Influence of bovine tuberculosis *(Mycobacterium bovis)* on condition and reproductive success of females African buffalo *(Syncerus caffer)* in Kruger National Park

by

Tania Leah Fairfax Bird

(Supervised by Prof. Johan T. du Toit
and Prof. Wayne M. Getz)

Submitted in partial fulfilment of the requirements for the degree
Magister Scientiae (African Mammalogy)

Mammal Research Institute
Department of Zoology and Entomology
University of Pretoria
Pretoria
0002
South Africa

November 2004

Influence of bovine tuberculosis *(Mycobacterium bovis)* on condition and reproductive success of females African buffalo *(Syncerus caffer)* in Kruger National Park

Tania Leah Fairfax Bird

Mammal Research Institute
Department of Zoology and Entomology
University of Pretoria
Pretoria, 0002
South Africa
tlfbird@zoology.up.ac.za

Supervised by

Prof. Johan T. du Toit

Mammal Research Institute
Department of Zoology and Entomology
University of Pretoria
Pretoria
0002
South Africa
jdutoit@zoology.up.ac.za

and

Prof. Wayne M. Getz

Department of Environmental Science Policy & Management
201 Wellman Hall
University of California, Berkeley
CA 94720-3112
USA
getz@nature.berkeley.edu

ACKNOWLEDGEMENTS

This research was funded by the United States National Science Foundation Ecology of Infectious Disease Grant DEB-0090323 to Wayne Getz, National Research Foundation in South Africa and the Mammal Research Institute at the University of Pretoria. Special thanks go to Paul Cross, for his mentorship and advice throughout the study. Many thanks to Craig Hay, Justin Bowers Julie Wolhuter, Augusta Mabunda and Khutani Bulunga, the BTb team in Satara, for their time, help, advice and company in the field. This project would not have been possible without their continued hard work and effort on this long-term project. Of course, thanks to Kruger Scientific Services for allowing me access to the amazing resource that is Kruger National Park.

I am indebted to J.W.H. Ferguson for his invaluable help with the statistical analysis, to Phillip Bateman, Adrian Schrader and Luke Verburgt for their advice and guidance, and to Mike Scantlebury for comments on the manuscript.

Many thanks also to all my friends in the Mammal Research Institute, and Conservation and Ecology Research Unit, at University of Pretoria, for making this masters so enjoyable, and for all the moral support through the hard times this year.

And of course, I could not have done it without my parents’ continued enthusiasm and support.

This work is dedicated to the memory of Neil Moore, Chris Reid, and Grant Cave, who loved Africa as I much as I do, to Monsieur, who never knew it, and to the recovery of Ari Bert and Ian Waterhouse, who will one day love it again.

ABSTRACT

The impact of the current bovine tuberculosis (BTb) epidemic in African buffalo (*Syncerus caffer*), in the central region of Kruger national park, South Africa, was investigated in relation to reproductive success of females. Over a four-year period, we compared the reproductive effort, calf survival and body condition of 72 marked adult females of known BTb status. The body condition of cows did not increase during the wet season as much for BTB positive cows than BTB negative cows. . Calved cows ended the dry season in significantly lower condition than barren cows, but tuberculosis did not significantly reduce the ability of infected cows to reproduce and did not reduce calf survival. This may have been because BTb positive cows that managed to reproduce suffered from subclinical symptoms. There was an indication of long-term reduction in reproductive output for infected cows, but this needs to be investigated further. A condition threshold may exist for reproductive ability, below which reproduction is inhibited. In the absence of sudden environmental change, which could otherwise breach the condition threshold, we suggest that BTb is unlikely to have a great impact on population dynamics of buffalo. African buffalo are therefore the perfect maintenance host for this chronic disease, which is of major concern for management and conservation of more susceptible species.

Key words: body condition threshold, buffalo, disease, maternal effects, fecundity, offspring survival, tuberculosis.

TABLE OF CONTENTS

TABLE LEGENDS

FIGURE LEGENDS

INTRODUCTION

African buffalo (*Syncerus caffer*) were first diagnosed with bovine tuberculosis (BTb) in Kruger National Park in 1990, although the disease may have been introduced by cattle (*Bos* sp.) as early as the 1960s (Bengis *et al.* 1996). Bovine tuberculosis is caused by the pathogen *Mycobacterium bovis,* and is usually transmitted through inhalation of infectious aerosol droplets from one animal to another (Menzies & Neill 2000). Ingestion of infectious material may provide another route of transmission (Chaddock 2002). Infection can occur *in utero* via the placenta (Morris, Pfeiffer & Jackson 1994), as well as via ingestion of infected milk (Huchzermeyer *et al.*1994).

A major aspect of disease ecology is understanding the impact of the disease on the host species (Anderson & May 1978). BTb is a chronic wasting disease that can allow the host to survive for many months, even years, without any clinical symptoms (Morris *et al.* 1994; De Lisle *et al.* 2002). In general, chronic diseases are difficult to investigate because they often affect life history traits such as fecundity and adult survival without causing obvious mortalities (Jolles 2004). This is particularly important in understanding the epidemiology of the disease as animals maybe infectious for long periods. Small lesions occur primarily in the lymphoid tissues and lung parenchyma, often causing chronic coughing and digestive failure (Huchzermeyer *et al.* 1994).

Bovine tuberculosis has now been found in many other species, from the greater kudu (*Tragelaphus strepsiceros*), to the lion (*Panthera leo*) and leopard (*Panthera pardus*) (Keet *et al.* 1996). Thus, the ramifications of the BTb epidemic reach well beyond buffalo ecology. Control of the epidemic is important for conservation management as well as for South Africa's livestock industry (De Lisle, Bengis, Schmitt & O'Brien 2002)

Diseases that target very young and very old individuals may not have much impact on population dynamics, as it is the survival and reproductive success of juvenile and adult individuals that determines the intrinsic population growth rate (Anderson & May 1978). If infected animals are able to reproduce successfully the host population may still survive, and the disease then becomes endemic. Conversely, a disease that directly reduces host density will simultaneously reduce the opportunity to infect a new host (Fenton, Fairbairn, Norman & Hudson 2002) resulting in a negative feedback system that impacts on the parasites success (Lafferty & Holt 2003). Thus, the impact of the disease on reproduction is key to its affect on population dynamics of the host species.

Evidence for an impact of BTb on reproductive success in African buffalo is inconclusive. Jolles (2004) found that BTb caused a 27% reduction in pregnancy rates of infected females. Conversely, Rodwell, Whyte & Boyce (2001) found no difference in pregnancy and lactation frequencies between BTb infected and uninfected buffalo, based on necropsies of over 3,700 culled buffalo between 1991 and 1998. However, both studies suggested that young animals were overrepresented in the infected subpopulation, which could indicate that adult mortality was affected, as more adults would have died. Population dynamics could then be affected by the disease, through removal of the breeding age group.

Maternal condition is also known to have an influence on reproductive success (Pike 1999; Benton, Ranta, Kaitala & Beckerman 2001; Gorman & Nager 2004). Here, reproductive success is defined as the contribution of a genotype to future generations. In this sense, reproductive success is contingent upon the ability of a female to produce offspring that survive to maturity and in turn produce successful offspring (Gorman & Nager 2004). Poor maternal condition has been shown to have an impact on fecundity for female buffalo (Grimsdell 1969; Prins 1996), and on offspring fitness in domestic cattle (Markusfeld, Galon & Ezra 1997) and other ungulates (reviewed by Benton *et al.* 2001).

