4. FACTORS AFFECTING PREVALENCE OF TUBERCULOSIS AND BRUCELLOSIS IN WBNP

The prevalence of introduced bovine pathogens in bison in WBNP has not been evaluated in a systematic manner. Tuberculosis and brucellosis prevalence levels were determined during bison slaughter operations (Choquette et al. 1961; Choquette et al. 1978; Fuller 1962) or on opportunistically sampled bison (Tessaro et al. 1990). These estimates of prevalence were questioned during Federal Environmental Assessment Review Office hearings in the late 1980s (e.g., Ferguson 1989), in particular whether these data were representative of the bison populations in WBNP (Ferguson and Burke 1994). Numbers of bison in WBNP have declined drastically since the 1960s, and so prevalence levels may have declined in response. Dobson and Meagher (1996) proposed a non-linear, density-dependent relationship between bison numbers and brucellosis prevalence. McCarty and Miller (1998) argued that tuberculosis prevalence in white-tailed deer might be a function of density. In contrast, Rodwell et al. (2001) found that tuberculosis prevalence in African buffalo (*Syncerus caffer*) did not vary among regions in Kruger National Park, South Africa, despite variation in population sizes among regions. It is not known if tuberculosis prevalence in bison is a function of density or population size.

Herein, I present the results of tuberculosis and brucellosis testing of bison in WBNP during February and March of 1997-1999. I was particularly interested in

whether the marked decline of bison numbers in the Delta population relative to other bison populations in WBNP was the result of increased pathogen prevalence. Specifically, I test whether prevalence is higher in the Delta than elsewhere in WBNP.

4.1 Methods

4.1.1 Blood sampling

Bison were captured and handled as described in section 2.1. Blood samples were taken from each bison from the caudal or carotid vein during the first handling period (section 2.1.2). Blood was collected in serum separator tubes to facilitate clotting, and prevented from freezing. Serum was removed by centrifuge within 12 hours, and serum samples were frozen until serological tests were conducted.

4.1.2 Testing for brucellosis

Two terms describe the ability of a test to correctly diagnose infection status correctly. A "specific" test will have few "false positives", while a "sensitive" test will have few "false negatives". The standard procedure for brucellosis testing in cattle is to use the buffered plate antigen test (BPAT) as a screening test, and the complement fixation test (CFT) as the confirmatory test for sera that agglutinate in the BPAT (Nielsen et al. 1996). A positive result in the BPAT is determined by visual confirmation of complete or partial agglutination between antigen and test serum on a glass plate, buffered to a pH of 4.1 (MacMillan 1990). Sera that agglutinated in the BPAT and agglutinated in the CFT at dilutions of 1:5 or more were considered

seropositive (Table 4.1; Joly et al. 1998). Testing was done at the Animal Disease Research Institute, Lethbridge, Alberta (Animal and Plant Health, Canadian Food Inspection Agency).

4.1.3 Testing for tuberculosis

I tested for tuberculosis using the caudal fold test with PPD tuberculin (Thoen et al. 1988; Monaghan et al. 1994). Bison were injected with 0.1 mL of PPD tuberculin intradermally in the caudal fold. A veterinarian (T. Shury) accredited by the Canadian Food Inspection Agency conducted the tuberculosis test. He inspected the injection site after 72 hours to determine pathogen exposure status (Monaghan et al. 1994). Bison were classified as positive, negative, or suspicious reactors, based on the degree of reaction at the injection site. As recently infected animals or those in the latter stages of infection may not react to the injection of tuberculin (e.g., Griffin and Buchan 1994), I supplemented the caudal fold test with the fluorescent polarization assay for tuberculosis (Lin et al. 1996). Lin et al. (1996) did not provide interpretation criteria for the fluorescent polarization assay. To determine the diagnostic threshold for the fluorescent polarization assay, I examined the millipolarization units (mp) values for 85 bison sera from Elk Island National Park, Canada (Om Surujballi, Canadian Food Inspection Agency, Nepean ON, unpublished data), a known tuberculosis-free herd. As the distribution of mp values was normal (Kolmogorov-Smirnov test, D max = 0.06, p = 0.67), I rearranged the formula for comparison of a single observation against a sample

Table 4.1 Criteria for determining status of bison sera with respect to exposure to *Brucella abortus*.

	Test	
Status	Complement fixation (Dilution Ratio)	Buffered plate antigen
Positive	1:10 - 1:1280	+ or -
Positive	1:5	+
Negative	1:5	-
Negative	0	+ or -

distribution (equation 9.5 in Sokal and Rohlf 1995) to determine the maximum mp value that would be consistent with a distribution of negative samples (one-tailed alpha = 0.05); any mp value greater than this threshold would have a low probability ($p < 0.05$) of not being infected with tuberculosis. Bison that tested positive on either the caudal fold or the fluorescent polarization tests were considered positive.

4.1.4 Statistical analysis

Factors affecting prevalence were determined using logistic regression analysis (Sokal and Rohlf 1995:767-776). Model selection was based on small sample size corrected Akaike Information Criteria, and parameter estimates were averaged among all possible models weighted by Akaike weights (Burnham and Anderson 1998: 119-140; see methods in section 2.1). The variables age, sex, and population were examined as possible factors affecting prevalence. Analysis was first conducted using age as a continuous variable; however, when preliminary analysis indicated nonlinearity in the relationship I grouped bison into the following age classes and entered age as a categorical variable in the model: <1, 1, 2, 3-5, 6-10, 11-19. I found in a preliminary analysis an interaction between age and sex for both pathogens, therefore I elected to conduct analyses on each sex separately. I did not include the variable "body condition" in this analysis as I felt that the causal relationships between infection status and body condition are not clear. Further, at least in the case of tuberculosis, evidence from cattle suggests that infection by *Mycobacterium bovis* is independent of body condition (Costello et al. 1998; Doherty et al. 1996). I am not aware of any data on the effect of

body condition on the risk of infection by brucellosis in bison. I did not include in the analysis test results from the second capture if a bison was caught in more than one year.

