition and animal density were represented as time-dependent variables. Survival analysis was conducted using the survival package (Fox & Weisberg 2011; Therneau 2012).

To test for an effect of brucellosis on fecundity, we used generalized linear mixed models (logit link, binomial errors) of two dependent variables: (i) the probability of having a calf and (ii) the probability of being pregnant. Calving is strongly age-dependent, with very few calves born to females younger than 4 years; therefore, only buffalo over 3 years old were included in this analysis (resulting in 677 samples from 140 buffalo). Because buffalo calves are often weaned after approximately 5–6 months (Winthrop *et al.* 1988), we expected calf observations to be less accurate just before or during birthing season and account for this by only including captures immediately following the birthing season (March–July). To test for condition-dependent effects of infection on calving, we consider host body condition at the previous capture. We use lagged condition because we expect the high energetic costs of lactation to drive current condition (the median body condition score during this period for buffalo with calves was 2.75 compared to 3.25 in buffalo without calves). We explore the direction of the correlation by reporting patterns of calving and host body condition at the current and previous time point (Table S3, Supporting information). Therefore, statistical models of the probability of having a calf included brucellosis infection status, age, host body condition and buffalo density at each capture period as fixed effects and animal ID as a random effect. Models of host pregnancy also included capture location and season as potential predictor variables. Mixed models were fit with the LME4 package (Bates *et al.* 2014).

IMPACT OF BRUCELLOSIS ON POPULATION GROWTH

To explore the consequences of brucellosis infection on population growth, we used the predicted effects of brucellosis on

individual-level survival and fecundity estimated in our statistical models (Table 2) to parameterize an age-structured, matrix population model. We model buffalo captured in the Lower Sabie region and the Crocodile Bridge region separately because of their different survival and fecundity rates. Vital rate parameters for the Lower Sabie region of KNP were estimated in a previous study (Cross & Getz 2006) and in Hluhluwe Imfolozi Park (Jolles 2007; Table 3). Vital rate parameters for buffalo captured in the Crocodile Bridge region were based on the proportional reduction in survival compared to buffalo in Lower Sabie, and vital rate parameters for brucellosis-positive buffalo were calculated from the proportional reductions in survival (Table 2) and fecundity (Table S4, Supporting information) indicated in the statistical models.

The population growth rate, λ , was determined by the dominant eigenvalue of the Leslie projection matrix (Caswell 2001). This model assumes exponential growth, that individuals with the same body condition score within an age class have the same vital rates and that female vital rates can be used to represent population growth. The distribution of infection was based on those in our parkwide disease survey and was assumed to be independent of infection prevalence within the range we observed. We explored the level of infection required to regulate population growth in both herds by modelling infection prevalence ranging from 0 to 50%. Because vital rates were dependent on host body condition, we incorporated the observed distribution of body condition values in two steps. First, we sampled from the disease-specific distribution of body condition scores in the area. Secondly, we calculated the survival and fecundity rates for each body condition score. Population growth was estimated using the median values for survival and fecundity. Bootstrap confidence intervals for population growth were estimated based on 1000 resamples of the observed distribution of body conditions. The model's predicted population growth rate was compared to the actual growth within KNP, calculated as the proportional increase or decrease in buffalo counted during annual, parkwide

large herbivore helicopter surveys (KNP megaherbivore censuses 2007–2012).

Results

Of the 983 buffalo sampled for brucellosis throughout KNP between 2001 and 2007 (Fig. 1), a total of 189 or 19% tested positive for brucellosis during the course of the study.

DEMOGRAPHIC PATTERNS OF INFECTION

Brucellosis infection increased with age until a maximum seroprevalence of 31% in adult buffalo (Fig. 2a). In female calf, juvenile and subadult buffalo (0–1, 2–3.5, 4–5.5 years, respectively), prevalence was significantly lower than in adult females (calf: $F_{2,962} = -2.318$, $P = 0.020$; juvenile: $F_{2,962} = -1.138$, $P = 0.001$; subadult: $F_{2,962} = -2.286$, $P = 0.022$). Mature adult females had a prevalence of 25.0% that did not differ significantly from adults ($F_{2,962} = 0.906$, $P = 0.365$) or subadults ($F_{2,962} = -1.517$, $P = 0.129$) but was higher than juveniles ($F_{2,962} = -2.696$, P -value = 0.007). The age-prevalence pattern in males differed from that in females in two distinct ways. First, male calves showed a significantly higher prevalence than both female calves (calf \times male: $F_{2,962} = 2.410$, $P = 0.0160$) and male juveniles ($F_{2,962} = -2.509$, $P = 0.012$). Passive antibodies in calves of seropositive cows decline after 5–6 months (Winthrop *et al.* 1988), but this pattern remained even when only considering buffalo at least 6 months old (4 seropositive/13 total male calves tested). Secondly, prevalence in adult male buffalo was lower than females ($F_{2,962} = -2.194$, $P = 0.028$) and prevalence in mature adults tended to be lower than females but this was not significant ($F_{2,962} = -1.192$, $P = 0.233$).