Body condition score is an indicator of nutrition and health, and is therefore directly correlated to environmental changes in food availability (Grimsdell 1969). Fat reserves are built up during wet season when resources are abundant, and are then utilised during the dry season, when food quality and quantity are both limited for buffalo (Sinclair 1977). The energetic costs of gestation and lactation for buffalo are great compared to other activities such as mating or foraging (Sinclair 1977). Births occur during the end of the wet season, allowing late gestation and lactation to coincide with peaks in nutrient availability (Prins 1996).

Milk quality and quantity are directly related to maternal condition in cattle and buffalo (Prins 1996; Markusfeld *et al.* 1997). Both quality and quantity are reduced by age in buffalo (Sinclair 1977) and by *Mycobacterium bovis* infection in domestic cattle (Hernandez & Baca 1998). *M. bovis* can also be directly transmitted in the milk to calves (Huchzermeyer *et al.* 1994) and therefore the potential for the disease to affect calf survival is two-fold.

Caron *et al.* (2003) found evidence to suggest that herds in high BTb prevalence zones had overall worse condition than herds in lower BTb prevalence regions of the park. Furthermore, they found that the nutritional stress experienced during the dry season (April through to October) was exacerbated by the disease. Jolles (2004) found evidence to suggest that BTb infection was affecting older cows' fecundity in the herd. In this study, I investigate the impact of BTb on reproductive success of females in relation to maternal condition and age at the individual level, in order to assess the potential affects of the disease on long-term population dynamics of African buffalo. I hypothesise that BTb has a negative effect on reproductive success of female African buffalo, through its impact on body condition, and that BTb does play a significant role in shaping population structure. I tested the following predictions: (1) older females will have a higher incidence of BTb infections; (2) infected female buffalo have a greater loss of condition over the dry season than BTb negative females; (3) BTb positive cows will have lower reproductive success (measured by calving events and calf survival) than BTb negative cows.

METHODS

Study area:

This study was part of a larger project on BTb in African buffalo in the central region of Kruger National Park, South Africa (Caron *et al.* 2003; Cross, Lloyd-Smith & Getz *In press a*; Cross *et al. In press b*). The study area is primarily composed of basaltic soils that are dominated by knob-thorn acacia and interspersed with riverine bushveld, as described by Venter, Scholes & Eckhardt (2003).

Data collection

Study animals

Female reproductive success was monitored using 72 marked adult female buffalo *(Syncerus caffer)* in Kruger National Park from 2001 until 2004. During the four-year project, over 300 females were captured from a helicopter or a vehicle, and branded with a unique number allowing individual identification in the field. Radiocollars and marker collars (with unique colour, letter and number sequences) were assigned to random individuals

Females were classified as adults once they had reached sexual maturity at five years of age (Sinclair 1977). We aged adult females using a Horn –Age Key that identified cow age based on size, length and angle of horn growth relative to age (Sinclair 1977). For captured individuals less than 5 years old, tooth eruption pattern was used for age determination (Pienaar 1969; Grimsdell 1969, Sinclair 1977). Sub-adult females were not included in the study until they reached maturity.

We located herds using radio telemetry. Herd membership was not considered in this study because previous studies indicate that females often form splinter groups and rejoin with other groups (Cross *et al. In press a*). Individuals were observed from a vehicle, or from a viewing point such as a tree or mound, with as little disturbance to the herd as possible. During the observation sessions, we recorded the date, herd, time, age, body condition, and reproductive status whenever possible. Hereafter female buffalo that had calves are referred to as 'calved cows' (CCs), and those without calves are referred to as 'barren cows' (BCs).

Body condition scores

Body condition was determined visually using a 1-5 scale according to Prins (1996) and Caron *et al.* (2003). Condition scores ranged from poor (1) to good (5) in increments of 0.5. I used condition scores during the dry season to examine possible effects of BTb on buffalo as this is the critical period for food availability (Sinclair 1977). I looked at change in condition over the wet season using condition score differences from the end of one dry season to the beginning of the next. I established every cow's condition at the start (April-May) and end (September-October) of each dry season using a mean score over the relevant months from all observers. This reduced potential subjective differences between observers. Observers were not aware of the disease status of individuals being monitored, which also helped to eliminate potential bias in assigning a score.

Reproductive status

I used reproductive status and calf survival in a given year as an indication of reproductive success. Reproductive status was split into seven categories: No Calf, Pregnant, 0-3 months calf, 3-6 months calf, 6-12 month calf, Calf and Unknown. 'Unknown' meant the observer was unsure whether an observed calf did, or did not, belong to a specific known female. Observers recorded 'Calf' when a calf was associated with a known female but they were unsure of its age. Calf age was determined when possible, based on horn development and shoulder height as described in Sinclair (1977).

Calf survival and mortality

We could not identify calf mortality in most cases; so instead, I inferred calf survival from the approximate age at last sighting. I calculated approximate calf age as the mean between the lengths of time the calf was physically observed, and the maximum age that the calf could have been at last sighting (e.g. if last sighted as '3-6 month calf', and the calf had been observed for 5 months, we record calf age as 5.5 months). I assumed that there was no difference in the likelihood of seeing infected and uninfected females, so that if BTb was affecting calf survival, one would find calf ages at last sighting would be significantly younger for BTb positive CCs.

Disease status

We collected blood samples from known individuals at either 6 or 12 months intervals to test for *M. bovis* infection. We used a modified gamma-interferon (IFNg) BOVIGAM^{TM} assay (Wood and Jones 2001), which has similar sensitivity (82-100%) and specificity (~99%) to the intradermal skin test (Wood & Jones 2001; Grobler, Michel, De Klerk & Bengis 2002; Cross *et al. In press a*). Some animals were difficult to recapture so may have only been tested once. Therefore, I only included years in which a cow had been tested. Positive animals were not recaptured, as there is no evidence that infected cows convert to BTb negative in domestic cattle (Chaddock 2002); and we assumed the same for *S. caffer*. Some results were suspect or came back as multiple reactors. These cases reacted positively for *Mycobacterium bovis,* as well as for *Mycobacterium avis,* and *Mycobacterium fortuitum*; related diseases that can cause false positive reactions to the BTb test (Cross *et al. In press b*). Only females with definitive BTb positive and negative test results were included to avoid misinterpretation of disease status.

Data analysis

To assess female reproductive success, each female's sighting history was evaluated and her reproductive status in each year determined. A cow was considered to have a calf if she was seen with a calf for two or more consecutive observations. Thereafter, she was assumed to have a calf until seen without the calf for two or more consecutive observations. Females were excluded from the analysis for a given year, if it was not clear whether a calf did or did not exist.

Only one record per cow was used in the analyses to meet the assumptions of independence and avoid pseudo-replication. In order to include data with regards to previous year parameters, I used the second year of observations as the present-year record and included the first-year observations as previous-year information. Some females were only observed for one year so they were excluded from specific analyses that included previous year parameters. Due to the small sample size (n = 72), I pooled all independent records (one per cow), irrelevant of the year in which they were recorded. (I don't think 2003 should be pooled with the rest. In 2003 only 10% of all females had calves whereas in the rest of the years around 50% of females had calves.)

Due to the limitations of opportunistic field observation techniques, data sets were not complete. For example, a female may have a condition score for the beginning of the dry season but not the end. Thus, sample sizes varied between tests in these analyses.

For statistical analysis, I used the software package STATISTICA 6.0 (StatSoft 2001). Parameters included in the analysis were condition at the end of the previous dry season, condition at the start of the present dry season, condition at the end of the present dry season, calf survival, female age, reproductive status in the previous year, BTb status, and present reproductive status. A summary of variables used in the results section is given in Table 1.