4.2 Results

4.2.1 Brucellosis prevalence

Overall, 30.9% (107/346) bison were seropositive for brucellosis. Sera that were anti-complementary (i.e., that reacted to the addition of complement in the absence of antigen) in the complement fixation test were excluded (n = 11). Inclusion of these sera drastically alters the sensitivity and specificity of this test, depending whether they are treated as positive or negative (see Gall et al. 2000).

Initial analysis indicated that brucellosis seroprevalence was a function of sex and age (Table 4.2), where prevalence increased with age and females were more likely to test positive than males (male vs. female odds ratio 0.70; 95% confidence interval 0.52-0.95). A model including sex and age as main effects as well as their interaction provided a reasonable compromise between bias and variance relative to the best model ($\Delta_i = 1.49$), although the odds ratio for the interaction term did not differ from one (Table 4.2).

Sex-specific analyses indicated that age was a good predictor of seroprevalence in males (Table 4.3). Few males <2 years of age were seropositive compared to females of the same age, yet after 2 years of age seroprevalence rose dramatically among males (Figures 4.1 and 4.2). There appeared to be a bimodal relationship between age and seroprevalence in males; males 2 - 5 years had high seroprevalence, then seroprevalence

Table 4.2 Comparison of models of brucellosis prevalence in male and female bison in WBNP (n = 346). 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
Sex, age	2	20.78	0.00	0.00	0.58
Sex, age, sex*age	3	21.34	0.00	1.49	0.27
Age	1	14.50	0.00	4.25	0.07
Age, sex*age	2	16.05	0.00	4.73	0.05
Sex, sex*age	2	12.56	0.00	8.22	0.01
Sex	1	10.31	0.00	8.44	0.01
Sex*age	1	10.25	0.00	8.50	0.01

[a] model-averaged odds ratios (95% CI): sex, 0.70 (0.59 - 0.83); age, 1.08 (1.01 - 1.16); sex*age, 1.00 (0.98 - 1.03).

Table 4.3 Relationship between age and brucellosis prevalence in males (n = 94). Odds ratios refer the increase in probability of testing positive for brucellosis relative to the youngest age class (e.g., male bison in the 3 - 5 year age class are 40.26 times more likely to test positive for brucellosis than male bison in the < 1 year age class).

Age Class	Odds Ratio	95% Conf. Int.	Wald[a]	p
< 2 y		Reference category		
2 y	48.22	4.08 - 569.81	9.46	0.002
3 - 5 y	40.26	3.89 - 416.72	9.60	0.002
6 - 10 y	4.82	0.269 - 86.29	1.14	0.29
11 - 14 y	45.00	3.73 - 543.1	8.97	0.003

