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Disease, predation and demography: assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels

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Summary

1. Understanding the effects of disease is critical to determining appropriate management responses, but estimating those effects in wildlife species is challenging. We used bovine tuberculosis (BTB) in the African buffalo *Syncerus caffer* population of Kruger National Park, South Africa, as a case study to highlight the issues associated with estimating chronic disease effects in a long-lived host.

2. We used known and radiocollared buffalo, aerial census data, and a natural gradient in pathogen prevalence to investigate if: (i) at the individual level, BTB infection reduces reproduction; (ii) BTB infection increases vulnerability to predation; and (iii) at the population level, increased BTB prevalence causes reduced population growth.

3. There was only a marginal reduction in calving success associated with BTB infection, as indexed by the probability of sighting a known adult female with or without a calf ($P = 0{\cdot}065$).

4. Since 1991, BTB prevalence increased from 27 to 45% in the southern region and from 4 to 28% in the central region of Kruger National Park. The prevalence in the northern regions was only 1·5% in 1998. Buffalo population growth rates, however, were neither statistically different among regions nor declining over time.

5. Lions *Panthera leo* did not appear to preferentially kill test-positive buffalo. The best (Akaike's Information Criterion corrected for small sample size) AIC*c* model with BTB as a covariate [$\exp(\beta) = 0{\cdot}49$; 95% CI = (0·24–1·02)] suggested that the mortality hazard for positive individuals was no greater than for test-negative individuals.

6. *Synthesis and applications*. Test accuracy, time-varying disease status, and movement among populations are some of the issues that make the detection of chronic disease impacts challenging. For these reasons, the demographic impacts of bovine tuberculosis in the Kruger National Park remain undetectable despite 6 years of study on known individuals and 40 years of population counts. However, the rainfall and forage conditions during this study were relatively good and the impacts of many chronic diseases may be a non-linear function of environmental conditions such that they are only detectable in stressful periods.

Key-words: bovine tuberculosis, *Syncerus caffer*, detection, predator, disease, population growth

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Introduction

Pathogens that cause acute disease, such as rinderpest and distemper viruses, can have extensive impacts on wildlife populations, but are often too fast for management to contain. On the other hand, managers may attempt to mitigate the spread or impact of chronic diseases post-introduction. However, assessing population-level impacts of chronic diseases is challenging (Jolles, Cooper & Levin 2005), particularly on the time-scale that management decisions need to be made. Therefore, it is difficult for managers to scale their efforts according to the expected impact. By definition, the immediate effects of a chronic disease on host vital rates will be small and population-level effects are likely to occur over years or decades (Albon *et al.* 2002; Stien *et al.* 2002). As a result, disease impacts may be confounded by, or act in synergy with, other factors such as density-dependence, competition, and climate variability. The ongoing epidemic of bovine tuberculosis (BTB) in the African buffalo *Syncerus caffer* population of the Kruger National Park (KNP) represents an opportunity to assess the impacts of an invasive chronic disease on a naïve ecosystem. The approximate timing and location of the introduction of BTB is known, aerial censuses of the buffalo population are conducted annually, and until recently the KNP was a closed system.

BTB, a chronic and progressive bacterial disease caused by *Mycobacterium bovis*, was first diagnosed in an African buffalo along the southern boundary of the KNP in 1990 (Bengis *et al.* 1996). Subsequent sampling of the buffalo population showed BTB increasing in prevalence and spreading northward (Fig. 1, Rodwell *et al.* 2000). Outbreak investigations suggested the disease was transmitted to buffalo from cattle in the southeast corner of KNP between 1950 and 1960 (Michel *et al.* 2006). As in cattle, most buffalo are infected with BTB via aerosol transmission, while vertical (intrauterine) and pseudovertical transmissions (through infected milk) can occur but appear to be rare events (Bengis 1999). Since 1990, the disease has been found in chacma baboons *Papio ursinus*, lions *Panthera leo*, greater kudus *Tragelaphus strepsiceros*, cheetahs *Acinonyx jubatus*, leopards *Panthera pardus*, and several other mammal species in the KNP (Michel *et al.* 2006). As with many introduced pathogens in a naïve ecosystem, limited data existed on the potential impacts needed to evaluate appropriate management actions.

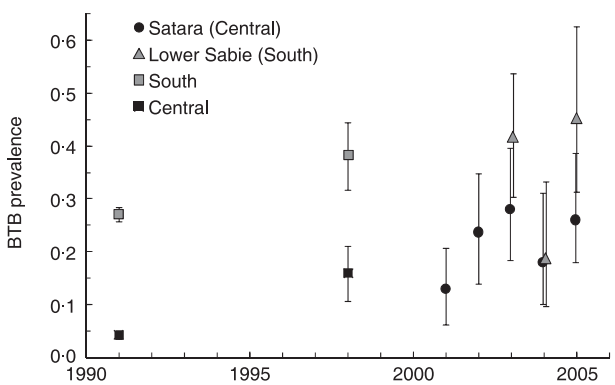

Fig. 1. Prevalence of bovine tuberculosis (BTB) increased over time in the buffalo population. BTB status in 1991 and 1998 was based on lethal samples, while the data from 2001–2005 are based upon blood tests of live animals. Bars represent 95% confidence intervals. Satara and Lower Sabie are areas within the central and southern regions, respectively.