Cut Table 1 for publication..

Table 1: Key to the variables used in the analyses

Variable	Variable type	Description	Categories/ range
BTb status	Binomial	Female tuberculosis status (IFNg blood test results):	positive OR negative
Reproductive status	Binomial	Whether a female was recorded with a calf or not in a given year.	Calved cow (CC) OR barren cow (BC)
Calf last year	Binomial	Whether a female was recorded with a calf or not in the previous year	CC last year OR BC last year
Condition end of last dry season	Multinomial	Condition score of female at the end (Sept –Oct) of the previous dry season	1 – 5 (with half-steps) 1= very poor 5= very good
Condition start of present dry season	Multinomial	Condition score of female at start (April-May) of dry season	1 – 5 (with half-steps)
Condition end of present dry season	Multinomial	Condition score of female at the end (Sept –Oct) of the present dry season	1 – 5 (with half-steps)
Cow age	Integer	Age of adult female	5 – 17 years

Fisher's exact test was used to analyse two categorical factors. Mann Whitney U-tests were performed when testing multinomial variables for differences among categorical factors. Simple regression analysis looked at the relationship between two multinomial variables. Analyses of covariance were used for testing continuous variables for covariance with categorical factors when the dependent variable was multinomial or integer.

The generalized linear model approach was used because it allows a binary dependent factor (calved cows/barren cows) to be interpreted, while incorporating both categorical and continuous (integer) independent variables. The interactions between conditions at various times of the year, BTb and reproduction needed to be included in the final model. It was not possible to incorporate all variables in a single generalized linear model so I examined links and interactions between various factors for significance before designing the final model. Several models were run using varying combinations of parameters. A goodness of fit test was applied to each model and I assumed that the model with the smallest scaled deviance was the most appropriate final model for my data (Venables & Ripley 1999).
NEED A TABLE WITH THE COEFFICIENTS AND P-VALUES ASSOCIATED WITH THIS EQUATION. This goes in the results section.

Reproductive status = BTb status +
+ condition start of dry season +
+ reproductive status last year +
*+ (BTb status*reproductive status last year).*

WHAT ABOUT THE CONSTANTS IN THIS EQUATION?

RESULTS

BTb status and reproductive status (I think this portion has to wait for one more year of data). Using all the data, this is a significant trend, but this pseudo-replicates by having the same female recorded multiple times. If you restrict the analysis to only one record per female, the results are not significant. And within any one year, the results are not significant. I think one more year will clinch the results.

Over the whole period, 58 calves were born to BTb negative CCs and 14 calves were born to BTb positive CCs. Five of the latter were born in the southern region of the park and so were not included in this study. Of the remaining nine BTb positive calves, two were excluded from the analyses because we used the second year of observations in order to include previous year factors, and the calves had been born in the first year. The proportion of CCs to BCs did not significantly differ between BTb positive and negative cows (Fisher's exact one-tailed p= 0.292) when a using a single record per cow (Table 2). Nevertheless, the total number of calves recorded over the whole study (including all records of all mature females) appeared to be different between BTb negative and BTb positive cows (Table 2).

Cow age

There was no significant trend between cow age and BTb status (Mann Whitney U= 521, p= 0.720), or cow age and reproductive status (U= 550, p= 0.438). One BTb positive CC had a calf at 15 years of age. Cow age was not correlated with condition at the start of the present dry season, when included in an ANCOVA as a covariate with BTb status or reproductive status ($F_{(1,46)}$= 2.56, p= 0.116, ($F_{(1,46)}$= 2.01, p= 0.163 respectively). ITALICS OF MATH SYMBOLS THROUGHOUT!

Cow age had no significant effect on condition at the end of present the dry season, when included as a covariate with BTb status, or with reproductive status ($F_{(1,46)}$= 1.602, p= 0.211, $F_{(1,46)}$= 0.735, p= 0.396 respectively). Age and BTb status did not have any significant effect on reproductive status. Cow age was not correlated with calf survival when included as covariate with BTb status. Cow age was therefore excluded from further analyses.

Table 2: Frequency of females in each reproductive category, for each BTb status

	BTb negative	BTb positive	Total	Fisher's Exact p test (one-tailed)
Calved cows	21 (58)	7 (14)	26 (72)	p= 0.292
Barren cows	29 (62)	15 (35)	46 (97)	
Total	50 (120)	22 (49)	72 (169)	

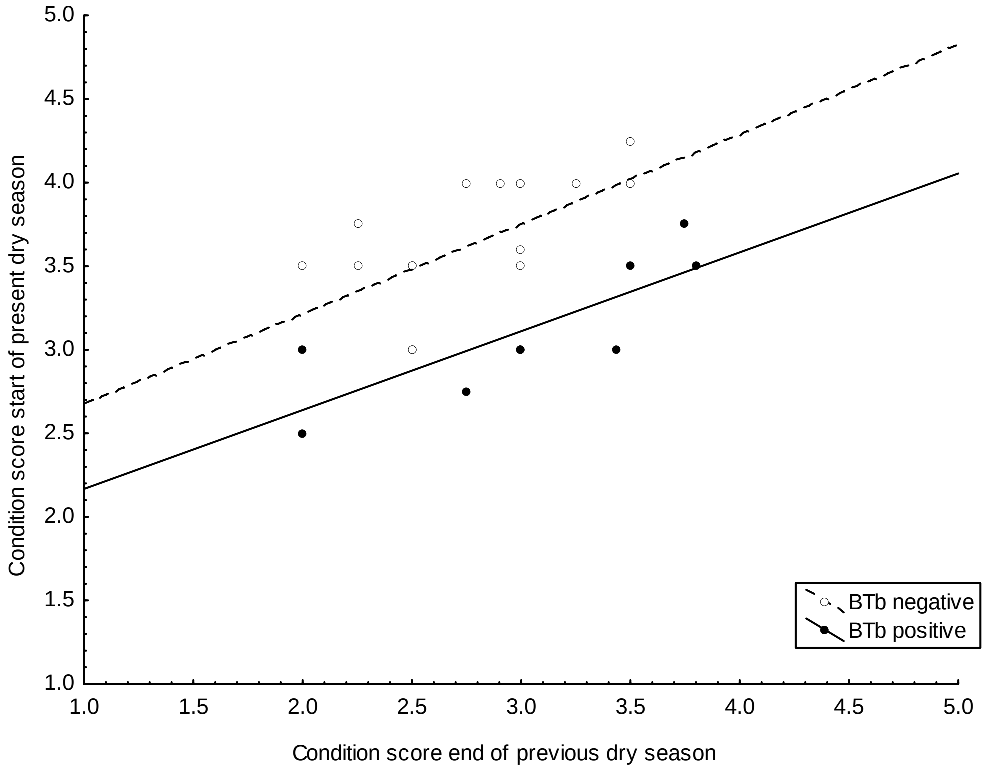
Numbers in brackets show the data for all records of all cows over the four-year study

This will confuse the readers to have two sets of numbers in one table.

BTb Status, Body Condition and reproduction

Both BTb status and condition score at the end of the previous dry season were strong predictors of the condition of an individual at the start of the present dry season (ANCOVA; $F_{(1,26)}$= 23.1, p= 0.000, $F_{(1,26)}$= 19.3, p= 0.000 respectively). This meant that BTb positive cows regained less condition over the wet season than BTb negative cows (Fig. 1). There was no significant influence of BTb status or condition at the start of the present dry season on the condition at the end of the present dry season (ANCOVA; $F_{(1,30)}$= 0.062, p= 0.806, $F_{(1,30)}$= 0.098, p= 0.757 respectively). There was no significant effect of BTb status or condition at the start of the present dry season on reproductive status (Generalised linear model (n=49); p= 0.714, 0.279 respectively) (Fig. 2).