[a] df = 1

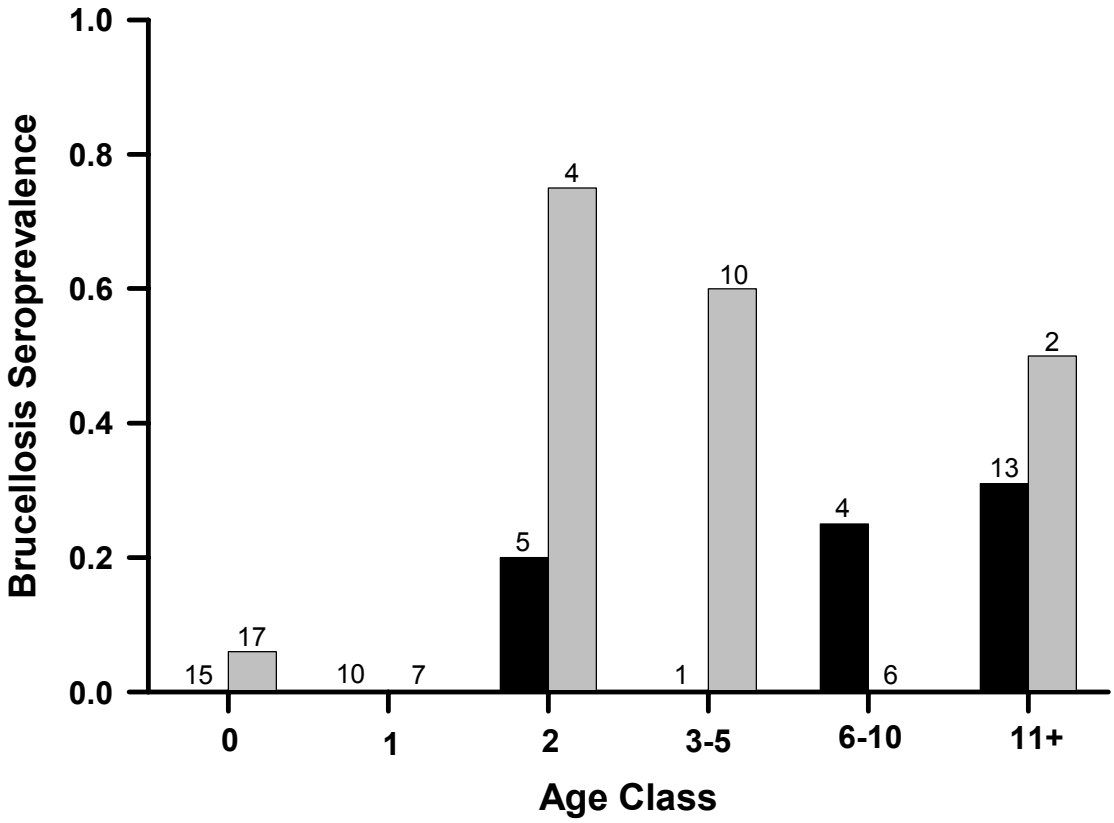

Figure 4.1. Prevalence of brucellosis in male bison from the Delta (dark bar) and Hay Camp (shaded bar); sample size is indicated at the top of each bar.

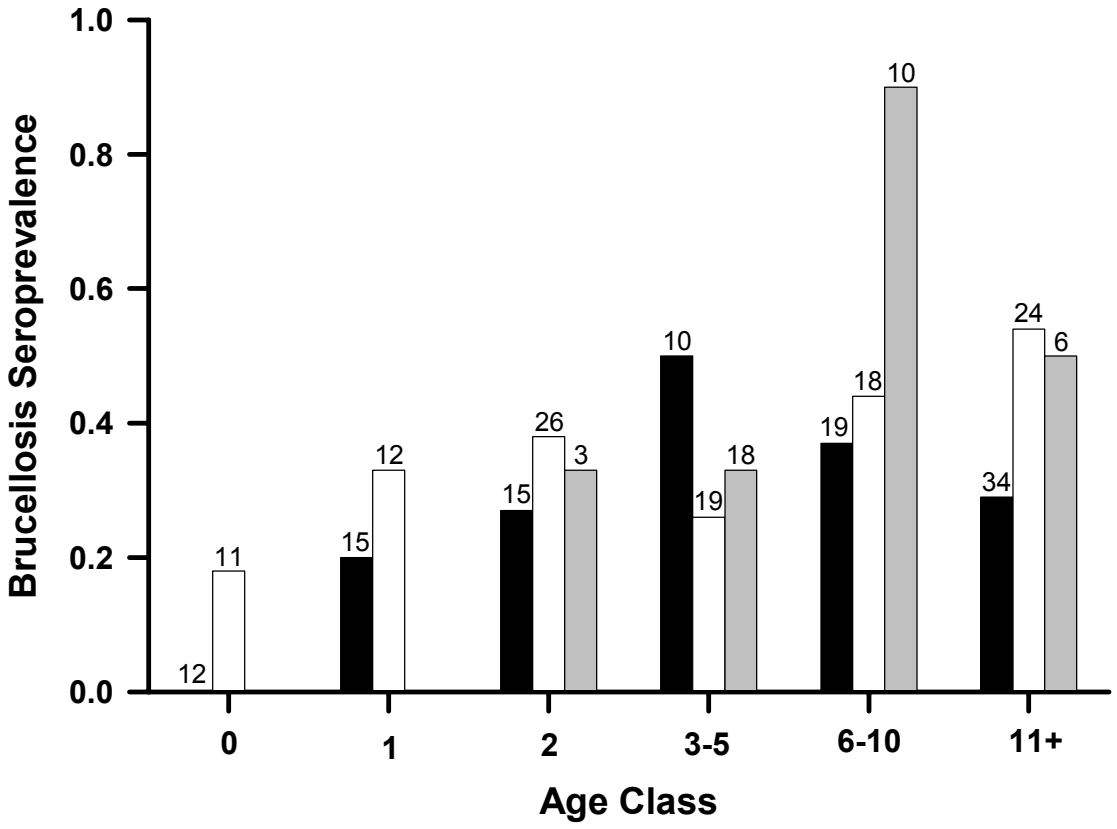

Figure 4.2. Prevalence of brucellosis in female bison from the Delta (dark bar), Hay Camp (white bar) and Nyarling River (shaded bar); sample size is indicated at the top of each bar.

declined in 6 - 10 year olds before increasing again in males >11 years of age (Table 4.3; Figure 4.1).

Brucellosis seroprevalence in females increased with age in the Delta population, but this effect was not detectable in either the Hay Camp or the Nyarling River populations (Table 4.4). Female bison in the Hay Camp region were 1.5 times more likely to be seropositive than in the Delta after controlling for the effects of age (95% confidence limits, 1.1 - 2.0; Figure 4.2). As I did not test females <2 years of age in the Nyarling River population, and only three 2 year olds were tested there, I repeated the analysis including only females >2 years of age and all three populations. After controlling for age, females in the Nyarling River were more likely to be seropositive than those in the Delta population (odds ratio, 1.7, 95% confidence interval, 1.0 - 2.7) but not the Hay Camp population (odds ratio, 1.0, 95% confidence interval, 0.6 - 1.6).

4.2.2 Tuberculosis prevalence

I examined the distribution of the fluorescent polarization assay results for bison from Elk Island National Park to determine a criterion for determining tuberculosis-positive status in the WBNP samples. The mean mp for these tuberculosis-negative bison was 166.5 (SE 0.48, n = 85), and less than 5% of a distribution of negative bison would have a mp result greater than 174 mp. All Elk Island National Park bison had sera with mp values less than this threshold. Therefore, I used the criteria of >174 mp to identify tuberculosis-positive bison based on the fluorescent polarization assay.