This outbreak differs from those in other ecosystems where BTB has been studied. First, the prevalence of BTB in buffalo is relatively high; 5–45% depending upon the year and region, compared to white-tailed deer *Odocoileus virginianus* in Michigan 0–4% (Schmitt *et al.* 1997). Secondly, most other systems with BTB are manipulated [e.g. test-and-removal of BTB-positive buffalo in Hluhluwe-iMfolozi Park (Jolles *et al.* 2005); hunting of white-tailed deer (O'Brien *et al.* 2002); culling of badgers *Meles meles* in Britain (Donnelly *et al.* 2003) and possums *Trichosurus vulpecula* in New Zealand (Ramsey *et al.* 2002)]. Thirdly, since BTB is still invading the KNP, variation in prevalence is most strongly dependent on time since introduction rather than other ecological factors that may vary regionally, allowing for stronger conclusions about the progression and impact of the disease in different regions.

Packer *et al.* (2003) argued that the selective predation of diseased individuals is likely to reduce the incidence and prevalence of microparasitic diseases and, in some cases, increase the total host population size. Many studies have shown how parasites alter host behaviour and increase predation, particularly when the parasite completes its life cycle in the predator (for review see Dobson 1988). But only a few studies of disease-induced predation are available (e.g. Hudson, Dobson & Newborn 1992; Lafferty & Morris 1996), particularly for ungulates. The current epidemic of BTB in KNP provides an opportunity to investigate the interaction of predation and disease because the predator community of the KNP remains intact and predation is the primary source of African buffalo mortality (this study; Radloff & du Toit 2004).

Here we used data from known individuals and population counts and build on previous studies that used data from culled individuals (Rodwell, Whyte & Boyce 2001; Jolles *et al.* 2005) and herd-level observations (Caron, Cross & du Toit 2003) to assess the impact of BTB on the buffalo population. We investigated the following three theoretical predictions of disease ecology, based on the premise that BTB is having a negative impact: (i) at the individual level, BTB infection reduces reproduction; (ii) BTB infection increases the vulnerability of individuals to predation; and (iii) at the population level, increased BTB prevalence causes reduced population growth.

Methods

The Kruger National Park, South Africa, is a fenced, wooded savanna covering 19 485 km^2. In 1993, it was enlarged by 449 km^2 through the removal of fences with adjoining private reserves on the western boundary, but nonetheless remained a closed system until

2004 when some fences were removed as a part of the creation of the Greater Limpopo Transfrontier Conservation Area.

KNP researchers conducted annual park-wide helicopter surveys of the buffalo population from 1969 to 2004 (Whyte 1996). Survey transects were along drainage lines to increase the probability of seeing individuals in these habitats of additional tree coverage. Due to the density of drainage lines, these surveys are likely to be reliable estimates of the buffalo population. Although the helicopter survey may be biased, we believe the consistent methodology resulted in a reliable index of population size, which we use to investigate changes in population growth over time. For the aerial census analyses, we divided KNP into four, roughly equal-sized regions on a north–south gradient (Owen-Smith & Mills 2006). Analysing each region individually assumes that net movement of buffalo among regions is equal. To test this assumption, we investigated the first and last sightings of buffalo that were captured in either the central or southern regions and were last seen more than 1 year after their first sighting. Seventy-six of the 126 central region buffaloes moved south, which is significantly different from 0·5 (exact binomial test; $\hat{p}$ = 0·61, 95% CI = [0·52–0·69], *P* value = 0·02), while 22 of the 33 southern region buffalo moved north (exact binomial test; $\hat{p}$ = 0·66, 95% CI = [0·48–0·82], *P* value = 0·08). We interpret this as a roughly equal exchange of individuals among the southern and central regions, but recognize that it is hard to draw strong conclusions from this evidence. Therefore, we present both regional and park-wide analyses.

KNP scientists and managers conducted park-wide lethal sampling to assess BTB prevalence in 1991–1992 and in 1998 (Rodwell *et al.* 2000). BTB status was determined via combination of macroscopic examination of the lungs, histopathology, and culture (Rodwell *et al.* 2000). To complement the 1991–1992 and the 1998 surveys, we conducted an intensive study of known and radiocollared buffalo in the central and southern regions. This study lasted from 2000 to 2006 in the central region and expanded in 2003 to include the Lower Sabie area of the southern region, where the prevalence of BTB was higher.