Females with and without calves did not start the present dry season in significantly different condition. However, CCs lost significantly more condition than BCs (U=123.5, p= 0.000), and CCs ended the present season in lower condition than BCs irrespective of BTb status (Generalised linear model (n =49); p = 0.003 for reproductive status, p = 0.677 for BTb status) (Fig. 4). The correlation between reproduction and condition at the end of the dry season was also significant using data from the previous year (U=74.0, p= 0.010). Sample sizes were too small to include the disease parameter in relation to reproduction in the previous year (not clear what you mean here, be explicit). Calves cows ended the dry season in significantly poorer condition than BCs, but BTb status and the interaction of BTb status* Reproductive status had no bearing on this condition score (respectively) (Fig. 4). Reproductive status in the previous year may well have an effect on reproduction in the present year (Fisher's exact p = 0.097), although sample size was too small for a sufficiently powerful statistical test of this phenomenon. (Calculate the Power of the test!)



Use larger font to decrease the amount of blank space in the graph (so that the graph will look better when it is reduced in size for publication)

Figure 1: Change in condition over the wet season (October – April) for female buffalo in different disease states; BTb positive cows gain significantly less condition between the end of one dry season and the start of the next, compared to BTb negative cows. Condition scores have a significant positive linear relationship from one dry season to the next.

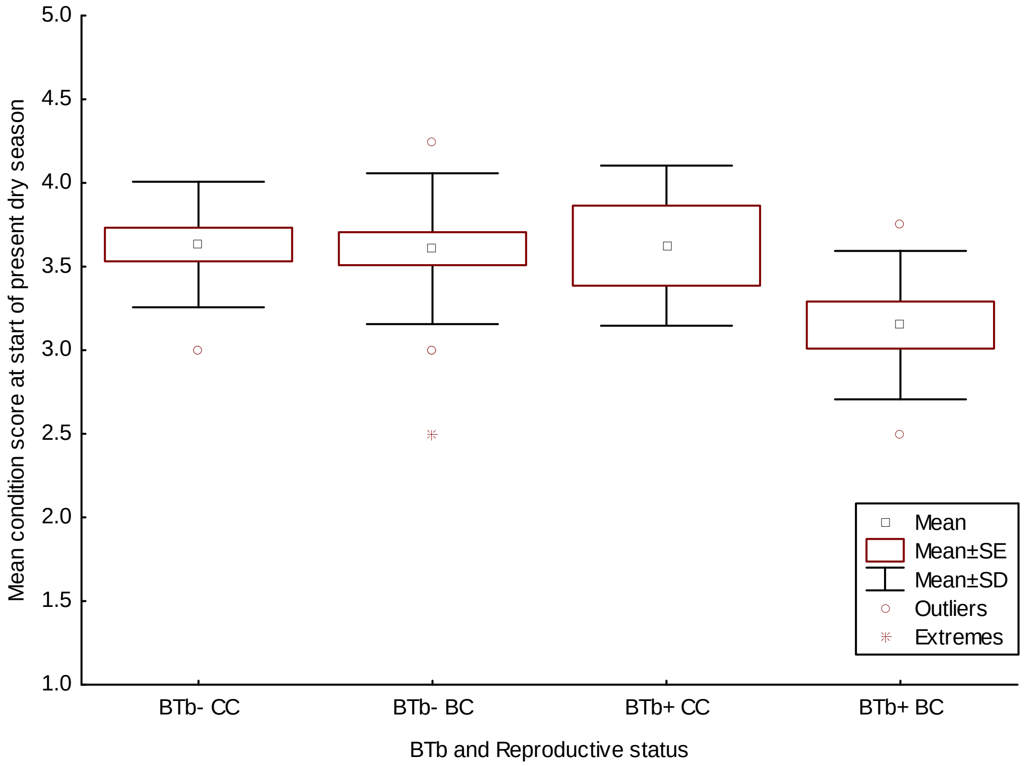


Figure 2: Mean condition scores at the start of the dry season (April-May). BTb positive (BTb+) cows start the dry season in significantly lower condition than BTb negative cows (BTb-), when calved cows (CC) and barren cows (BC) are considered together.

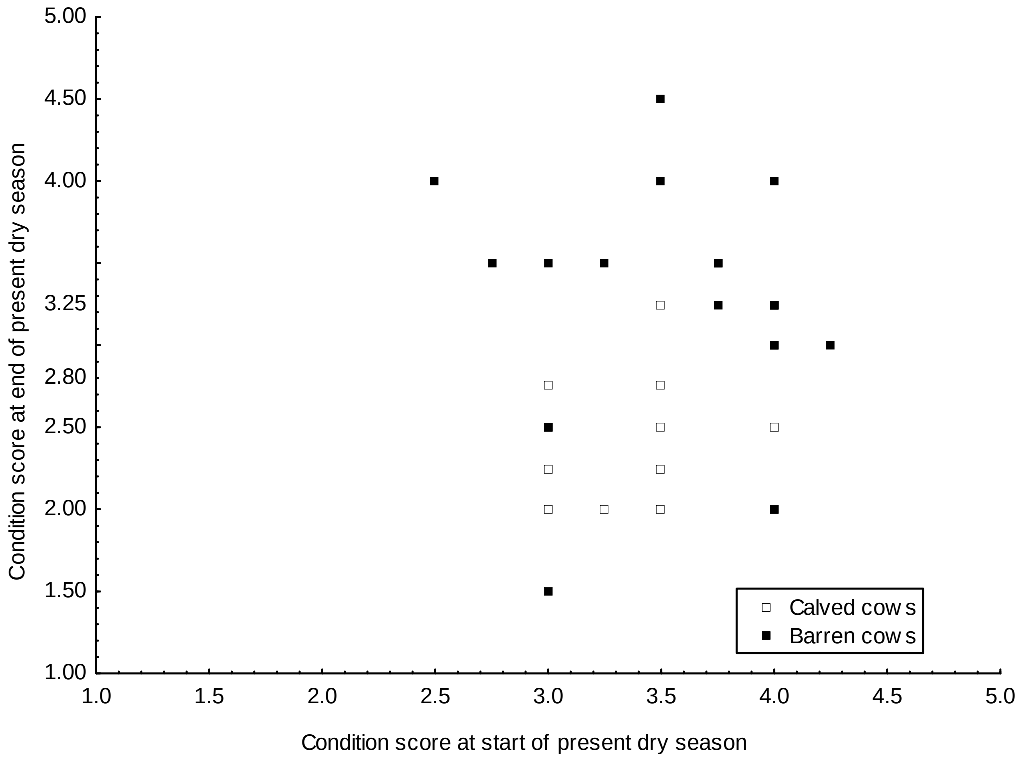


Figure 3: Change in condition over the dry season (April – October) for female buffalo in different reproductive states; all cows start the season in relatively good condition, but calved cows lose significantly more condition over this period than barren cows.

So, females with calves loose more condition, but less BTB positive females have calves than BTB negatives. This may result in no difference in condition between BTB positive and negative adult females.

