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


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

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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
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 foetuses 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 (Ryhan 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, geographic 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).
impacts of bovine tuberculosis (Cross et al. 2009). Buffalo were captured as part of a study assessing the serological survey of 983 buffalo from 2001 to 2007 throughout 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 Kruger National Park (KNP). 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 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 (Niehens 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002). These tests have been validated in cattle (Nielsen 2002).

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.

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.
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 accounted 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 censuses (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).

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

<table>
<thead>
<tr>
<th></th>
<th>South</th>
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<th>Central</th>
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<th>North</th>
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<tr>
<td>N</td>
<td>19</td>
<td>N</td>
<td>73</td>
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<td>2001</td>
<td>21</td>
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<td>24</td>
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<td>45</td>
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<td>22-22</td>
<td>2004</td>
<td>8-70</td>
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</table>

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, \( \lambda \), 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 growth in both herds by modelling infection prevalence ranging 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 scores 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 replications 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% 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 × male: \( F_{2,962} = 2.410, P = 0.016 \)) 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). Second, 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 × 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 (\( F_{2,654} = -0.218, P = 0.392 \)). 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

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**Table 2.** Associations of host body condition and mortality with age, capture site and season. Parameter values (\( \beta \)), standard errors (SE) and significance test values (\( \chi^2 \) and \( P \)-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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \beta )</th>
<th>SE</th>
<th>Statistic</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body condition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brucella</td>
<td>-0.483</td>
<td>0.102</td>
<td>-4.727</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
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<td>0.032</td>
<td>-4.727</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Season – wet</td>
<td>-0.965</td>
<td>0.198</td>
<td>-4.874</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site – CB</td>
<td>-0.269</td>
<td>0.132</td>
<td>-2.042</td>
<td>0.050</td>
</tr>
<tr>
<td>Calf</td>
<td>-0.168</td>
<td>0.089</td>
<td>-1.883</td>
<td>0.061</td>
</tr>
<tr>
<td>Season × Brucella</td>
<td>0.423</td>
<td>0.145</td>
<td>2.925</td>
<td>0.004</td>
</tr>
<tr>
<td>Season × age</td>
<td>0.077</td>
<td>0.045</td>
<td>1.697</td>
<td>0.091</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brucella</td>
<td>0.674</td>
<td>0.322</td>
<td>2.093</td>
<td>0.036</td>
</tr>
<tr>
<td>Age</td>
<td>-0.878</td>
<td>0.302</td>
<td>-2.910</td>
<td>0.004</td>
</tr>
<tr>
<td>Age²</td>
<td>0.062</td>
<td>0.025</td>
<td>2.496</td>
<td>0.013</td>
</tr>
<tr>
<td>Condition</td>
<td>-3.213</td>
<td>0.934</td>
<td>-3.440</td>
<td>0.001</td>
</tr>
<tr>
<td>Condition²</td>
<td>0.394</td>
<td>0.164</td>
<td>2.409</td>
<td>0.016</td>
</tr>
<tr>
<td>Site – CB</td>
<td>0.689</td>
<td>0.379</td>
<td>1.815</td>
<td>0.069</td>
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</table>
There was suggestive evidence that buffalo captured in the 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), and 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 = 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; \( F = 2.317, P = 0.022 \)).

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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...
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 (Treonor 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 ability (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 ability (granite) and lower body condition (south) would be associated with increased brucellosis prevalence.
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, confinement) 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.

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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).

References


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**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.