Forty-nine percent of bison tested in late winter of 1997, 1998, and 1999 tested positive on either the caudal fold test or the fluorescent polarization assay (n = 342).

Table 4.4 Relationship of age to brucellosis seroprevalence for females in the Delta and Hay Camp populations (n = 215). There was no effect of age on brucellosis seroprevalence for females 2 years and older in the Nyarling River population (Wald Statistic 5.18, df = 3, p = 0.15).

	Odds Ratio (95% Confidence Interval)[a]	
Age Class (y)	Delta	Hay Camp
<1		Reference Category
1[b]	Reference category	2.25 (0.32 - 15.76)
2	1.92 (0.24 - 15.26)	2.81 (0.50 - 15.77)
3-5	8.33 (1.23 - 56.67)	1.61 (0.26 - 10.14)
6-10	7.29 (1.31 - 40.53)	2.86 (0.47 - 17.35)
>11	4.50 (0.88 - 22.95)	5.32 (0.94 - 30.0)

[a] odds ratios refer to the increase in odds of a female bison in a particular age class testing positive for brucellosis relative to the youngest age class (<1 year for Hay Camp and <2 year for Delta).

[b] Delta 1 year age class includes 12 seronegative females <1 year of age as none in that age class tested positive.

Tuberculosis prevalence was a function of age, and prevalence increased faster for males than females (Table 4.5). Sex-specific analyses indicated that age was the predominant factor associated with tuberculosis prevalence in males, with the probability of testing positive for tuberculosis increasing 1.26 times for each additional year of age (95% confidence interval, 1.03-1.54; Table 4.6). There was some indication that tuberculosis prevalence may increase with age faster in the Hay Camp area, and that males in general may have a higher tuberculosis prevalence in the Hay Camp population, although neither odds ratio differed from one (Table 4.6). The most parsimonious model predicted that tuberculosis prevalence in males increased to almost 70% by the age of nine (Figure 4.3).

The most parsimonious model of tuberculosis prevalence in females indicated that prevalence varied as a function of age, population, and an interaction between age and population (Table 4.7). Prevalence in Hay Camp females was higher than Nyarling River, which in turn was higher than Delta (Table 4.7). Prevalence increased with age for females, with the probability of testing positive increasing 1.1 times with each year of age (95% confidence interval, 1.00 - 1.2); however this effect of age was not similar among the populations (Table 4.7). I repeated the analysis on separate populations to facilitate interpretation of these data, including age as a categorical variable (Figure 4.4). Tuberculosis prevalence in females rose with age in both Delta and Hay Camp populations (Hay Camp Wald Statistic 14.3, df = 5, p = 0.01; Delta Wald Statistic 19.51, df = 5, p = 0.002). Hay Camp population females in the 11-19 year age class had lower tuberculosis prevalence than those in the 6-10 year age class (Wald Statistic 7.26, df = 1,

Table 4.5 Comparison of models of tuberculosis prevalence in male and female bison in WBNP (n = 351). 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
Sex, age, sex*age	3	46.93	0.00	0.00	0.54
Age, sex*age	2	44.55	0.00	0.34	0.46
Age	1	32.65	0.00	10.20	0.00
Sex, age	2	33.82	0.00	11.06	0.00
Sex*age	1	0.65	0.42	42.21	0.00
Sex	1	0.08	0.77	42.77	0.00
Sex, sex*age	2	0.74	0.69	44.15	0.00

[a] model-averaged odds ratios (95% CI): sex, 1.16 (0.95 - 1.43); age, 1.25 (1.14 - 1.36); sex*age, 0.89 (0.81 - 0.97).

Table 4.6 Comparison of models of tuberculosis prevalence in male bison in WBNP (n = 97). 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
age[b]	1	33.06	<0.001	0.00	0.52
age, population[c]*age	2	33.24	<0.001	1.95	0.20
age, population	2	33.21	<0.001	1.98	0.19
age, population, population*age	3	33.87	<0.001	3.49	0.09
age*population	1	2.15	0.14	30.91	<0.001
population	1	0.91	0.34	32.15	<0.001
population, population*age	2	2.15	0.34	33.04	<0.001

[a] model-averaged odds ratios (95% CI): age, 1.26 (1.03 - 1.54); population, 1.02 (0.98 - 1.06); age*population 1.01 (0.99 - 1.03).

[b] age of bison determined by tooth eruption (< 3 years) or cementum annuli

[c] population as determined in chapter 3. Note that males were only tested in the Delta and Hay Camp populations.

Table 4.7 Comparison of models of tuberculosis prevalence in female bison in WBNP (n = 260). 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
Age[b], population[c], age*population	3	31.46	<0.001	0.00	0.72
Population, age*population	2	26.19	<0.001	3.20	0.14
Age, population	2	23.92	<0.001	3.47	0.13
Age	1	13.79	<0.001	9.55	0.01
Age, age*population	2	16.14	0.001	11.25	0.002
Age*population	1	9.76	0.01	15.59	<0.001
Population	1	7.45	0.02	17.90	<0.001

[a] model-averaged odds ratios (95% CI): age, 1.09 (1.02 - 1.16; population: Delta vs. Nyarling River 0.48 (0.23 - 0.97; i.e., Delta females were 0.48 times as likely to be positive than Nyarling River); Hay Camp vs. Nyarling River, 2.29 (1.18 - 4.45); age*Delta vs. Nyarling River 1.09 (1.00 - 1.19; i.e., probability of infection increased 1.09 times faster with age in the Delta than Nyarling River); age*Hay Camp vs. Nyarling River 0.96 (0.88 - 1.04).