Disease status was determined by the interferon-gamma (IFNg) BOVIGAM™ assay (Wood & Jones 2001). Unlike the skin test, the IFNg assay does not require the recapture of animals 48–72 h later, and as a modified triple comparative assay, has a specificity of 99% and sensitivity of 85% (Michel *et al.* 2000; Grobler *et al.* 2002; Cousins & Florisson 2005). Suspect, multiple, and avian reactor tests were not considered as BTB positive (De Klerk *et al.* 2006). Due to the high specificity of the IFNg assay (Grobler *et al.* 2002) and the lack of any evidence that infected cattle recover (Chaddock 2002), we assumed that positive individuals remained positive for the duration of the study. We considered BTB-negative status, however, to be a time-varying covariate and thus recaptured negative individuals every 6–12 months so that we could periodically update their disease status (Fig. 2). From November 2000 to July 2006, we captured 1053 buffalo (593 different individuals) for prevalence estimates and radiotracking (Fig. 2). We conducted annual prevalence surveys in the study area by using a helicopter to capture random subsets of 10–14 individuals at a time. All of these individuals were uniquely branded and given subcutaneous microchips for identification purposes and a subset was fitted with either radiocollars or GPS collars.

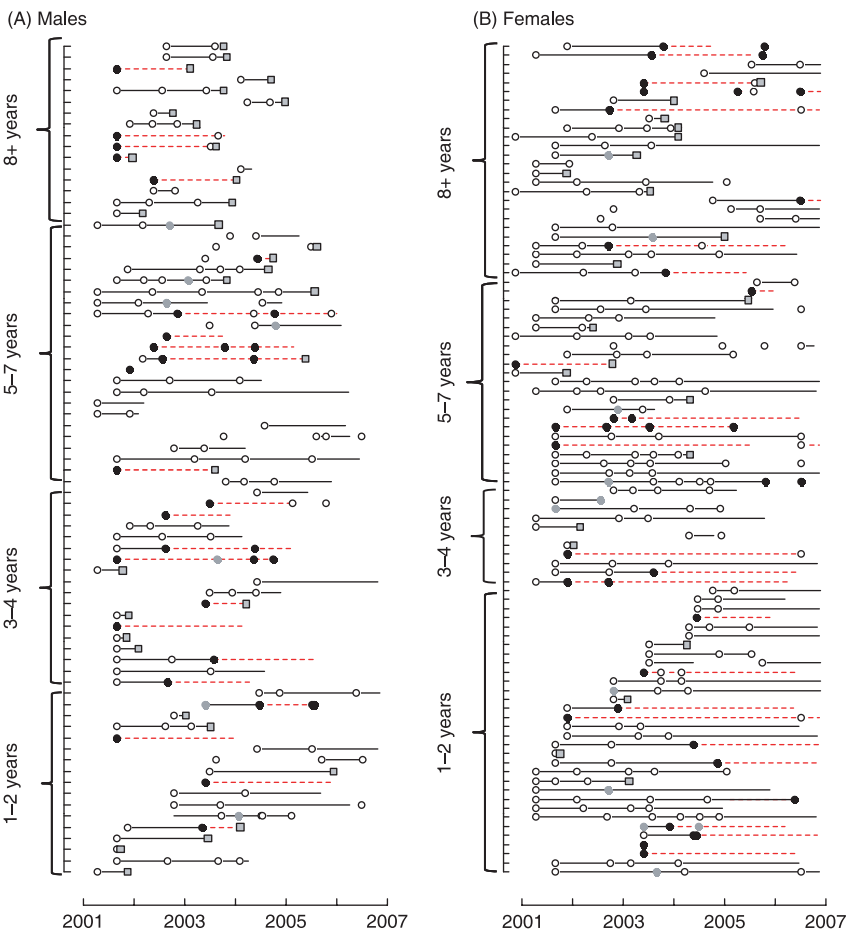

Fig. 2. Mortality and disease testing data on male (A) and female (B) buffalo. Circles represent BTB tests (black, positive; white, negative; grey, suspect). Dotted and solid lines correspond to collared individuals that were test-positive or negative, respectively. Lines begin when the collar was placed on the animal and end when the collar was removed, failed, or the individual died (squares). Individuals were assumed to be BTB-positive after their first positive test.

This capture protocol makes two major assumptions. First, individuals do not become infected and then die as a result of infection prior to being recaptured. Given the chronic nature of BTB (Jolles *et al.* 2005; Joly & Messier 2005), such an event is unlikely within 6–12 months. On average, we caught all negative individuals at least once per year and a concurrent vaccine study showed that none of 29 experimentally infected buffaloes died prior to slaughter at the end of 1 year (De Klerk-Lorist 2005). Secondly, we assume that the capture process does not affect the mortality rates of negative individuals. Only six of the 1053 individuals captured died during a capture event. These six were right-censored from the analysis (i.e. removed) on the capture date. We did not find any indication of delayed capture effects on either mortality or reproduction (Oosthuizen *et al.* in press).

