5. IMPACT OF TUBERCULOSIS AND BRUCELLOSIS ON REPRODUCTION

An important effect of bovine pathogens on bison demography may be a reduction in reproduction (Tessaro 1987). Brucellosis in cattle can cause abortion, stillborn calves, and retained placentas (Fraser et al. 1991:668). Of 12 bison exposed experimentally to *Brucella abortus* strain 2308, 6 aborted their fetus 38-65 days post exposure (Davis et al. 1990). Fetal and placental lesions paralleled those found in goats, sheep and cattle. Abortions and post-parturition calf mortality due to brucellosis have been observed in wild populations of bison (Williams et al. 1993; Rhyan et al. 1994, 2001). Tuberculosis may affect reproduction of bison in a direct fashion. Choquette et al. (1961) implicated tuberculosis in the deaths of three bison fetuses. Further, tuberculosis has been shown to affect the reproductive tract of domestic cattle, with as many as 23.8% of cattle with advanced tuberculosis showing uterine lesions (see reviews by Francis 1947; O'Reilly and Daborn 1995). Plum (1924, 1937) reported that abortion in cattle due to tuberculosis takes place late in gestation, 25 of 74 of the aborted fetuses showed no macroscopic tuberculous lesions, and the aborting cow appeared to be otherwise in good health. Tuberculosis may also affect reproduction indirectly. For example, I would expect to see lower pregnancy rates in tuberculosis-positive females if tuberculosis reduces weight gain by diverting energy to immune function or by hindering effective foraging due to debilitation. Further, tuberculosis may increase mortality risk in bison, especially among older animals; such an effect would translate into a shorter life span and reduced lifetime reproduction (Rodwell et al. 2001).

5.1 Methods

5.1.1 Pregnancy and recruitment assessment

Bison were captured and handled as described in chapter 2 in late February and March of 1997-1999. Pregnancy status of female bison was determined by testing for the presence of pregnancy specific protein B (PSPB) (Biotracking, Moscow, Idaho; Haigh et al. 1991). I used a 93% binding criterion in the PSPB test (Noyes et al. 1997). I validated the PSPB test in 2000 by palpating females at the same time as sampling for the PSPB (n = 87). The test correctly identified 69/70 pregnant females (sensitivity = 98.6%) and 11/12 barren females (specificity = 90.5%).

Between June 22 and July 22, 1998 and May 25 and June 30, 1999, I attempted to visually determine if radio-collared bison that were pregnant at capture had a calf at heal. Bison were located using radio-telemetry, and were observed from the air or on foot.

5.1.2 Statistical analysis

I examined the following variables as predictors of pregnancy rate in bison: body condition, age, and population (Delta, Hay Camp, and Nyarling River), as well as tuberculosis and brucellosis status. The immunological response of a bison varies with time since first infection with brucellosis, increasing at the time of first abortion then declining with time (see Cheville et al. 1998). Although the serological tests for *Brucella* infection are highly sensitive and specific (Gall et al. 2000), Roffe et al. (1999) found that a complement fixation titre of 1:40 correlated better with culture results (and therefore with a current rather than previous infection), than a threshold of 1:5. Therefore, I decided to compare pregnancy rates for bison with a complement fixation titre equal or greater than 1:40. Hereafter I refer to bison with a complement fixation titre ≥1:40 as "high titre".

As it was not practical, nor informative, to consider all of the 63 possible models than could be generated by the above variables and two-way interactions, I made an *a priori* decision to restrict the number of terms considered in the AICc analysis. I used backward elimination multiple logistic regression (SPSS 10.05, Chicago, IL) to determine which four terms (main effects or two-way interactions) had the highest value in predicting pregnancy rate. Independent variables were sequentially removed from the global model at $p > 0.10$. Olden and Jackson (2000) showed that with large sample sizes ($n = 60$), backward elimination multiple regression was among the least biased in selecting models of multiple regression. The bias was to include "extra" variables rather than miss significant parameters; thus I believe this approach guards effectively against a type I error. I used the small sample-size corrected Akaike information criteria (AICc) to rank models of pregnancy rate in bison (STATISTICA 5.5, Tulsa, OK), and calculated model-averaged confidence intervals to estimate the effect of each parameter on pregnancy rate (Burnham and Anderson 1998: 46-48, 123-140; Anderson et al. 2000). Results are presented as odds ratios and confidence intervals to facilitate interpretation of data. Model fit was assessed using likelihood ratio tests (Sokal and Rohlf 1995: 686-697).