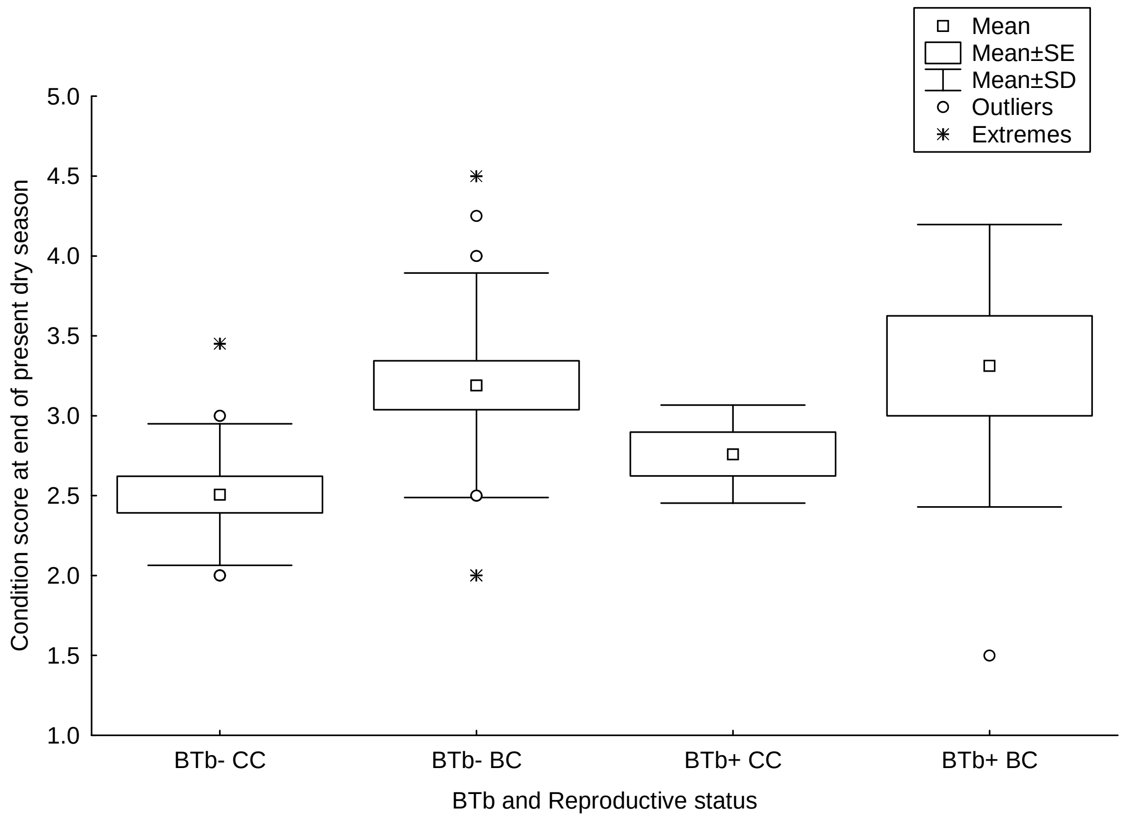


Figure 4: Mean condition scores at the end of the present dry season (September - October). BTb positive (BTb+) and BTb negative (BTb-) cows that had calves (CC) end the dry season in lower condition than barren cows (BC).

i.e. BTB has no effect upon condition

Calf survival

Condition at the start of the present dry season, and BTb status were not significant predictors of calf survival (ANCOVA; $F_{(1,17)}$= 1.96, p= 0.180, $F_{(1,17)}$= 0.631, p= 0.438 respectively). There was also no relationship between calf survival and BTb status or condition at the end of the present dry season (ANCOVA; $F_{(1,17)}$= 0.124, p= 0.281, $F_{(1,17)}$= 0.096, p= 0.761 respectively).

The Final Model

Condition at the start of the present dry season, BTb status, reproductive status in the previous year and the interaction between reproduction last year and BTb status, were not significant predictors for reproductive status in the present year (Generalised linear model, (n=46) p= 0.244, 0.996, 0.905, 0.604 respectively) despite this model showing the least scaled deviance (1.12) (Table 3).

DISCUSSION

Our findings described above did not all agree with the original hypotheses I made. I expected cow age, BTb status and poor condition scores to reduce the reproductive success of female buffalo by reducing reproductive capacity and calf survival during the dry season. The expected and observed interactions are depicted in Fig. 5.

Cow age effects on the population

As with any chronic disease, the impact of infection on individuals is difficult to assess. Previous studies have found that risk of BTb was positively correlated to age in buffalo (De Vos *et al.* 2001; Rodwell *et al.* 2001; Jolles 2004), and cattle (Kazwala *et al.*) although this did not necessarily have effect on the age structure of the herd (Jolles 2004). Caron *et al.* (2003) did not find any effect of BTb prevalence on age structure of buffalo herds, in a sample size of over 3000 animals. I find no evidence of increased prevalence of BTb in older cows. The few studies that have investigated age and reproduction in ungulates have found reduced fecundity in only the oldest cows

Table 3: Results of the final generalised linear model showing BTb, reproductive status last year or condition scores at the start of the present dry season had no effect on reproductive status of cows in the present year.

Effect	Degrees of freedom	Wald Statistic (3 s.f)	p-value (3 s.f)
BTb status	1	1.36	0.244
Reproductive status last year	1	0.000	0.996
Condition at start of present dry season	2	0.199	0.905
BTb status*Reproductive status last year	2	1.012	0.603