To monitor survival, we located each radiocollared animal a minimum of once a month. The majority of the individuals remained in the focal study area and were seen once per week. Those individuals not found after 6 weeks were located again on observation from aircraft. We distinguished between mortality events and radiocollar failures using lion spoor, signs of a carcass, as well as the condition of the collar (Hay, Cross & Funston 2008). We assumed that the radiocollar had fallen off if the belting was broken and no carcass was present. This was confirmed in a number of instances when individuals were re-sighted later. Animals younger than 5 years were aged according to incisor eruption patterns (Grimsdell 1973). Animals older than 5 years at the first capture were subjectively aged according to a photographic reference collection in Pienaar (1969), and were calibrated according to individuals of known age in a neighbouring reserve (C. U. Knechtel, personal communication). Since buffalo frequently moved among herds and herds were difficult to frequently count, we were unable to assess the effects of group size in these analyses (Cross, Lloyd-Smith & Getz 2005).

We monitored female reproductive success by observing 165 females, of known BTB status and older than 4 years, 1313 times from January 2002 until October 2005 to see whether or not they were accompanied by a calf (within a few metres). Calves stay in close contact with their mothers until the next calf is born (Sinclair 1977). Misidentifications reduce the magnitude of the estimated effect, but are unbiased if they are randomly distributed among positive and negative individuals, which we accept as a reasonable assumption. The presence or absence of a calf is a crude index of reproductive success, since it incorporates fertility, calf survival, time of weaning, and sightability.

STATISTICAL ANALYSES

We used the counting version of a Cox proportional hazards (CPH) approach to assess survival differences between BTB-positive and negative individuals (Cox 1972; Therneau & Grambsch 2000), thereby removing the confounding effects of seasonal fluctuations by allowing the baseline mortality hazard to vary freely over time and accounting for both left-truncation resulting from delayed entry and right-censoring due to collar failure (Therneau & Grambsch 2000; Heisey & Patterson 2007). We used Akaike Information Criterion (AIC) to rank models first with only age and sex as explanatory variables (Burnham & Anderson 2002), and then we added BTB status in several different ways. We modelled the effect of age in four ways: a linear effect, a categorical effect (AgeCat1: 1–4, 5–7, 8+; AgeCat2: 1–2, 3–4, 5–7, 8–12, 12+), and by fitting a penalized spline that was chosen based on the lowest AIC value. Splines are a method of fitting non-linear effects where the degree of flexibility can be balanced by the amount of data using AIC (Therneau & Grambsch 2000). We estimated male and female survival rates independently using the survfit function in R with age as a categorical variable. Ninety-five per cent confidence intervals were based upon the log hazard (Therneau & Grambsch 2000; R Development Core Team 2005).

We conducted two different analyses of reproductive success. Since observations within a short period of time on the same individual are likely to be correlated, we first used a general linear mixed-effect logistic regression models incorporating individual identity as a random effect. In the second analysis, we considered all explanatory variables to be fixed effects, but also included the number of observations of an individual as a blocking factor. Additionally, we conducted each of these analyses with all the available data as well as with a subset that excluded BTB-negative individuals if they had not been retested within a year of the observation. In all cases, our conclusions remained the same.

We assessed the ability of the buffalo population to recover from droughts by comparing population growth rates, $\ln(N_{t+1}/N_t)$, among regions and among three inter-drought periods (1969–1980; 1985–1990; 1995–2005). We hypothesized that population growth rates should be lower in the southern regions where BTB prevalence was highest (Rodwell *et al.* 2000). Since BTB was increasing over time, we also expected growth rates to be higher in earlier inter-drought periods (e.g. 1969–1980, 1985–1990 compared to 1995–2004). The far northern region of KNP had much more variability in population growth and sample sizes among time periods were unequal. Therefore, we used a randomization test to assess the associations among region, time period and population growth. We compared ANOVA *F*-statistics derived from the original data to 1000 randomized data sets. Significant levels of culling occurred from 1969 to 1991, which we accounted for by assuming that these culled individuals would have survived to be counted in the next census. Our conclusions remained the same, however, when we repeated the analyses without accounting for culling. All statistical analyses were conducted in R version 2·5 (R Development Core Team 2005).

Results

Throughout the study period, BTB generally increased in prevalence and spread northwards (Fig. 1). In the Lower Sabie area of the southern region, there was a drop in prevalence in 2004, which was mirrored by a coincident drop in the central region; but in both cases, the prevalence rebounded the following year. The difference in the 2003 and 2004 prevalence in the southern region is statistically significant when $\alpha = 0{\cdot}05$ (P value = 0·01, two-sample test of proportions with Yates correction), but since this test was conducted *post hoc* after seeing the data, α should be reduced (arguably to 0·005 considering 10 possible comparisons from 1 year to the next within a region).