5.2 Results

The overall pregnancy rate of female bison ≥2 years was 72.2% (n = 205). All but the following terms were excluded by the backward elimination multiple regression: body condition, tuberculosis status, an interaction between tuberculosis and high brucellosis titre, and an interaction between population and tuberculosis status (remaining variables and interaction terms $p > 0.10$). The latter interaction indicated that tuberculosis status may affect pregnancy rate differently among the populations; and further examination of the data indicated the effect of tuberculosis on pregnancy was likely different in the Nyarling River population than in the Hay Camp (Wald Statistic 3.30, df = 1, p = 0.07) and Delta (Wald Statistic 3.11, df = 1, p = 0.08) populations, but the effect was similar in the Delta and Hay Camp (Wald Statistic 0.001, df = 1, p = 0.98). Therefore, I repeated the analysis for Nyarling River separately from a pooled sample involving Delta and Hay Camp bison.

The most parsimonious model of pregnancy in the Delta and Hay Camp populations included body condition and an interaction between tuberculosis and brucellosis (Table 5.1). Bison that were in good body condition were 1.7 times more likely to be pregnant than bison in poor body condition (95% confidence interval, 1.2 - 2.4). Odds of pregnancy for tuberculosis-positive bison did not differ from that for tuberculosis-negative bison (odds ratio 1.0, 95% confidence interval 0.9 - 1.2), and I was

Table 5.1 Comparison of models of pregnancy rate in female bison in the Hay Camp and Delta populations of WBNP (n = 167). The χ^2 and p-value refer to the likelihood ratio goodness of fit test. Relative AIC_c is presented as Δ_i, and the Akaike weight (ω_i) refers to the probability that the model is the Kullback-Liebler best model, given the data (see Anderson et al. 2000).

Model[a]	df	χ^2	p	Δ_i	ω_i
Body[b], br*tb[c]	2	14.07	0.001	0.00	0.52
Body, tb, br*tb	3	14.08	0.003	2.09	0.18
Body, tb	2	11.26	0.004	2.81	0.13
Body	1	9.19	0.002	2.80	0.13
Br*tb	1	5.59	0.02	6.41	0.02
Tb, br*tb	2	5.59	0.06	8.48	0.01
Tb	1	1.95	0.16	10.04	0.00

[a] model-averaged odds ratios (95% CI): body condition, 1.69 (1.17 - 2.44); tb, 1.04 (0.90 - 1.20); br*tb, 0.74 (0.55 - 1.00).

[b] body condition score (see section 2.1).

[c] brucellosis: complement fixation titre ≥ 1:40; tuberculosis: FP ≥ 174 mp and/or caudal fold test positive. Br*tb indicates bison that test positive for both diseases.

unable to detect a difference in pregnancy rate between bison with low (< 1:40) or high complement fixation titre for brucellosis (Wald Statistic 0.13, df = 1, p = 0.72). However, bison that tested positive for tuberculosis and had a high titre for brucellosis were 0.7 times as likely to be pregnant (95% confidence interval, 0.6 - 1.0; Figure 5.1) than bison with one or neither pathogen.

The most parsimonious model of pregnancy in Nyarling River bison suggests that body condition, tuberculosis status, and an interaction between tuberculosis and brucellosis are important factors determining pregnancy (Table 5.2), although there was not clear separation among the first four models in terms of predictive power. Tuberculosis status was a strong predictor of pregnancy in these bison, where positive bison were 0.26 times as likely to be pregnant as negative bison (95% confidence interval, 0.08 - 0.82; Figure 5.2). Bison that tested positive for both pathogens also were less likely to be pregnant although the odds ratio did not differ from one (odds ratio 0.61, 95% confidence interval, 0.31 - 1.23). This seemingly contradictory result is an artifact of sample size and the older age distribution of female bison in the Nyarling River population sample.