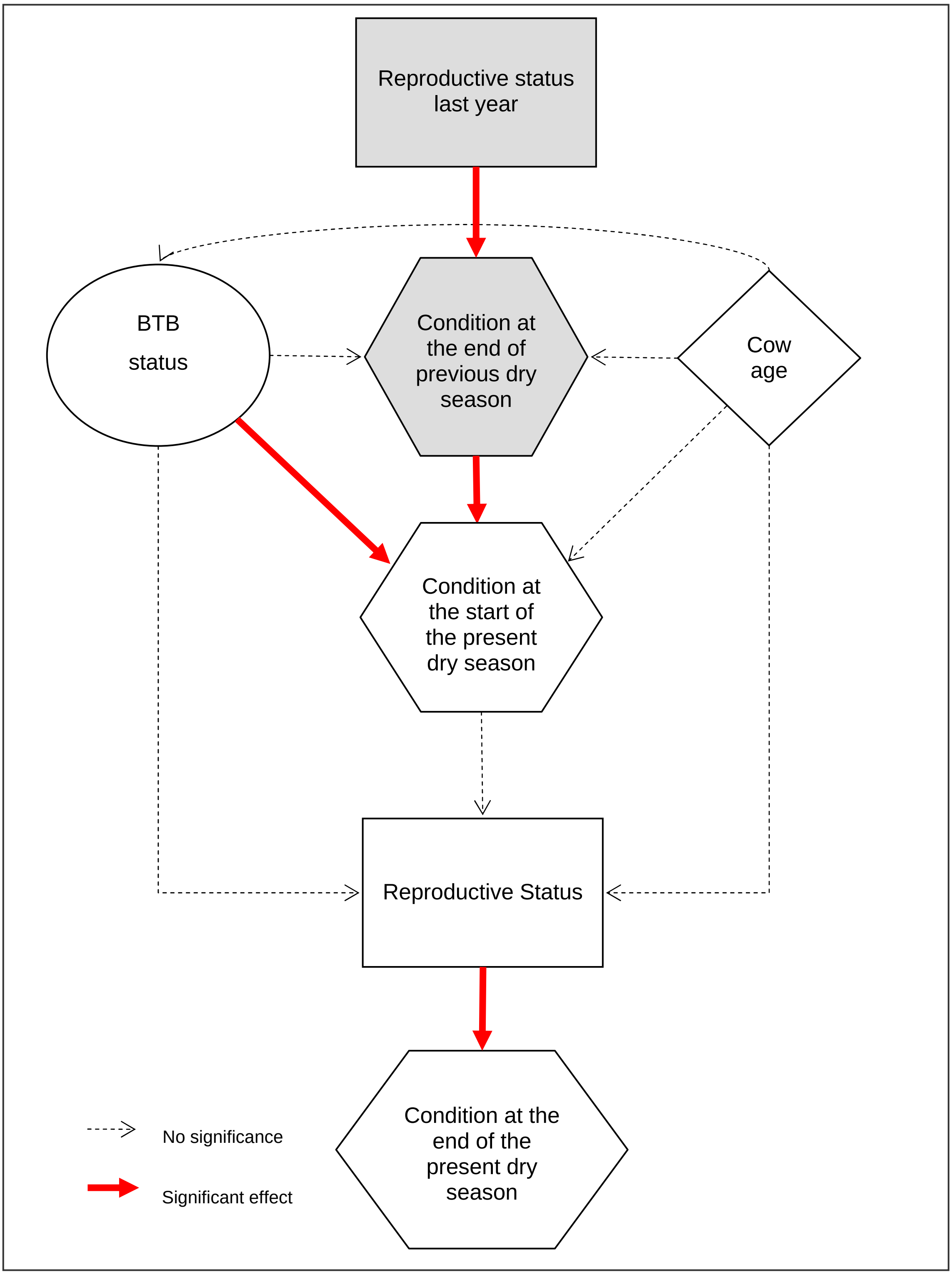


Figure 5: A model of the expected (dashed arrows) and observed (bold arrows) interactions between BTb, condition, age and reproduction in female buffalo ($p<0.05$). Grey boxes indicate factors that are repeated, illustrating the cyclic chain of events from season to season.

(Nichols 1978; Berger & Cunningham 1994; Gaillard, Andersen, Delorme, & Linnell 1998; Gaillard *et al.* 2000). I found no evidence for reproductive senescence in female buffalo, although this could have been because the individuals we saw were not old enough (5-15 years old).

Body condition responses to tuberculosis

Prins (1996) described the cycle of body condition for buffalo throughout the year. In general, animals tend to lose condition over the dry season and gain condition during the wet season. On average, female buffalo did follow this pattern of condition loss and gain. BTb positive cows were less able to regain condition over the nutrient-rich wet season than BTb negative cows (Fig.1), and therefore began the following dry season in poorer condition (Fig. 2). However, I did not find any effect of BTb on change in condition over the dry season. This did not support the findings of Caron *et al.* (2003) that herd-level reduction in condition over the dry season was greater in higher BTb prevalence herds. Possibly, the differences in condition between high and low prevalence herds they observed may have been due to factors other than BTb, such as regional differences in habitat quality.

Although the sample sizes were small, and the ultimate cause is unclear, I suggest that the wet season may be the critical period for buffalo infected with *Mycobacterium bovis.* Tuberculosis is known to reduce appetite in infected individuals, which results in weight loss in domestic cattle (Chaddock 2002). Also, parasitic infection can affect body condition through its influence on host grazing patterns (Gunn & Irvine 2003). Forage near faecal deposits in often highly nutritious but it carries a risk of infection from parasites (Scantlebury, Hutchings, Allcroft & Harris 2004). Adaptive foraging strategies in ungulates can occur as a parasite-avoidance mechanism, such that infected individuals will avoid highly nutritious forage as a trade-off for reduced parasite risk (Hutchings, Kyriazakis & Gordon 2001). A similar behavioural response to *M. bovis* infection may occur in buffalo, with the result that over the wet season, infected individuals avoid the highest quality forage and therefore gain less weight than naïve, uninfected animals.

Body condition and reproductive success

Experiences in early life can alter growth rates of offspring and future reproductive fitness (Grimsdell 1969; Sinclair 1977; Benton *et al.* 2001). Food availability in the year of birth has been shown to affect subsequent breeding success of the calf in female red deer (*Cervus elaphus,* Albon, Mitchell, Huby & Brown 1986). Maternal condition can also have knock-on effects on offspring reproductive success (Benton *et al.* 2001).

I did not find any affect of condition score on calf survival in buffalo, regardless of the mother's BTb status. Other vital rates such as fecundity and fitness of the surviving calves may have been affected, but it was not possible to determine these effects in the context of the present study. Future longer-term investigations may well reveal a link between maternal condition and reproductive success across generations.

In domestic cattle, cows that are seen losing weight at the time of conception are less likely to conceive than those that were gaining weight (Gordon 1996). It has been reported that a condition threshold exists for moose and other ungulates, below which reproduction is inhibited (Stephenson 2003 and references therein). Yet African buffalo mostly gained condition over the period of conception during the wet season, and I found no effect of condition score on reproductive status. On close inspection of the data, CCs at the start of the present dry season had a narrower range of conditions scores between 3.0 and 4.0, while BC conditions ranged from 2.5 to 4.25 (Fig. 3). The majority of buffalo in the current study may well have been able to maintain their body condition above this minimum threshold even when infected with BTb. Those that did not exceed the threshold score remained BCs. If this threshold does exist, an extreme disturbance that reduces herd condition, such as drought, may result in collapse of the buffalo population in Kruger, despite the paucity of any apparent population effects to date. This supposition is in agreement with the conclusions of Jolles (2004). Cows that were in good condition and yet still didn't calve may have been in early pregnancy (and so appear fat), or may have been having a calving interval (Grimsdell 1969) to maintain condition in preparation for conception at the end of the year.

Rearing offspring puts huge energetic demand on a cow and studies have shown that body condition decreases during lactation in African buffalo (Prins 1996), domestic cattle (Markusfeld *et al.* 1997), and domestic buffalo, *Bubalus bubalis* (Gordon 1996). My study confirms their findings showing a greater loss in condition over the dry season for CCs than for BCs (Fig. 3). I expected a greater loss in condition for BTb positive CCs, however found no difference in condition at the end of the dry season compared to BTb negative CCs (Fig 4).

Body condition, reproductive success and BTb infection

The interaction between body condition, disease and reproduction in all animals is complex (Anderson & May 1978). Malnourished animals become immuno-compromised, making them susceptible to disease (Nelson & Demas 2004). Once they are infected, loss of appetite and failure to put on weight during the wet season results in further nutrient deficiencies producing a positive feedback mechanism that allows the parasite to proliferate (Lafferty & Holt 2003; Kristan 2004). The poorer the mother's health, the worse her ability to conceive, gestate or lactate and therefore the lower her reproductive success (Benton *et al*. 2001)

Gestation occurs during the wet season (Sinclair 1977), so infected females that are struggling to maintain or regain their condition during this time will find it even harder to cope with pregnancy than uninfected cows. Thus, if a BTb positive cow is able to give birth, perhaps this is an indication that the disease is in a subclinical phase; she can lactate as normal and hence we see no difference in calf survival. One might expect BTb positive cows that do not manage to give birth are suffering more severely than BTb positive CCs, and the former group would therefore regain less condition over the wet season than the latter. Larger sample sizes are needed to test this prediction although our data does suggest a trend of this sort (Fig 2).

Tuberculosis in cattle can reduce milk yield (Hernandez and Baca 1998), and can contaminate the milk itself (Huchzermeyer *et al.*.1994). Mother to calf transmission of BTb via the milk is one of the most common methods of calf infections (up to 5%) besides the respiratory route (Adlakha & Sharma 1992; Huchzermeyer *et al.* 1994;). Poor maternal body condition and BTb infection are also found to result in loss of milk fat content (Adlakha & Sharma 1992; Markusfeld *et al.* 1997). Thus, the combination of infection and poor condition at the start for the dry season would be expected to reduce reproductive success in BTb positive CCs, through reduction in milk quantity and quality, or by direct infection of the calf with contaminated milk. However, Jolles (2004) found that no buffalo under the age of one-year old had contracted the disease, and we found that calf survival was not affected by BTb infection in CCs.