We observed 55 natural mortalities of radio-collared buffalo, 13 of which were of unknown cause. One individual burned in a grass fire and all the others were presumed to be killed by lions on the basis of seeing lions or lion spoor near the carcass as well as an intact radiocollar. Other mortalities of unmarked buffalo included one potential drowning and one vehicle collision. During the 6-year study, we observed only three buffaloes (all uncollared) die as a direct result of BTB infection (i.e. they were not victims of predation and had extensive pulmonary lesions). We did not find any significant effect of being test-positive for BTB on the mortality rates of

Table 1. Cox proportional hazards models for 55 deaths of 163 African buffalo of known disease status from May 2001 to November 2006. AIC, Akaike Information Criterion; AIC_c, AIC corrected for small sample size. The model in bold had the lowest AIC_c value

Model	Parameters	AIC	AIC_c	exp (β)[1]	Lower CI	Upper CI
Age	1·0	491·2	491·3	–	–	–
AgeCat1	2·0	491·3	481·5	–	–	–
AgeCat2	4·0	486·3	487·1	–	–	–
spline(Age)[2]	4·2	479·9	480·8	–	–	–
AgeCat1 + Sex	3·0	468·9	469·3	–	–	–
AgeCat2 + Sex	5·0	473·1	474·3	–	–	–
AgeCat1 × Sex	5·0	470·1	471·3	–	–	–
AgeCat2 × Sex	9·0	470·8	474·7	–	–	–
spline(Age) + Sex	**5·4**	**466·4**	**467·8**	**–**	**–**	**–**
spline(Age) × Sex	13·9	469·7	480·1	–	–	–
spline(Age) + Sex + BTB	6·3	464·3	466·2	0·49	0·24	1·02
spline(Age) + Sex + BTB(males only)	6·4	467·1	469·1	0·65	0·29	1·45
spline(Age) + Sex + BTBtime[3]	6·3	468·2	470·1	0·90	0·57	1·41
spline(Age) + Sex + BTBtime[2]	6·3	468·3	470·3	0·98	0·81	1·19
spline(Age) + Sex + BTB1yr[4]	6·3	468·1	470·0	0·80	0·35	1·83
spline(Age) + Sex + BTB2yr[5]	6·3	468·2	470·1	0·72	0·16	3·16

[1]estimated proportional hazard associated with being BTB+.
[2]penalized splines were fit to age and the degrees of freedom were chosen based upon the lowest AIC value.
[3]BTBtime refers to the minimum time an individual was known to be BTB positive.
[4]BTB1yr: only those individuals known to be infected for over 1 year are considered BTB+.
[5]BTB2yr: only those individuals known to be infected for over 2 years are considered BTB+.

either male or female buffalo (Table 1). We considered alternative ways of modelling BTB: (i) only considering those individuals known to be infected for at least 1 or 2 years, and (ii) using minimum time infected as a covariate. In none of these cases, were test-positive individuals estimated to be at greater risk than test-negative individuals (Table 1). When all test-positive individuals were included, the best estimate of the BTB effect was a counterintuitive halving of the mortality rate [Table 1, exp(β) = 0·49, 95% CI = (0·24–1·02)]. The upper confidence limit on the hazard ratio was 1·02, indicating that test-positive buffalo are very unlikely to have a higher mortality rate than test-negative buffalo. Out of 40 test-positive female buffalo years (i.e. the sum of the observation time across all individuals in that category), we observed only two mortalities, compared to 21 test-negative female deaths in 85·3 buffalo years. We observed 13 test-positive individuals for over 3 years and five test-positive buffalo for over 4 years (Fig. 2; Supporting Information, Fig. S1). None of these individuals died during the study.

To minimize our impacts, we did not frequently recapture test-positive individuals. However, over the course of the study, we recaptured previously test-positive individuals 46 times. Four of 16 that were recaptured within 1 year back-converted to test-negative, and 18 of 46 back-converted overall. In the absence of post-mortem culture results on these animals, it is difficult to ascertain whether these tests were false positives or false negatives. As a result, we conducted two further analyses. First, we compared survival rates irrespective of disease status to other published studies and we compared survival rates of individuals in Lower Sabie to those in Satara, hypothesizing that survival may be lower in Lower Sabie where BTB has been present for longer and is at higher prevalence. Our estimated survival rates were very similar to Sinclair (1977) but slightly less than Jolles (2007) in the older age categories (Fig. 3). Models with region included were not statistically better than those without (reduced model: spline(Age) + Sex vs. full model: spline(Age) + Sex + Region: $\chi^2_1 = 0{\cdot}04$, *P* value = 0·84).

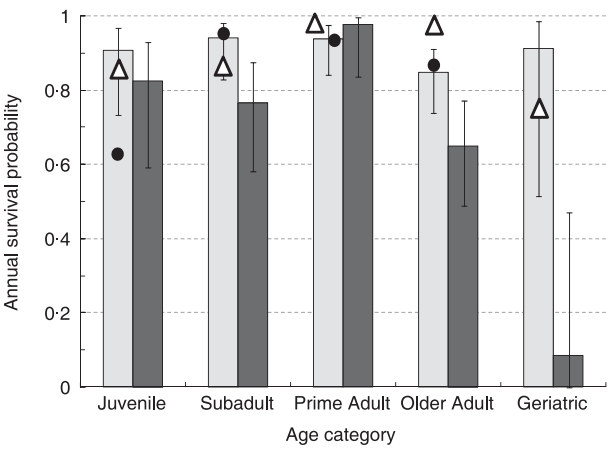

Fig. 3. Annual female (light bars) and male (dark bars) survival estimates with 95% confidence intervals. This study categorized ages as follows: juveniles 1–2 years; subadults 3–4 years; prime adults 5–7 years; older adults 8–12 years; geriatrics 12+ years. Filled circles and open triangles are female survival estimates from Sinclair (1977) and Jolles *et al.* (2005), respectively. Studies differed in the way ages were estimated and categorized.