Sixty-three radio-collared bison were pregnant at capture in 1998 and 1999. Of these, I was able to determine that 16 of 41 bison had a calf at heel in early summer (median date 27 June, range 28 May - 19 July), indicating a minimum summer recruitment rate of 39% (95% confidence interval, 26% - 56%; Figure 5.3).

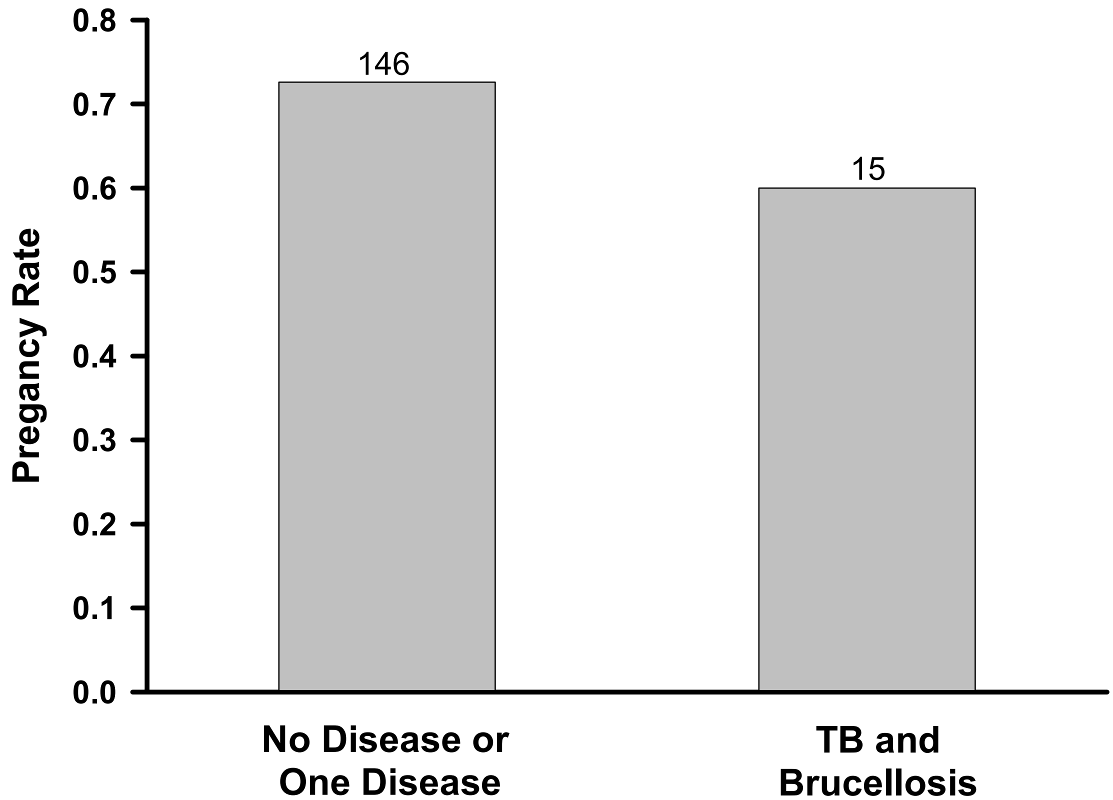

Figure 5.1. Pregnancy rate in relation to disease status for bison in the Hay Camp and Delta populations. Sample size is indicated at the top of each bar.

Table 5.2. Comparison of models of pregnancy rate in female bison in the Nyarling River population of Wood Buffalo National Park (n = 36). The χ^2 and p-value refer to the likelihood ratio goodness of fit test. Relative AIC_c is presented as Δ_i, and the Akaike weight (ω_i) refers to the probability that the model is the Kullback-Liebler best model, given the data (see Anderson et al. 2000).

Model[a]	df	χ^2	p	Δ_i	ω_i
Body, tb, br*tb	3	10.65	0.01	0.00	0.30
Body, tb	2	7.36	0.03	0.37	0.25
Tb	1	5.42	0.02	0.69	0.22
Tb, br*tb	2	7.59	0.02	1.06	0.18
Body	1	0.68	0.41	5.04	0.02
Br*tb	1	0.12	0.73	5.99	0.02
Body, br*tb	2	0.85	0.65	6.87	0.01

[a] model-averaged odds ratios (95% CI): body condition, 1.63 (0.80 - 3.35); tb, 0.26 (0.08 - 0.82); br*tb, 0.61 (0.31 - 1.23).