Rodwell *et al.* (2001) found no difference in occurrence of pregnancy or lactation between BTb negative and positive buffalo cows that were culled between 1991 and 1998. It may be that the culled cows in this study were continuing to lactate despite having lost their calves, or that pregnant females might not have carried their calves to term. Therefore, real affects on reproductive success may have been missed, because late abortions or early calf mortalities could not be identified post-mortem.

I suggest tuberculosis may affect fecundity over a lifetime, although this remains to be shown statistically. When one record per female was used, the number of BTb positive CCs was not significantly smaller than BTb negative CCs, possibly due to small sample sizes (Table 2). Across all females and all records (repeated measures), there was an indication that there may be some difference in reproductive capacity of BTb positive and negative cows (Table 2) but it was not possible to test this statistically due to the assumptions of independent samples in statistical tests. It is interesting to note that of the females for which I did have repeated measures (i.e. consecutive year history), 10 BTb negative buffalo (n= 25) had calves in two consecutive years. In comparison, only one BTb positive female (n=8) produced more than one calf over the four-year study period, with a 25-month calving interval between these. This further suggests that BTb may have some influence on reproduction in the long term. Larger sample sizes across more than one generation may find significant reduction in reproduction due to BTb infection.

Maternal condition effects on reproductive success have been reported for birds (2002; Gorman & Nager 2004), fish (Bagamian, Heins & Baker 2004; Koops, Hutchings & McIntyre 2004), and a variety of mammals including the red kangaroo *(Macropus rufus;* Moss & Croft 1999), moose (*Alces alces,* Stephenson 2003), red deer (Albon *et al.* 1986) and African buffalo (Grimsdell 1969) to name a few. Sex ratios are well documented as being determined by maternal and environmental conditions for many species including ungulates (Trivers & Willard 1974; Sheldon & West 2004), and it may be that disease incidence can also alter sex ratios. BTb infected buffalo may be able to produce offspring, but there may still be a reduction in the fitness of the calves produced. Calf survival in this study did not appear to be affected by the disease but other factors such as poor growth rate, calf sex ratios and future fecundity of the offspring may have all been affected.

Modelling of vaccination efficiency, under the assumption that a competent vaccine exists, has suggested that 70% of calves would need to be inoculated each year in order to reduce the prevalence of BTb to background rates (Cross *et al. In press b*). Abortion rates, calf mortalities and incidence of vertical transmission are important factors that need to be included when calculating the viability of control strategies such as vaccination or culling. Future research should concentrate on these, and other calf parameters such as timing of birth, growth rate and condition, sex, age of weaning, and fecundity in response to BTb prevalence.

CONCLUSIONS

Body condition score at the start of the dry season was influenced by BTb status and the condition of the cow at the end of the previous dry season. Cows that were infected with BTb gained less weight (as inferred by lower condition scores) and therefore started the dry season in poorer condition than uninfected animals. Furthermore, reproductive status during the dry season determined the condition of the cow by the end of the dry season. However, condition of the cow at the start of the dry season did not have any bearing on her reproductive status that year. I suggest a condition threshold for reproduction may exist that was not breached by these animals. Other environmental factors such as drought might have to occur in synchrony with BTb proliferation, in order for any effect of the disease on mortality or reproductive success to be realised (Jolles 2004).

I conclude that fecundity and calf survival may not be affected by tuberculosis in the short term, but that over a breeding lifetime, some reduction in reproductive output in infected females may occur. The only evidence of an impact of the disease on individuals was on condition change during the wet season, thus the costs of reproduction may be critical during gestation and not (as is the case for healthy animals; Sinclair 1977) during lactation.

Other species are more vulnerable and can have much more severe responses to the disease (Keet *et al.* 1996), so there is urgent need for eradication of BTb. Overall, the presence of BTb did not seem to reduce reproductive success in females enough to influence population growth rates under normal environmental conditions. (THIS IS SIMPLY NOT TRUE—With more data you are very likely to find and affect that reduces growth rates but not enough to make them negative unless a serious of drought years is encountered. Thus, BTb is likely to persist as a subclinical chronic disease in buffalo in the absence of human intervention. African buffalo are therefore a reservoir host for bovine tuberculosis.

REFERENCES

ADLAKHA, S.C. & SHARMA, S.N. 1992, Infectious Diseases. In: Tulloh, N.M. & Holmes, J.H.G. (Eds.). Buffalo Production: World Animal Science, C6. (pp. 271-288). Elsevier Science Publishers, Amsterdam.

ALBON, S.D. MITCHELL, B., HUBY, B.J. & BROWN.1986. Fertility in female red deer (*Cervus elaphus*): the effects of body composition, age, and reproductive status. *J. Zool.* 209:447-460.

ANDERSON, R.M. & MAY, R.M. 1978. Regulation and stability of host-parasite population interactions I. Regulatory processes. *J. Anim. Ecol.* 47: 219-247.

BAGAMIAN, K.H., HEINS, D.C. & BAKER, J.A. 2004. Body condition and reproductive capacity of three-spined stickleback infected with the cestode *Schistocephalus solidus. J. Fish Biol.* 64: 1568-1576.

BENGIS, R.G., KRIEK, N.P.J., KEET, D.F., RAATH, J.P., DE VOS, V. & HUCHZERMEYER, H.F.K.A. 1996. An outbreak of bovine tuberculosis in a free-living buffalo (Syncerus caffer) population in the Kruger National Park- A preliminary report. *Onderstepoort J. Vet.* 63:15-18.

BENTON, T.G., RANTA, E., KAITALA, V. & BECKERMAN, A.P. 2001. Maternal effects and the stability of population dynamics in noisy environments. *J. Anim. Ecol.* 70: 590-599.

BERGER, J. & CUNNINGHAM, C. 1994. Bison: Mating and Conservation in Small Populations. Columbia University Press, New York.

CARON, A., CROSS, P. C. & DU TOIT, J.T. 2003. Ecological implications of bovine tuberculosis in African Buffalo herds. *Ecol. Appl*.13:1338-1345.

CHADDOCK, M.H. 2002. Tuberculosis. In: Smith B.P. (Ed.). Large animal internal medicine. 3rd Edition. (pp. 588-589). Mosby Publications, London.

CROSS, P.C., LLOYD-SMITH, J.O. & GETZ, W. M. In press *a*. Disentangling association patterns in fission-fusion societies using African buffalo as an example. *Behav. Ecol.*

CROSS, P.C., LLOYD-SMITH, J.O., BOWERS, J., HAY, C., HOFMEYR, M. &. GETZ, W. M . In press *b*. Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park. *Ann. Zool. Fenn.*

DE LISLE, G.W., BENGIS, R.G., SCHMITT, S.M.& O'BRIEN, D.J. 2002. Tuberculosis in free-ranging wildlife: detection, diagnosis and management. *Rev. Sci. Tech. O.I.E.* 21:317-334.

DE VOS, V., BENGIS, R.G., KRIEK, N.P.J. MICHEL, A., KEET, D.F., RAATH, J.P. HUCHZERMEYER H.F.KA. 2001. The epidemiology of tuberculosis in free-ranging African buffalo (*Syncerus caffer*) in the Kruger National Park, South Africa. *Onderstepoort J. Vet.* 68: 119-130.