SPATIAL AND TEMPORAL PATTERNS OF INFECTION

Soil type and park section were significantly associated with brucellosis prevalence (drop in deviance test, $P < 0.0001$, Table S1, Supporting information). Buffalo captured on granite soils were associated with 2.0 (95% CI = 1.1–3.8) times higher odds of brucellosis than buffalo captured on basalt soils ($F_{2,656} = 2.020$, $P = 0.028$). Although the main effect of park section was significant, the magnitude of this effect varied with soil type (Fig. 2b). Buffalo captured on granite soils in southern KNP had the highest prevalence compared to all other sections, 32.7% (soil \times park section, $F_{2,656} = -2.881$, $P = 0.004$). Brucellosis prevalence in buffalo captured in southern KNP on basalt soils was not significantly different than in central KNP ($F_{2,656} = -0.218$, $P = 0.392$). Buffalo density was not a significant predictor of brucellosis prevalence (Table S1, Supporting information). Brucellosis prevalence only varied in some years, with the highest prevalence in 2002, (2002 > 2003, $F_{2,654} = -2.005$, $P = 0.045$; 2002 > 2004, $F_{2,654} = -3.153$, $P = 0.002$; and

Table 2. Associations of host body condition and mortality with age, capture site and season. Parameter values (β), standard errors (SE) and significance tests (χ^2 and F -values) are shown for each independent variable. A positive parameter value for season indicates host condition/mortality was higher in the wet season, and a positive association with capture site indicates body condition/mortality was higher in the Crocodile Bridge region

Parameter	β	SE	Statistic	P -value
Body condition				
<i>Brucella</i>	-0.483	0.102	-4.727	<0.0001
Age	-0.140	0.032	-4.727	<0.0001
Season – wet	-0.965	0.198	-4.874	<0.0001
Site – CB	-0.269	0.132	-2.042	0.050
Calf	-0.168	0.089	-1.883	0.061
Season \times <i>Brucella</i>	0.423	0.145	2.925	0.004
Season \times age	0.077	0.045	1.697	0.091
Mortality				
<i>Brucella</i>	0.674	0.322	2.093	0.036
Age	-0.878	0.302	-2.910	0.004
Age ²	0.062	0.025	2.496	0.013
Condition	-3.213	0.934	-3.440	0.001
Condition ²	0.394	0.164	2.409	0.016
Site – CB	0.689	0.379	1.815	0.069

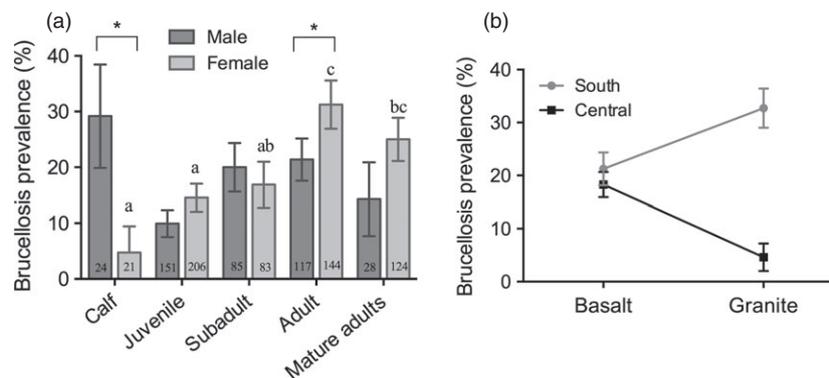


Fig. 2. Brucellosis infection patterns. (a) Male and female age specific prevalence (number infected/total sampled \times 100%) and standard error. Numbers indicate the sample size of each category, letters define significance between age groups, and sex differences are indicated with an asterisk, *. (b) Brucellosis prevalence was higher in buffalo captured in southern Kruger National Park (KNP) compared to central KNP on granite soils but not basalt soils (southern, basalt $N = 174$, southern granite $N = 162$, central basalt $N = 268$, central granite $N = 65$).