[b] body condition score (see section 2.1).

[c] brucellosis (br): complement fixation titre ≥ 1:40; tuberculosis (tb): FP ≥ 174 mp and/or caudal fold test positive. br*tb indicates bison that test positive for both diseases.

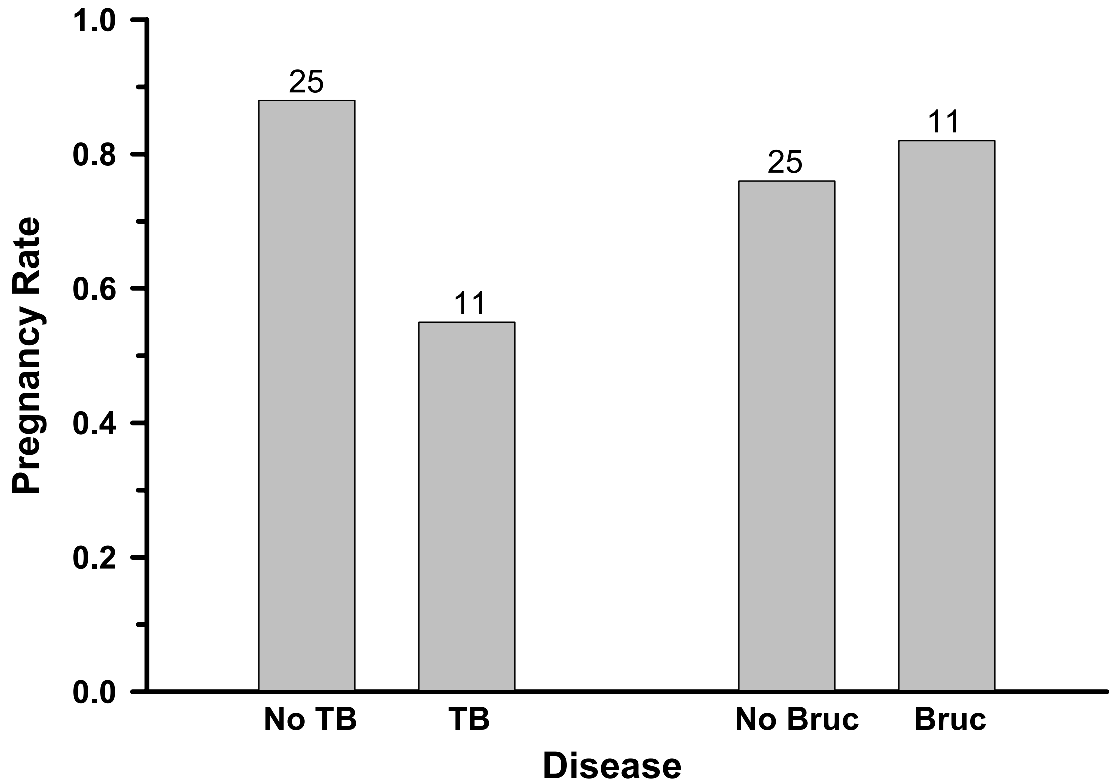

Figure 5.2. Pregnancy rate in relation to disease status for bison in the Nyarling River population. Sample size is indicated at the top of each bar.