The probability of observing a calf with a mother of known BTB status peaked from April to August, coinciding with the dry season and a reduction in grass biomass and height, with a concurrent improvement in ground-level visibility (Fig. 4). After August, calf sightings declined, probably due to a

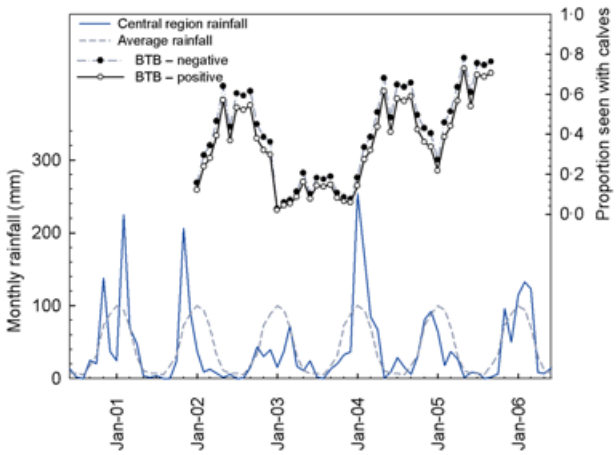

Fig. 4. Mean monthly rainfall for the central region of KNP (solid line) and the park-wide average from 1920 to 2006 (dotted line) compared to model estimates based upon a logistic regression of the presence/absence of calves with female African buffalo (see Supporting Information, Model 6 in Table S3). Dotted lines with solid circles are BTB-negative individuals and solid lines with open circles are BTB-positive individuals, respectively. Estimates assume that the female is seven years old and seen between 20–30 times.

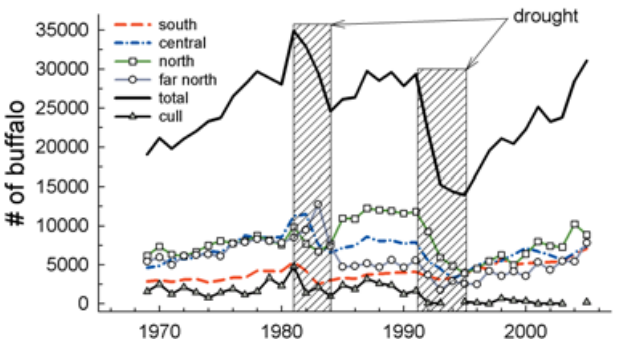

Fig. 5. Total number of buffalo counted and culled (dotted line) in the different regions of KNP. Major droughts are noted in as hashed boxes. Number of buffalo culled is the park-wide total.

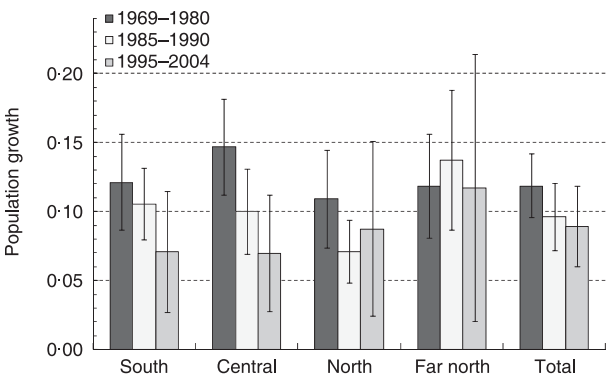

Fig. 6. A comparison of buffalo population growth by region during inter-drought periods. Population growth, $\ln(N_{t+1}/N_t)$, was adjusted for culling by assuming that removed individuals would have survived to the next census. Bars represent standard errors.

combination of calf mortality, weaning and reduced calf visibility during the rainy season. Reproductive success was depressed in 2003 due to a lack of rainfall during the preceding wet season (Fig. 4). Relative to the larger seasonal and annual variability of calf sightings, there appeared to be only marginal evidence of a BTB effect. Logistic regression analyses suggested that test-positive females were less likely to be seen with calves (P value = 0·065 for the best AIC model, Supporting Information, Tables S1–S4). This effect, however, was not significant in the models where individual identity was entered as a random effect (P value = 0·14 for the best AIC model, Supporting Information, Tables S1–S4). Further, when we removed negative individuals that had not been retested within a year of the observation, BTB was no longer a significant predictor.