2002 > 2005, $F_{2, 654} = -2.680$, $P = 0.007$) and lowest prevalence in 2004 (2001 > 2004, $F_{2, 654} = 1.881$, $P = 0.060$).

BODY CONDITION

Buffalo were, on average, in lower body condition after testing positive for brucellosis ($F = -6.139$, $P < 0.0001$), the effect of brucellosis remained significant after accounting for capture year, age, park region, buffalo density and if the buffalo was observed with a calf (Table 2). The most parsimonious model of body condition included an interaction between brucellosis and season (Table S2, Supporting information). The interaction between brucellosis and season indicates that the condition consequences of infection were limited to the dry season (Fig 3a). In the dry season, acquiring brucellosis was associated with decrease in body condition score by -0.48 (95% CI = -0.29 to -0.68), compared to a minimal decrease in the wet season by -0.06 (95% CI = -0.28 to 0.17). Buffalo in the Crocodile Bridge region and buffalo with a calf were suggestively associated with lower body condition scores. The interaction term, season \times age, provides suggestive evidence that seasonal fluctuations were less pronounced in older buffalo (Table 2).

SURVIVAL AND FECUNDITY

We observed a total of 43 mortalities out of the 146 buffalo monitored from 2008 to 2012. Eighteen of the mortalities were buffalo initially captured in the Lower Sabie region and 25 were buffalo initially captured in the Crocodile Bridge region. At the time of initial capture, brucellosis prevalence was 30% (16/53) in Lower Sabie and 27% (14/52) in Crocodile Bridge.

The most parsimonious model explaining mortality included brucellosis, capture site, body condition and age (Table S2, Supporting information). The probability of mortality in buffalo with brucellosis was estimated to be

2.0 (95% CI = 1.1–3.7) times greater than uninfected buffalo (Table 2). There was suggestive evidence that buffalo captured in Crocodile Bridge had higher mortality compared to buffalo captured in Lower Sabie (Site, $\chi^2 = 1.815$, $P = 0.069$). After accounting for brucellosis infection status and capture site, host body condition had the largest influence on buffalo mortality (Fig. 3b).

The probability of having a calf was not statistically different in buffalo that tested positive or negative for brucellosis ($P = 0.884$; Table S3, Supporting information). Host condition at the previous time period was positively associated with calving ($F = 2.680$, $P = 0.007$; Table S4, Supporting information); an increase in body condition score by one was associated with 4.6 times higher odds of having a calf (95% CI = 1.5–13.7). We also did not detect an association of brucellosis with pregnancy (Table S3, Supporting information, $P = 0.606$). However, the odds of a buffalo being pregnant were positively associated with host body condition ($F = 3.208$, $P = 0.001$). After accounting for host condition, pregnancy remained lower in buffalo captured in the Crocodile Bridge region ($F = 2.929$, $P = 0.003$) and in the dry season (Table S3, Supporting information; $F = 2.317$, $P = 0.022$).

PREDICTED IMPACT OF BRUCELLOSIS ON POPULATION GROWTH

To evaluate whether the fitness costs of infection at the individual level translate into population-level consequences, we parameterized a matrix population model based on estimates of the influence of park region (Lower Sabie vs. Crocodile Bridge, Table 3), host condition and brucellosis infection in our statistical models. The model-estimated mortality rates in the Crocodile Bridge region were estimated as 2.0 (95% CI = 0.9–4.2) times the rate in the Lower Sabie region. We could not include an estimate of how fecundity rates varied between regions because we limited our statistical models of calving to include only the region captured after the birthing season.

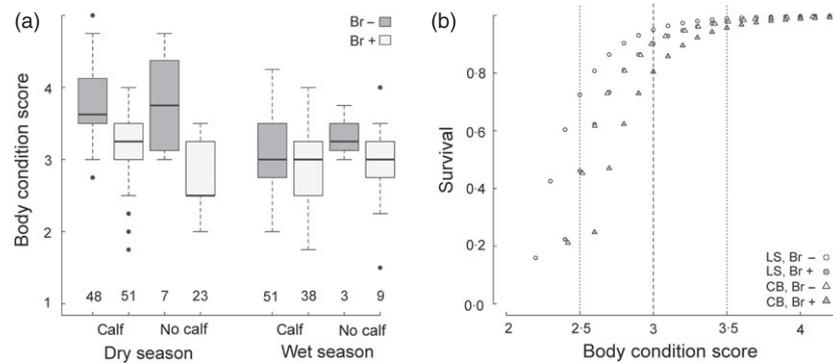


Fig. 3. Brucellosis is negatively associated with host condition and host survival. (a) Host body condition was lower in buffalo after testing positive for brucellosis and in buffalo with a calf; the association between condition and brucellosis was only observed in the dry season. (b) Predicted annual survival probability for brucellosis-positive (Br+) and brucellosis-negative (Br-) buffalo in the Lower Sabie (LS) and Crocodile Bridge (CB) region. Displayed are estimates for the body condition scores observed during the study; vertical lines represent the 25th, 50th and 75th percentiles of the distribution of body condition scores.