[b] age of bison determined by tooth eruption (< 3 years) or cementum annuli.

[c] population as determined in section 3. Females were tested in the Delta, Hay Camp and Nyarling River populations.

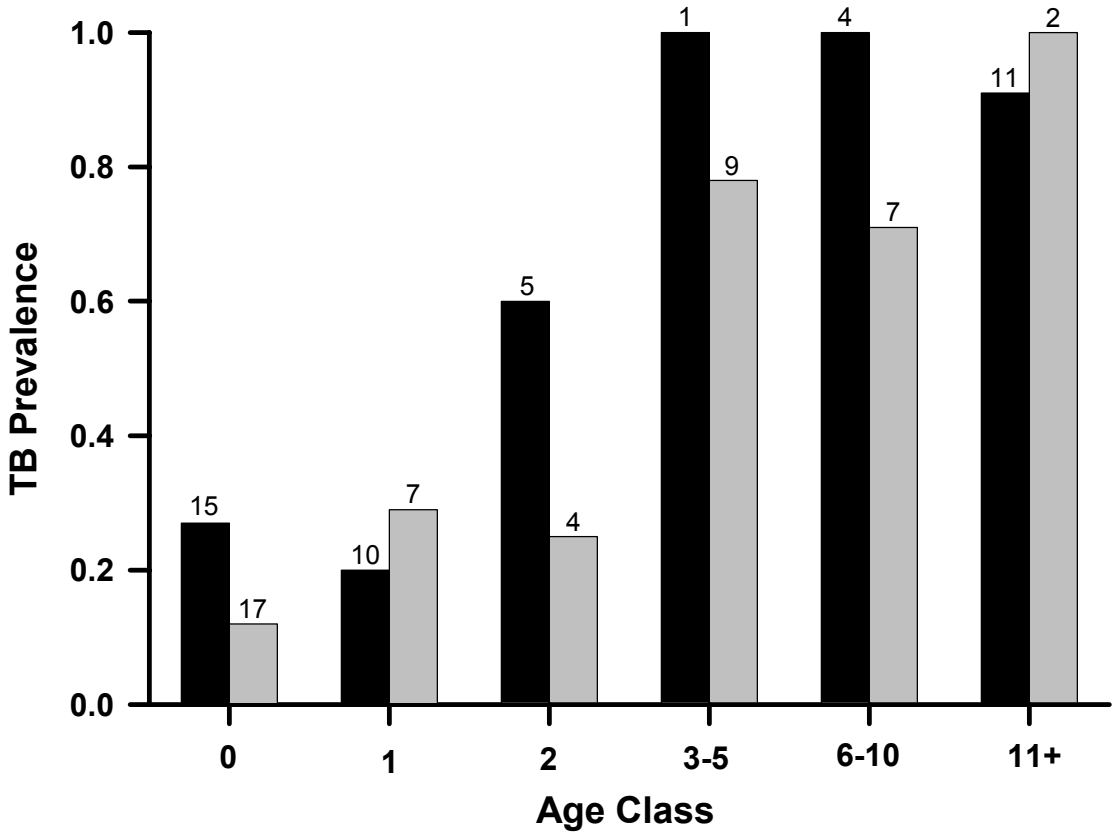

Figure 4.3. Prevalence of tuberculosis in male bison from the Delta (dark bar) and Hay Camp (shaded bar); sample size is indicated at the top of each bar.