Preliminary analyses suggested that population growth rates may be declining over time particularly in the south and central regions. In addition, population growth since 1993 appeared to be less in the south, which may be indicative of a disease effect (Fig. 6). However, randomization tests indicated population growth rates were not significantly different among regions or inter-drought periods and there was no interaction between regions and periods (Supporting Information, Figs S2–S3). The observed population growth of KNP, as a whole, from 1995 to 2004 was 8·9% per year, and population growth showed a non-significant increase over time since 1993, suggesting that density dependence is not yet affecting population growth [linear regression with year as a continuous variable, slope = 0·0179, 95% CI = (–0·002, 0·037), $F_{1,11}$ = 3·28, P value: 0·098]. Further, population growth in the southern region was not statistically different from other regions since the 1991–1992 drought [linear model with region (categorical) and year (continuous) as explanatory variables, $F_{7,40}$ = 0·316 P value = 0·94].

Discussion

We investigated the demographic impacts of BTB on African buffalo in KNP by combining an intensive study of known individuals with almost 40 years of aerial census data. Over a 6-year period, we monitored 163 individuals of known disease status for a total of over 360 buffalo years, but found no detectable adverse effect of BTB on the survival of these individuals (Table 1), and the upper confidence limit of 1·02 is equivalent to rejecting the null hypothesis that the mortality hazard of test-positive buffalo was more than 2% higher than test-negative buffalo (Hoenig & Heisey 2001). There was a marginal effect of BTB on reproductive success, as indexed by the probability of sighting known females with associated calves. Population growth in the south, however, did not differ from other regions and the population growth in the KNP as a whole remained strong (Figs 5 and 6). Each of these analyses has limitations and, independently, they do not yield a definitive conclusion on the overall impact of BTB. However, all the analyses we conducted support an overall conclusion that the impacts of BTB on buffalo have thus far been relatively minor.

Our conclusion that BTB has a minor impact upon buffalo survival depends on several assumptions worth further

discussion because they are common among studies intended to detect the impacts of a chronic disease on a long-lived species. First, study subjects will change disease status over time requiring a rigorous retesting programme. We attempted to recapture individuals every 6 months (Fig. 2). Due to logistics as well as inconclusive tests (i.e. suspect, avian, or multiple reactors), our team of 6–7 people caught the ~100 known-status buffalo often enough to get a usable test result, on average, every 355 days. We believe this is acceptable for the case of bovine tuberculosis where disease progression is probably slow (De Klerk-Lorist 2005; Jolles *et al*. 2005). Secondly, the sample size must be high enough to observe sufficient mortality. To have an 80% chance of detecting a doubling of mortality, one would need to observe over 50 deaths (p. 64, Therneau & Grambsch 2000). When survival rates are high, say 90% per annum, this will take approximately 500 subject years, a formidable task given that we could only manage 360 subject years in our intensive 6 years of study.

Perhaps most importantly, however, detecting disease effects depends upon accurate diagnosis. Data from captive exposures suggest that the IFNg assay is very good at diagnosing buffalo that are infected with high doses of BTB (De Klerk-Lorist 2005). However, the counter-intuitive decrease in the mortality hazard for test-positive individuals is at least suggestive that some test-negative individuals may be heavily infected. Some cattle with severe or generalized disease may not react to tuberculin (Lepper, Pearson & Corner 1977; Monaghan *et al*. 1994). Interestingly, Joly & Messier (2005) also found that BTB increased the survival of bison, but considered this result either spurious or due to anergic animals testing negative (D.O. Joly, personal communication). Further research on this issue is needed since there are very few studies on this phenomenon in either cattle or wildlife because it requires the monitoring of individuals known to be infected for long periods of time. Nonetheless, even if some of the mortalities of test-negative individuals are due to disease, the overall survival of female buffalo in the KNP is comparable to other published studies (Fig. 3).

Packer *et al*. (2003) argued that predation might reduce the prevalence of disease in the host due to selectively removing infected individuals or reducing overall density. Few studies of large mammals, however, have estimated the amount of predation on diseased versus healthy individuals (but see Rau & Caron 1979). Testing this hypothesis in large mammals will be challenging for several other reasons. Many diagnostic tests only indicate exposure rather than the extent of disease. As a result, the effect of disease on heavily infected individuals may be undetectable when mixed with other test-positive individuals that may have recovered. Our data suggest that lions did not appear to selectively prey upon buffalo that were positive on the IFNg assay. However, the lack of selection for test-positive buffalo may be due to some heavily infected individuals being test-negative. In addition to diagnostic difficulties, few studies are able to capture and recapture known individuals so that the disease status of monitored individuals is consistently updated. We hypothesize that cursorial predators, such as wild dogs *Lycoan pictus* and wolves *Canis lupus* are more likely to target weaker prey than ambush predators like lions and leopards. In addition, species that use herd defence may shelter some infected individuals from disease-induced predation.

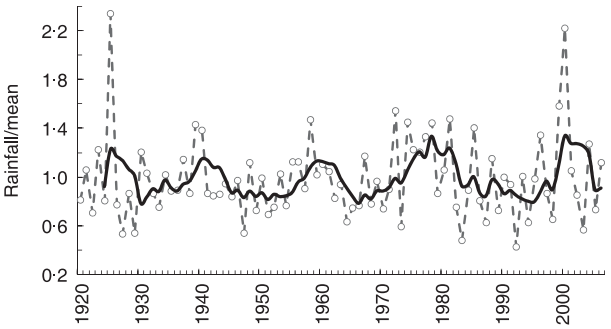

Fig. 7. Deviations in the long-term mean of annual rainfall in KNP. The dotted line represents annual data while the solid line is the 5-year running mean.