Table 3. Buffalo baseline vital rates, elasticities of the population growth rate and data sources for each parameter in the Lower Sabie and the Crocodile Bridge region. Elasticity values are the summed elasticities of λ for each age within an age category (calves, subadults or adults, mature adults and senescent buffalo)

	Value	Elasticity	Explanation	Sources
S_c	0.74	0.263	Annual survival probability for calves in LS	3
	0.48	0.245	Annual survival probability for calves in CB	
S_a	0.95	0.530	Annual survival probability for buffalo <8 years in LS	1, 2
	0.90	0.551	Annual survival probability for buffalo <8 years in CB	
S_m	0.86	0.073	Annual survival probability for buffalo 8–14 years in LS	1, 2
	0.72	0.079	Annual survival probability for buffalo 8–14 years in CB	
S_s	0.74	0.004	Annual survival probability for buffalo >14 years in LS	1,3
	0.48	0.002	Annual survival probability for buffalo >14 years in CB	
F_{sa}	0.51	0.021	Annual probability of a female aged 3 bearing a calf in LS	1, 4
	0.18	0.012	Annual probability of a female aged 3 bearing a calf in CB	
F_a	0.64	0.041	Annual probability of a buffalo aged 4–5 bearing a calf in LS	1, 4
	0.28	0.034	Annual probability of a buffalo aged 4–5 bearing a calf in CB	
F_m	0.60	0.070	Annual probability of a buffalo aged 6+ bearing a calf in LS	1, 4
	0.24	0.076	Annual probability of a buffalo aged 6+ bearing a calf in CB	

1 = Estimated in this study, 2 = Cross & Getz (2006), 3 = Jolles (2007) and 4 = Funston (1999).

In both regions, we represent the effects of brucellosis on survival as additive to the effects of condition. This assumption is supported by the additive relationship found in the CPH survival analysis (Table S2, Supporting information). We did not include an effect of brucellosis on fecundity because calving was not significantly associated with infection status. However, host body condition varied between regions and decreased after infection with brucellosis (Fig. 3). The condition consequences of brucellosis infection could result in lowered fecundity rates in animals with brucellosis because body condition was positively associated with calving. To capture the observed distribution of host body condition scores, we sampled from the disease- and region-specific body condition scores. This allows survival and fecundity rates to indirectly reflect the condition consequences of infection.

Based on the observed distribution of body condition scores, the predicted population growth rate was higher in Lower Sabie than in Crocodile Bridge, reflecting the low

body condition, survival and fecundity of buffalo captured in Crocodile Bridge (Fig. 4a). The model also estimated a negative relationship between infection prevalence and growth. Annual population growth at 30% prevalence was predicted to be 7% lower than growth in uninfected populations, reducing the growth rate from 1.20 (95% CI = 1.19–1.21) to 1.13 (95% CI = 1.13–1.16) in the Lower Sabie region and from 1.02 (95% CI = 1.01–1.05) to 0.85 (95% CI = 0.85–0.90) in the Crocodile Bridge region. These estimates are similar to the range of annual growth rates observed in southern KNP during our study (Fig. 4b). Elasticity analysis of the model showed population growth responded most to changes in adult and calf survival (age < 8).