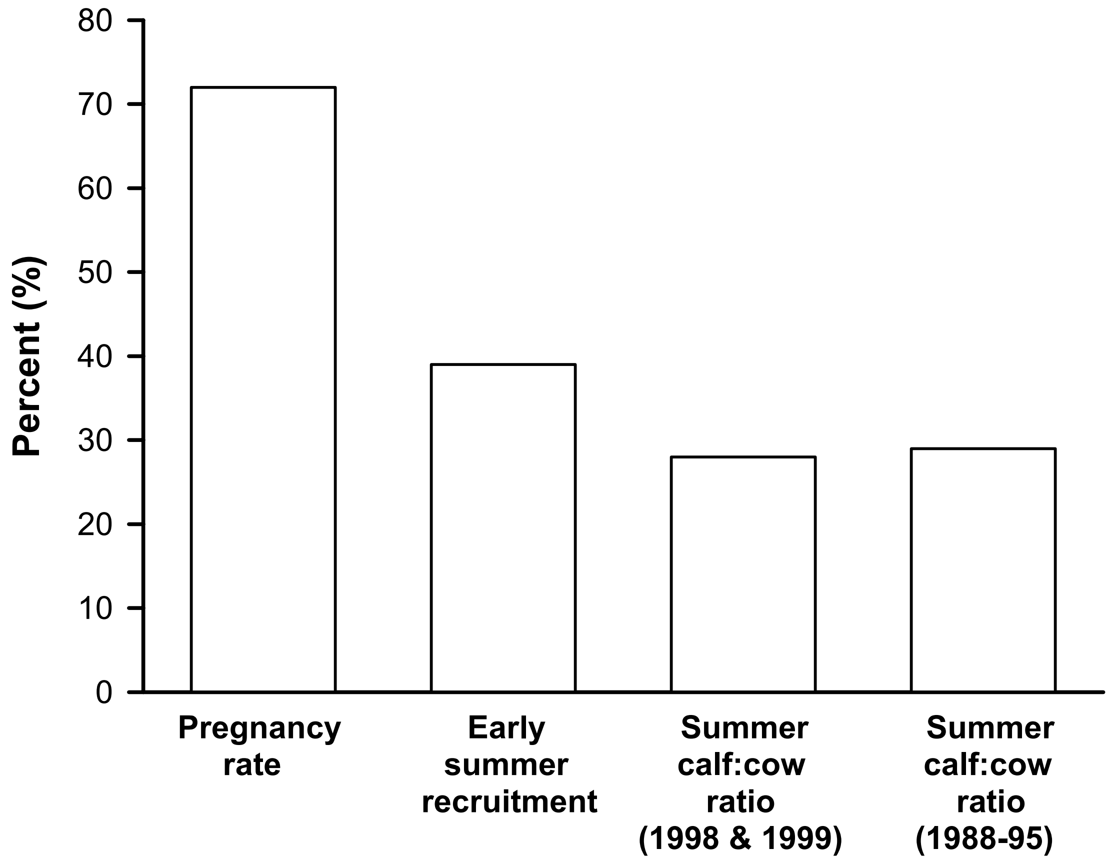

Figure 5.3. Reproductive parameters for radio-collared bison in Wood Buffalo National Park (>2 years of age, 1999). Data for 1988-95 are from Carbyn et al. (1998).

5.3 Discussion

The effect of tuberculosis on pregnancy rate was stronger in the Nyarling River population than in the Hay Camp and Delta populations, after controlling for body condition. I believe that this was a consequence of the lower autumn and winter survival rates in the Hay Camp and Delta populations (see below), and the inability to distinguish between bison with generalized tuberculosis and those that test-positive but have yet to develop pathological signs. Reduced survival in autumn and winter associated with tuberculosis would result in a decrease in the proportion of test-positive bison that have generalized tuberculosis over the winter. If bison with generalized tuberculosis have a lower pregnancy rate, the average pregnancy rate for surviving test-positive bison would increase over the winter, thus decreasing the ability to detect an effect. However, winter survival rates in the Nyarling River population were significantly higher, likely as a consequence of reduced wolf predation. Consequently, I speculate that the effect of the pathogen on pregnancy may have been more evident in the Nyarling River area as more bison in later stages of infection could survive the winter, related to less predation by wolves.

Previous surveys in WBNP have failed to detect an effect of tuberculosis on pregnancy in bison. For example, Fuller (1962) found in the Hay Camp area that 51% of 331 "healthy" cows were pregnant relative to 55% of 323 tubercular cows. Similarly, in the Peace-Athabasca Delta, 66% of 95 apparently tuberculosis-free cows were pregnant relative to 81% of 26 tubercular cows. It is difficult to explain why these results conflict with mine. The simplest explanation is a spurious correlation with a variable I have not controlled for. The sum of the Akaike weights for models including tuberculosis is 0.95 (Table 5.2), indicating very strong support for inclusion of this factor among the models examined, but this does not preclude existence of a lurking variable. Methodological differences may also be responsible for the conflicting results. First, Plum (1924, 1937) reported that tuberculosis induces abortion in cattle in late gestation. Fuller (1962) conducted his examinations during December and January slaughters. If the effect of tuberculosis on bison reproduction mirrors that in cattle as it does in other aspects (e.g., Tessaro et al. 1990), then the timing may have been premature to detect an effect. It is important to note that Plum (1924, 1937) noted that 25 of 74 of aborted fetuses showed no macroscopic lesions and the cows otherwise appeared to be in good health. In any case, experimental studies of the effect of tuberculosis on reproduction of bison would aid in clarifying this relationship.