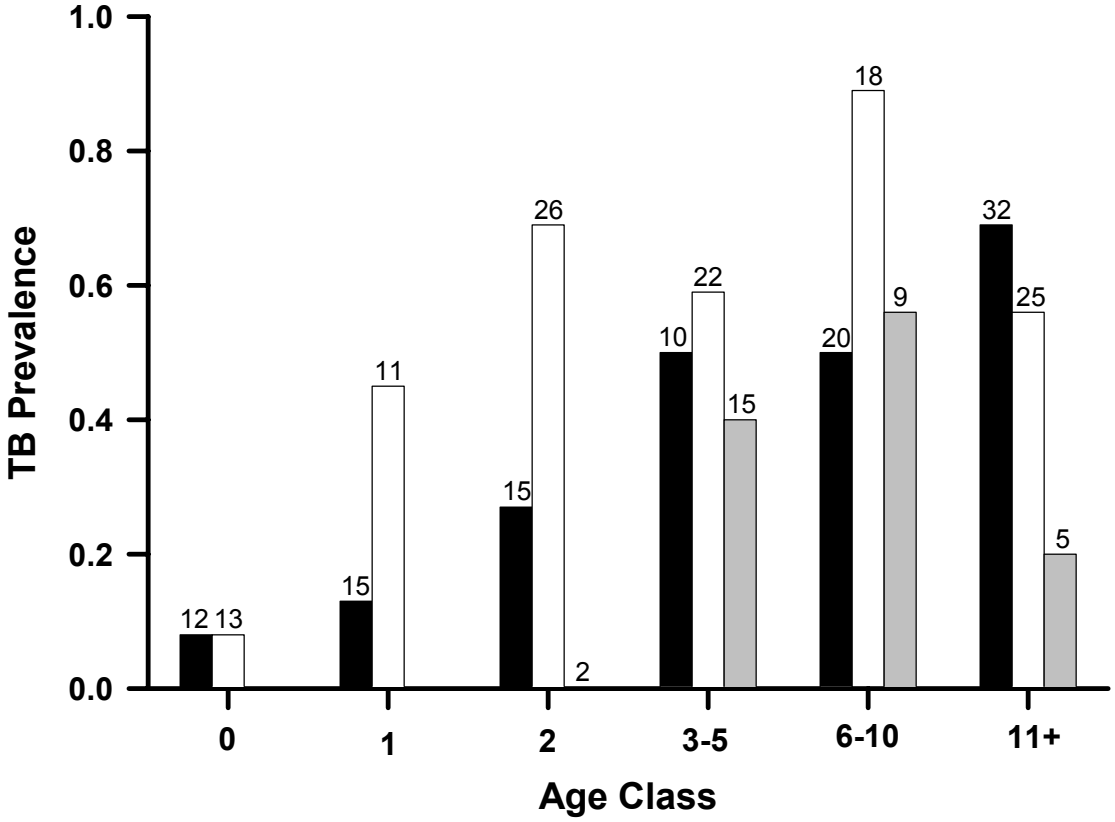

Figure 4.4. Prevalence of tuberculosis in female bison from the Delta (dark bar), Hay Camp (white bar) and Nyarling River (shaded bar); sample size is indicated at the top of each bar.

p = 0.007); whereas the opposite was true for the Delta population (Figure 4.4). I was unable to detect an effect of age on tuberculosis prevalence in the Nyarling River population for females 2 years of age or older (Figure 4.4; Wald Statistic 3.79, df = 3, p = 0.28).

4.3 Discussion

4.3.1 Pathogen testing

The buffered plate antigen test for brucellosis has a reported specificity in bison of 91.7% and a sensitivity of 92.1% (Gall et al. 2000). The complement fixation test has a specificity of 95.5% and sensitivity of 89.5% in bison (Gall et al. 2000). There are no controlled studies evaluating the sensitivity or specificity of the caudal fold test with PPD tuberculin in bison, although Thoen et al. (1988) found that bison reacted similarly to the test as domestic cattle. Reanalysis of data in Choquette et al. (1961) by Tessaro (1989) showed the caudal fold test using OT tuberculin has a sensitivity of 66.7% and specificity of 89.6% in bison. The sensitivity and specificity of OT tuberculin and PPD tuberculin in domestic cattle were similar when compared at similar dosages (OT vs. PPD tuberculin: sensitivity, 84% vs. 85%; specificity 99% vs. 98%; recalculated from Legg et al. 1940; Francis et al. 1978). I assumed the accuracy of OT tuberculin in bison as assessed by Tessaro (1989) approximates the accuracy of the present testing.

I determined the diagnostic threshold for tuberculosis in the fluorescent polarization assay by examining the distribution of test results for a tuberculosis-free bison population. Although I could evaluate the specificity of this test in diagnosing

tuberculosis in bison as the sample of known-negative bison was used to derive the threshold, none of the known-tuberculosis-free Elk Island National Park sera were greater than this threshold, nor were any of the control bison in Lin et al. (1996). This suggests the fluorescent polarization assay has a high specificity in bison, although I recognize the circular reasoning in this statement. The sensitivity of the fluorescent polarization assay in bison is unknown and will be assessed in future work (Om Surujballi, Canadian Food Inspection Agency, Nepean ON, personal communication). Note that the diagnostic threshold based on serological methods should be based on knowledge of the distribution of results for both infected and non-infected animals (e.g., Wobeser 1994a: 92-93). Future research addressing this shortcoming is necessary.

The probability that a test accurately reflects the infection status of an individual is a function of the sensitivity and specificity of a test as well as the parametric prevalence of infection (see Sokal and Rohlf 1995: 69-71 and references therein). I applied Bayes' theorem to determine the probability that a test-positive bison was actually infected (i.e., the positive predictive value) and whether a test-negative bison was actually infection-free (i.e., the negative predictive value) based on the sensitivity and specificity values above and my test criteria. As I interpreted both sets of tests in parallel (i.e., bison that tested positive on either test were considered positive except for a minor alteration in the case of brucellosis; see Table 4.1), I used the minimum sensitivity and specificity for the brucellosis tests. This conservative approach should allow estimation of the minimum positive predictive value for brucellosis. As I had no estimate of accuracy for the fluorescent polarization assay in bison, I used the values for the caudal fold test using OT tuberculin noted above. Prevalence estimates for

tuberculosis (50.2%) and brucellosis (31.2%) are from Choquette et al. (1961) and Choquette et al. (1978) respectively as these represent the previous most extensive surveys from WBNP. Applying Bayes' theorem (Sokal and Rohlf 1995: 70), I calculated an 83% chance that a bison that tests positive for brucellosis (based on my criteria) was actually infected with the bacteria. I calculated an 80% chance that an individual that tested negative for brucellosis was actually infection-free. There was a 73% chance that a bison that tests positive for tuberculosis based on my criteria was actually infected with the bacteria and an 85% chance that an individual that met my criteria for tuberculosis-negative was actually infection-free. Despite these inherent test errors, I believe my data provide the best possible estimates of prevalence given the state of pathogen testing of bison. Further, the tests used in this study are the same tests used to determine the presence of these pathogens in other bovine populations. Thus, these results are comparable to other studies.