Discussion

Our results suggest heterogeneous costs and patterns of brucellosis infection, consistent with the expectation that

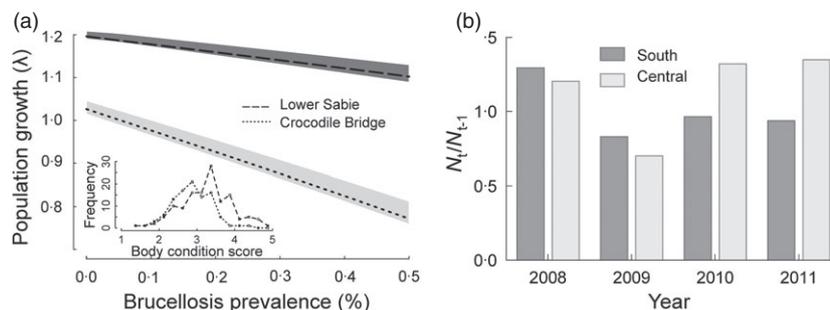


Fig. 4. Comparison of model predictions with the observed population growth rates in Kruger National Park (KNP). (a) Model predicted population growth in Lower Sabie and Crocodile Bridge based on the distribution of body condition scores observed in each region. The inset figure shows the distribution of body condition scores in each region. (b) Observed population growth rates in KNP based on the 2007–2012 megaherbivore censuses.

the effects of persistent infections are likely to vary with environmental conditions. Throughout KNP, brucellosis infection showed geographic variation, with increased prevalence in the south and western areas of the park where buffalo were, on average, in lower body condition and have lower forage quality. At the individual level, brucellosis was associated with increased mortality but was not associated with changes in host fecundity. Brucellosis infection also resulted in decreased host body condition in a season-dependent manner. Because condition was strongly correlated with host survival and fecundity, the heterogeneous effects of brucellosis on condition may contribute to the dynamics of brucellosis. When we represented the distribution of body condition values in the study population, the model predicted reduced population growth in infected populations. At a prevalence of 30%, population growth was predicted to be 7% lower than in uninfected populations (Fig. 4a).

DEMOGRAPHIC PATTERNS OF INFECTION

The epidemiology of brucellosis in African buffalo showed similar age and sex patterns of infection as livestock (Gomo *et al.* 2012) and wildlife in more temperate climates (Joly & Messier 2004; Etter & Drew 2006; Rhyan *et al.* 2009). The age patterns are typical of an endemic disease, with serological detection increasing with age (Treanor *et al.* 2011). We also observed lower infection prevalence in older buffalo (age > 9). This age-prevalence pattern may indicate mortality, reduced exposure or loss of antibodies in older animals (Wilson *et al.* 2002). Male calves had high brucellosis seroprevalence, compared to juvenile males and female calves and juveniles. It is not likely that a spurious association drives this pattern because equal numbers of males and females were captured across the variables measured in this study: season (July $N = 18/22$; August–September $N = 4/2$ for females/males), capture year (2005 $N = 12/14$; 2006 $N = 1/2$; 2007 $N = 8/8$ for females/males) and park region (north $N = 10/10$; south $N = 12/14$ for females/males). It also remains unclear whether this pattern reflects maternal

antibodies or infected calves. Passive antibodies in calves of seropositive cows decline after 5–6 months (Winthrop *et al.* 1988; Rhyan *et al.* 2009), and our study included only nine calves between 7 and 12 months. Due to these sample size constraints, additional investigations into the sex ratio of calves born to brucellosis-positive mothers and the dynamics of maternal antibodies in male and female calves are required to illuminate the mechanism underlying this finding.

SPATIO-TEMPORAL PATTERNS OF INFECTION

Brucellosis infection prevalence at KNP showed strong geographic variation. Buffalo on granite soils in southern KNP had the highest prevalence and buffalo on granite soils in the central KNP had the lowest prevalence. This striking pattern of high spatial variation in brucellosis infection prevalence on granite soils is consistent with our hypothesis that buffalo in areas with lower resource availability (granite) and lower body condition (south) would be associated with increased brucellosis prevalence. The south-western granites of KNP are dominated by unpalatable grasses that cannot sustain grazers during the dry season (Smit 2011). Southern KNP is also associated with overall higher grazer densities; therefore, an alternative explanation for the spatial variation in infection could be that hosts in poor condition reflect differences in grazer densities (Owen-Smith & Mills 2006). During this study, buffalo densities were higher in the southern and granite sections (south/granite $\mu = 2.68/\text{km}^2$; south/basalt $\mu = 1.47/\text{km}^2$; central/granite $\mu = 1.21/\text{km}^2$; central/basalt $\mu = 0.924/\text{km}^2$, KNP megaherbivore census). However, we did not observe an association between buffalo density and infection. Additional experimental data are required to draw causal inferences about the role of resources, density and disease in driving patterns of brucellosis prevalence. This study is the first description of the spatiotemporal patterns of brucellosis in African buffalo; follow-up work should consider the mechanism behind this pattern because at the parkwide scale, we could not control for individual host condition or potentially impor-

tant variation in buffalo behaviour that mediates patch selection, forage quality (Winnie, Cross & Getz 2008) and disease transmission (Cross, Lloyd-Smith & Getz 2005).