FENTON, A. FAIRBAIRN, J.P., NORMAN, R. & HUDSON, P.J. 2002. Parasite transmission: reconciling theory and reality. *J. Anim. Ecol.* 71: 893-905.

GAILLARD, J.M., ANDERSEN, R., DELORME, D. & LINNELL, J.D.C. 1998. Family effects on growth and survival of juvenile roe deer. *Ecology.* 79:2878-2889.

GAILLARD, J.M., FESTA-BIANCHET, M., YOCCOZ, N.G., LOISON, A. & TÖIGO, C. 2000.Temporal variation in fitness components and population dynamics of large herbivores. *Annu. Rev. Ecol. Syst.* 31:367-393.

GORDON, I. 1996. Controlled reproduction in cattle and buffaloes: controlled reproduction in farm animals series, Vol. I. CAB international. Cambridge.

GORMAN, H.E.& NAGER R.G. 2004. Prenatal developmental conditions have long-term effects on offspring fecundity. *Proc. R. Soc. Lond. B.* 271:1923-1928.

GRIMSDELL, J.J.R.1969. Ecology of the buffalo, *Syncerus caffer*, in Western Uganda. PhD. Thesis, University of Cambridge, Cambridge.

GROBLER, D. G., MICHEL, A. L, DE KLERK, L. & BENGIS, R. G.2002. The gamma-interferon test: its usefulness in a bovine tuberculosis survey in African buffaloes (Syncerus caffer) in the Kruger National Park. *Onderstepoort J. Vet.* 69: 221-227.

GUNN, A. & IRVINE, R.J. 2003. Subclinical parasitism and ruminant foraging strategies – a review. *Wildlife Soc. B.* 31:117-126.

HERNANDEZ, J. & BACA, D.1998. Effect of tuberculosis on milk production in dairy cows. *J. Am. Vet. Med. Ass.* 213: 851-854.

HUCHZERMEYER, H.F.K.A., BRÜCKNER, G.K.M., VAN HEERDEN, A.,KLEEBURG, H.H., VAN RENSBURG, I.B.J., KOEN, P. & LOVEDAY, R.K. 1994. Tuberculosis. In: Coetzer J.A.W., Thomson G.R. & Tustin R.C. (Eds), Infectious diseases of livestock with special reference to southern Africa. Vol II. (pp.1425-1428). Oxford University Press, Oxford.

HUTCHINGS, M.R., KYRIAZAKIS, I. & GORDON, I.J. 2001. Herbivore physiological state affects foraging trade-off decisions between nutrient intake and parasite avoidance. *Ecology.* 82:1138-1150.

JOLLES, A.E. 2004. Disease ecology of tuberculosis in African buffalo. Ph.D. Thesis, Princeton University, Princeton.

KAZWALA, R.R., KAMBARAGE, D.M., DABORN, C.J., NYANGE, J., JIWA, S.F.H. & SHARP, J.M. 2001. Risk factors associated with the occurrence of Bovine Tuberculosis in cattle in the southern highlands of Tanzania. *Vet. Res. Commun.* 25:609-614.

KEET, D.F., KRIEK, N.P.J., PENRITH, M.L., MICHEL, A. & HUCHZERMEYER, H. 1996. Tuberculosis in buffaloes (*Syncerus caffer*) in the Kruger National Park: Spread of the disease to other species. *Onderstepoort J. Vet.* 63:239-244.

KOOPS, M.A. HUTHCINGS, J.A. & McINTYRE, T.M. 2004. Testing hypotheses about fecundity, body size and maternal condition in fishes. *Fish Fish.* 5:120-130.

KRISTAN, D.M. 2004. Intestinal nematode infection affects host life history and offspring susceptibility to parasitism. *J. Anim. Ecol.* 73: 227-238.

LAFFERTY. K.D. & HOLT, R.D. 2003. How should environmental stress affect the population dynamics of disease? *Ecol. Lett.* 6: 654-664.

MARKUSFELD, O., GALON, N. & EZRA, E.1997. Body condition score, health, yield and fertility in dairy cows. *Vet. Res.* 141:67-72.

MENZIES, F.D. & NEILL, S.D. 2000. Cattle-to-Cattle transmission of bovine tuberculosis. *Vet. J.* 160: 92-106.

MOE, B.,LANGSETH, I., FYHN, M., GABRIELSEN, G.& BECH, C. 2002. Changes in body condition in breeding kittiwakes *Rissa tridactyla. J. Avian Biol*. 33: 225-234.

MORRIS, R.S., PFEIFFER, D.U. & JACKSON, R. 1994. The epidemiology of Mycobacterium bovis infections. *Vet. Microbiol*. 40: 153-177.

MOSS, G.L. & CROFT, D.B.1999. Body condition of the red kangaroo (*Macropus rufus*) in arid Australia: The effect of environmental condition, sex and reproduction. *Aust. J. Ecol.* 24: 97-109.

NELSON, R.J. & DEMAS, G.E. 2004. Seasonal patterns of stress, disease, and sickness responses. *Curr. Dir. Psychol. Sci.* 13: 198-201.

NICHOLS, L. 1978. Dall sheep reproduction. *J. Wildlife Manage.* 42:570–80.

PIENAAR, U.1969. Observations of developmental biology, growth and some aspects of the population ecology of African buffalo in Kruger National Park. *Koedoe*. 12:29-52.

PIKE, I.L. 1999. Age, reproductive history, seasonality, and maternal body compostition during pregnancy for nomadic Turkana of Kenya. *Am. J. Hum. Biol.* 11: 658-672.

PRINS, H.H.T. 1996. Ecology and behavior of the African buffalo. Social inequality and decision making. Chapman & Hall, London.

RODWELL, T.C., WHYTE, I.J. & BOYCE, W.M. 2001. Evaluation of population effects of bovine tuberculosis in free-ranging African buffalo (*Syncerus caffer). J. Mammal.* 82:231-238.

SCANTLEBURY, M., HUTCHINGS, M.R., ALLCROFT, D.J. & HARRIS, S. 2004. Risk of disease from wildlife reservoirs: badgers, cattle and bovine tuberculosis. *J. Dairy Sci.* 87: 330-339.

SHELDON, B.C. & WEST, S.A. 2004. Maternal dominance, maternal condition and offspring sex ratio in ungulate mammals. *Am. Nat.* 163:40-54.

SINCLAIR, A.R.E. 1977. The African Buffalo. University of Chicago Press, Chicago.

STATSOFT INC. 2001. STATISTICA (data analysis software system), version 6. Http://www.statsoft.com

STEPHENSON, T. 2003. Physiological ecology of moose: nutritional requirements for reproduction with respect to body threshold conditions. Alaska Department of Fish & Game. Federal aid in wildlife restoration research final performance report, 1 July 1997- 30 June 2003. Project 7.16 Juneau, Alaska.

TRIVERS R.L. & WILLARD, D.E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90-92.

VENABLES, W. & RIPLEY, B. 1999. Modern Applied Statistics with S-PLUS, 3rd Edition. Springer-Verlag, Berlin.

VENTER, F.J., SCHOLES, R.J. & ECKHARDT, H.C. 2003. The abiotic template and its associated vegetation pattern. In: Du Toit, Biggs, H.C. & K.H. Rogers (Eds). The Kruger experience; ecology and management of Savanna heterogeneity (pp. 83-129). Island Press, Washington.

WOOD, P. R., AND S. L. JONES. 2001. BOVIGAM™: an in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis.* 81:147-155.