I must stress that error in testing result in an underestimation of the effect of pathogens on demographic parameters such as reproduction and survival, which reduces my ability to detect this effect (Figure 1.3). True effect size is the difference between demographic rates for known infected and non-infected individuals. Effect size is underestimated when there are errors in testing as some individuals are misclassified as positive, when they are actually infection-free and experience the demographic rate of a pathogen-free individual. This results in an overestimate of the true demographic rate for infected individuals. Conversely, some individuals are misclassified as negative when they are actually infected, and possess the demographic characterisitics of an infected individual. This results in an underestimate of the demographic rate of non-

infected individuals. The sum of these biases results in an overall underestimate of the difference in demographic rate between infected and non-infected individuals (Figure 1.3), and consequently reduces the ability of a statistical test to detect this difference (e.g., Peterman 1990). Therefore, estimates of the effect of a pathogen on a demographic parameter in which testing is used to identify infected individuals should be viewed as conservative estimates. Results where no effect of a pathogen is detected should also be interpreted cautiously.

4.3.2 Brucellosis prevalence

Few males <2 years of age were brucellosis seropositive compared to females of the same age, yet after 2 years of age seroprevalence rose dramatically among males. The most likely route of transmission of brucellosis is contact with an infected fetus and/or fetal material (Cheville et al. 1998), and so the rate of contact with parturient or aborting females is likely a determinant of prevalence. Young plains bison males (<2 years) initiate few interactions with females older than 2 years of age (Rothstein and Griswold, 1991), which may reduce their exposure to the pathogen. However, the most frequent visitors to a parturient female and newborn calf are 2-year old bulls who sniff and lick placental material and consequently are exposed to large numbers of bacteria (Rhyan 2000).

I found a decline in brucellosis seroprevalence for males in the 6 - 10 year age class. Perhaps this pattern is the result of a decline in antibody titre to non-detectable levels in this age class after initial exposure as young-mature bull. I can offer some support for this decline in antibody titres by comparing complement fixation titres for

bison when I removed the radio-collars relative to the first capture (Figure 4.5). The titres for all bison that were seropositive for brucellosis at first capture declined or stayed stable in the 1, 2, or 3-year interval between collar deployment and collar removal. The increase in brucellosis seroprevalence in the 11-14 year age class may be related to the development of arthritic lesions associated with brucellosis (e.g., Tessaro et al. 1990). Specifically, I propose that bulls in this age class have been infected since first infection as a young bull; however, the antibody titres declined in intermediate ages until development of brucellar arthritis late in life.

4.3.3 Tuberculosis prevalence

Tuberculosis prevalence increased with age in both males and females in the Hay Camp and Delta populations. This is consistent with previous data for infected bison herds in and around WBNP (Fuller 1962; Novakowski 1965). The inability to detect an increase in prevalence with age in the Nyarling River sample is likely a consequence of smaller sample sizes and having sampled primarily adult females. I found that males may have had a higher prevalence than females, and that prevalence increased at a higher rate with age in males than females (Table 4.5). I hypothesize that high prevalence in males is related to long periods of nose-to-nose contact in aggressive encounters during the rut. This fact is important in regards to pathogen containment programs, as mature males with little access to females during the rut have larger home ranges, and are often associated with range expansion (e.g., Gates and Larter 1990; Larter and Gates 1994). These males had relatively high prevalence in this study (40 - 70%; Figure 4.3). If males that wander come into contact with pathogen-naïve populations during periods