In our study, the rainfall in 2003 was 43% below the long-term average and coincided with a dramatic reduction in calf sightings (Fig. 4). In 2004, BTB seroprevalence appeared to drop in both the central and southern regions (Fig. 1). This may be due to a lagged effect of the 2003 drought, whereby many BTB-positive individuals did not survive to be tested in 2004. However, this hypothesis is not supported by the survival data on radiocollared individuals and does not explain the dramatic rebound in seroprevalence the following year. Thus, we view sampling error as a more parsimonious explanation, but believe that the interaction of drought and BTB will remain an open question until a drought similar to 1982–1983 and 1991–1992 occurs again. If the past bi-decadal oscillations in rainfall continue, this drought is likely to occur within the next 10 years (Fig. 7, Jury 2002).

Estimating the impact of chronic diseases on wildlife populations is logistically and statistically challenging, particularly for long-lived species where the effects are likely to occur over time-scales coincident with natural successional and anthropogenically induced changes. In some cases, researchers are forced to estimate host vital rates using known individuals and then use models to predict population-level effects. In these models, even small effects on adult mortality or reproduction may have large impacts on population size and resilience (Joly & Messier 2004; Jolles, Etienne & Olff 2006). Our aerial census data, combined with the spatial variation in BTB prevalence, suggest that overall population growth and resilience did not appear to be reduced by BTB during this time period of average precipitation. Our analysis of population counts indicated that the regional growth rates were not significantly different and the park-wide growth rate appeared to be increasing since 1993 (Fig. 6), assuming that movement of individuals among the regions is negligible. Analyses of the population counts are confounded by several factors. We expected the southern region population to decline more than northern regions during drought conditions and recover more slowly afterwards due to the higher prevalence of BTB. However, due, in part, to an outbreak of anthrax in the

north, the southern region population declined less in the 1991–1992 drought than that of the northern regions (Bengis, Grant & de Vos 2003; Supporting Information Figs S2–S3). As a result, the marginal reduction in population growth rate in the south may be due to greater intra-specific competition in the south rather than a minor disease impact. Movement among regions is likely to obscure BTB effects and a more accurate assessment of the full impact of BTB on the KNP might not be possible until all the regions have roughly the same prevalence.

The BTB epidemic in the KNP epitomizes many of the difficulties that arise in the management of wildlife disease. Early action would have improved the likelihood of disease control or eradication, but limited data existed on the long-term impacts of the pathogen, making it difficult to argue for aggressive management. Almost 20 years later, it is still unclear how BTB might affect other hosts, and in particular, whether BTB-induced reductions in lion densities could have cascading impacts upon the ecosystem. Furthermore, management options remain limited and complete eradication is unlikely, given the challenges associated with the currently large size of the KNP buffalo population, the multiple alternative hosts that may either serve as spill-over hosts or maintain the infection independent of buffalo (Keet *et al.* 1996), and the limited efficacy of the BCG vaccine (De Klerk-Lorist 2005; Cross & Getz 2006). What is apparent is that the KNP buffalo population undergoes steep declines during severe droughts (Fig. 4), and animals in herds with high BTB prevalence lose condition fastest during dry conditions (Caron *et al.* 2003). It might thus be expected that a post-drought population will be greatly reduced in size and exhibit a reduced BTB prevalence, two conditions that improve the chances of holding BTB prevalence down (e.g. through aggressive test-and-slaughter/vaccination campaigns) as the population recovers in size.

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Supporting Information

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

Fig. S1. Histograms of the amount of time BTB negative (A) and positive (B) individuals were monitored for survival with radiocollars

Fig. S2. Results from permutation tests using region (south, central, north, and far north) and period (1969–1980, 1985–1990, and 1995–2004) to predict average population growth. The solid black lines are the density of *F*-statistics from 1000 randomizations. The solid red vertical lines are the 95th percentiles and the dashed vertical lines represent the results from the original data

Fig. S3. A comparison of population growth from 1993 to 2005, BTB prevalence in 1998, and the percent decline in population size from 1991–1993 due to drought and an outbreak of anthrax. Population growth is estimated as the natural log of the geometric mean

Table S1. Model comparisons of the mixed effect logistic regressions of 1313 observations of the presence/absence of calves for 165 female African buffalo 4 years of age and older. Buffalo identity (BufID) was included in all the models as a random effect

Table S2. Parameter estimates from the best AIC logistic regression model to estimate presence/absence of calves based upon 1313 observations of 165 adult females. Individual identity was a random effect

Table S3. Model comparisons of logistic regressions of 1313 observations of the presence/absence of calves for 165 female African buffalo 4 years of age and older

Table S4. Parameter estimates from the logistic regression model to estimate whether African buffalo will be observed with or without calves based upon 1313 observations of 165 adult females

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