HETEROGENEOUS EFFECTS OF INFECTION ON HOST BODY CONDITION AND ASSOCIATIONS WITH SURVIVAL

The associations of brucellosis infection with host survival and fecundity observed in this study were not consistent with previous studies of brucellosis in elk and bison (Thorne *et al.* 1978; Joly & Messier 2005; Fuller *et al.* 2007; Rhyan *et al.* 2009). For example, brucellosis in African buffalo was negatively associated with survival after accounting for the effects of host body condition and region. Previous research on survival in elk and wood bison have not identified a strong effect of brucellosis on survival (Joly & Messier 2005; Fuller *et al.* 2007). In addition to species-specific differences, this could be due to the higher levels of predation in Kruger compared to previous studies. Buffalo which became infected with brucellosis also had lower body condition scores after testing positive (Fig. 3a). As far as we are aware, this is the first study to explore the condition costs of brucellosis infection in African wildlife. Our longitudinal study and frequent disease testing allowed us to identify heterogeneities in the condition costs of infection. Because these costs were dependent on season, it is not clear to what extent they will occur in other regions or species. We also did not find a relationship between brucellosis and either the probability of being pregnant or the probability of having a calf (Table S3, Supporting information). This result is in contrast to patterns observed in livestock and some wildlife (bison and elk), where brucellosis infection is associated with increased abortion (Thorne *et al.* 1978; Rhyan *et al.* 2009). Therefore, these results suggest that the reproductive effects of brucellosis may not be uniform across all species or ecological contexts and highlight the importance of studying brucellosis in a greater diversity of species.

PREDICTED POPULATION-LEVEL EFFECTS

The modelling results reflect the negative consequences of brucellosis on survival and the effects of condition on host survival and fecundity. Brucellosis was associated with reduced model-predicted population growth. Previous work has shown region-specific differences in body condition (Caron, Cross & Du Toit 2003) and vital rates (Ezenwa & Jolles 2015), which resulted in the lowest growth occurring in the Crocodile Bridge region. Although brucellosis did not regulate population growth below $\lambda = 1$ in Lower Sabie, growth was regulated below $\lambda = 1$ in the Crocodile Bridge. The model-predicted growth rate in both regions combined drops from $\lambda = 1.11$ (95% CI = 1.02–1.21) to $\lambda = 1.00$ (95% CI = 0.85–1.16) at a brucellosis prevalence of 30%. Thus,

potential perturbations that generate artificially high prevalence (aggregation from supplemental feeding or boreholes) or low body conditions (drought, coinfection) may result in a reduced population size (Hines *et al.* 2007; Jolles *et al.* 2008). The population model was designed to be intentionally simplistic, in order to reflect the data in our statistical analyses. In reality, both host body condition and infection prevalence vary dynamically and may interact to influence the overall impact of infection on the population. Future empirical and theoretical work in this system will be needed to further our understanding of the relationship between condition and disease.

Acknowledgements

Buffalo captures were conducted by SANPark's Veterinary Wildlife Services, the Department of Agriculture, Forestry and Fisheries, Directorate of Animal Health, State Veterinary Office, and Rob Spaan, Jo Spaan, Kristie Thompson and Brianna Beechler. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the US Government. We thank Brian Henrichs and Heather Broughton for their insight on this work. This research was approved by Oregon State University and University of Georgia IACUC (Protocol numbers: OSU No.3822; UGA No.A2010 10-190-A1) and by KNP's Scientific Services. The work was supported by a National Science Foundation Ecology of Infectious Diseases award to V. Ezenwa and A. Jolles (EF-0723918, DEB-1102493/EF-0723928) and a NSF-GRFP and NSF-DDIG award to E. Gorsich (DEB-121094).

Data accessibility

Data are available from Dryad digital repository <http://dx.doi.org/10.5061/dryad.p6678> (Gorsich *et al.* 2015b), and; <http://dx.doi.org/10.5061/dryad.5hh3h> (Cross *et al.* 2008).

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Received 21 June 2014; accepted 5 February 2015

Handling Editor: Andrew Fenton

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Model selection table for the spatio-temporal patterns of brucellosis infection in southern and central KNP from 2000 to 2005.

Table S2. Model selection table of host body condition and survival.

Table S3. Model selection tables of host fecundity.

Table S4. Results of best fitting models of host fecundity.