I did not detect a main effect of brucellosis on pregnancy. This result is not surprising as these data reflect pregnancy rates before the period in which *Brucella*-induced abortions are expected to occur in bison (Williams et al. 1993; Rhyan et al. 1994), although some abortions do occur earlier (Rhyan et al. 2001). Further, I determined pregnancy by measuring levels of pregnancy-specific protein B, which has been shown to have a half-life post-calving or abortion of 7-8 days in dairy cattle (Semambo et al. 1992; Kiracofe et al. 1993). Bison that aborted shortly before capture may have been misclassified as pregnant. Although I did not find a main effect of brucellosis, bison in the Hay Camp and Delta populations who had a high brucellosis titre and tuberculosis were less likely to be pregnant. Perhaps bison with tuberculosis infection are more likely to suffer brucellosis-induced abortion during winter as a result of overall weakened immune function. Alternatively, as the effect of tuberculosis on pregnancy in the Nyarling River population exceeded the effect of brucellosis, perhaps the presence of brucellosis increases the risk of tuberculosis-induced reproductive failure. Further research may be necessary to elucidate the potential for an interactive effect of tuberculosis and brucellosis on reproductive success of bison.

I was able to determine that 16 of 41 pregnant cows had a calf at heel in early summer (May 25), indicating a minimum summer recruitment rate of 39% (95% confidence interval, 26% - 56%; Figure 5.3). This recruitment rate takes into account possible sources of mortality such as predation, starvation, late-term abortions, and drowning. From this figure I can estimate the calf: cow ratio for early summer to be 28:100 (number of pregnant cows per 100 cows times number of calves at heal in summer per 100 formerly pregnant cows). This value is consistent with the average calf: cow ratio for Wood Buffalo National Park from 1988-1995 (29:100 cows, Wood Buffalo National Park unpublished data; 32:100; Carbyn et al. 1998).

Although I cannot specifically evaluate the effect of brucellosis and tuberculosis on calf recruitment due to small sample sizes, I can compare WBNP calf: cow ratios to other northern bison populations. The Mackenzie Bison Sanctuary, NT bison population (MBS) grew from an introduced population of 16 bison in 1963 to approximately 2400 bison in 1989 and is regulated by density-dependent dispersal and the availability of high quality forage (Larter et al. 2000). The Mink Lake, NT bison population (ML) originated in the mid-1980s when bison migrated from MBS, and increased in population size to 824 by 1998 (Larter et al. 2000). Neither of these bison populations is currently infected with brucellosis or tuberculosis (Tessaro et al. 1993). The most recent summer segregation surveys in MBS and ML indicate calf: cow ratios of 44.0 (± SE 3.0) and 58.2 (± SE 5.5) per 100 cows respectively (Larter et al. 2000). The calf: cow ratio estimated from the present study (28 calves per 100 cows) was significantly lower than the ratio in ML (Test for proportions, $Z = 3.2$, $p = 0.001$; Zar 1996:552-554) and likely lower than in the Mackenzie Bison Sanctuary ($Z = 1.8$, $p = 0.07$; sample sizes: ML = 141, MBS = 507; J. Nishi, Department of Renewable Resources, Wildlife, and Economic Development, Government of the Northwest Territories, personal communication). It is important to note that juvenile bison formed the bulk (~75%) of wolf kills in MBS and a significant portion (~35%) in ML (Larter et al. 1994), comparable to that reported by Carbyn et al. (1993) for the Hay Camp and Delta populations of WBNP. Thus the reduced calf: cow ratio in WBNP relative to *Mycobacterium*- and *Brucella*-free populations cannot strictly be attributed to differences in wolf predation.