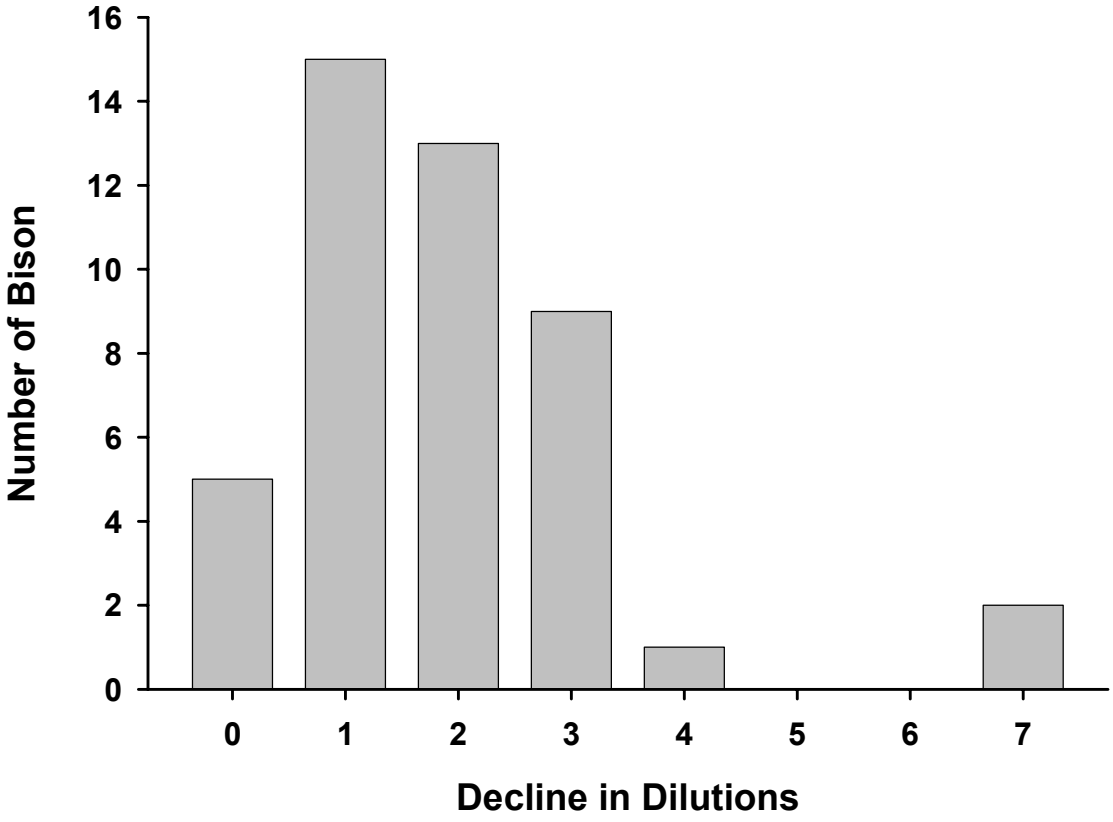

Figure 4.5. Change in complement fixation titre from first to last capture.

of nomadism or emigration excursions, they are likely to transmit tuberculosis to males in these populations.

In the Hay Camp population, tuberculosis prevalence in females declined in females ≥11 years of age relative to younger age classes. Rodwell et al. (2001) suggested a decline in prevalence of tuberculosis in aged African buffalo relative to younger individuals due to a decline in relative survival in tuberculosis-positive buffalo in older age classes. I believe this interpretation also explains the similar results for the Hay Camp population. It is puzzling that I did not see the same pattern among Delta females. Carbyn et al. (1993: 184-186) found that bison in this age class were disproportionately represented in wolf kills in the Hay Camp area (≥11 year-old bison formed 20.8% of wolf kills vs. 10.8% of the total population). This trend was not as striking in the Delta area (≥11 year-old bison formed 14.0% of wolf kills vs. 10.8% of the total population). If wolves in the Hay Camp area target aged bison, then it is logical to hypothesize that infected bison in this age class would be at greatest risk of predation, resulting in a decline in tuberculosis prevalence relative to younger age classes. An alternate explanation is that environmental conditions in the Delta area are such that general body condition of bison is higher, and so progression of the disease is slowed by the immune system.

4.3.4 Density relationships

Due to differing testing methods, the present results are not directly comparable to previous pathogen surveys in Wood Buffalo National Park. However, Tables 4.8 and 4.9 suggest that pathogen levels have not declined in the last 40 years for either

tuberculosis or brucellosis. The decline in bison abundance for the Delta population has been substantially greater in magnitude than elsewhere in WBNP (Carbyn et al. 1998). Therefore, if prevalence was related to bison abundance, I would expect that the difference in prevalence between the Delta and Hay Camp populations would change as well. I found that tuberculosis prevalence was greater for females in the Hay Camp population relative to the Delta population. Historical data from WBNP indicate that the Hay Camp population may have had a higher prevalence of tuberculosis than the pre-decline Delta population: 38.4% of 1567 bison slaughtered in Hay Camp area from 1950 to 1966 had tuberculous lesions compared to only 28% of 722 bison slaughtered in the Delta population from 1951 to 1970 ($\chi^2 = 22.2$, d.f. = 1, $p < 0.001$; data from Carbyn et al. 1993: p. 30). Note that these inferences require the assumption that the historical data are representative of the bison population at the time, and the age structures of the samples were similar between the Hay Camp and Delta populations. Tuberculosis prevalence in the Hay Camp population was 8% higher than the Delta population in 1997-1999, which is comparable to the 10% difference seen in 1950-1970. This stability in relative prevalence suggests that transmission of tuberculosis may be a non-linear function of density, where a decline in transmission does not occur until very low densities. This could be a consequence of the gregarious nature of bison, and may also explain the lack of a relationship between density and tuberculosis prevalence in African buffalo (Rodwell et al. 2001). More evidence for a non-linear density-transmission relationship is found in the fact that tuberculosis prevalence did not differ among young females in the Delta and Nyarling River populations (<6 years; Figure 4.4), despite the Delta population being on average 5 times larger during recent years. As the number of

Table 4.8. Results of various tuberculosis surveys in the Greater Wood Buffalo National Park Ecosystem.

Year(s)	N	Prevalence	Method	Reference
1952-56	1,508	40%	post-mortem examination	Fuller (1962)
1959	1,116	13.5%	OT tuberculin	Choquette et al. (1961)
1983-85	72	21%	various	Tessaro et al. (1990)
1997-99	324	49%	PPD tuberculin/FP	this study

Table 4.9 Results of various brucellosis surveys in the Greater Wood Buffalo National Park Ecosystem.

Year	N	Prevalence	Method	Reference
1959-74	2,365	31%	serology	Choquette et al. (1978)
1983-85	72	25%	various	Tessaro et al. (1990)
1997-99	342	31%	serology	this study

bison counted in the latter population has not exceeded 236 bison since 1990, I suggest that tuberculosis will persist at very low densities.

Dobson and Meagher (1996) proposed a non-linear relationship between brucellosis prevalence and density, and suggested that a minimum population size of 200 was required to sustain brucellosis in bison populations. The degree of transmission of brucellosis is likely a function of group size during the third trimester of gestation, as this is the period in which most brucellosis-induced abortions occur (Cheville et al. 1998). I determined the typical group size (Jarman 1982) of bison during bison total count surveys conducted by WBNP staff in February and March during each year from 1981 - 1999 (excluding 1986 and 1993) for each of the Hay Camp, Delta, and Nyarling River populations, and used an analysis of covariance (Sokal and Rohlf 1995: 499-521) to test the effect of population numbers and population on typical group size (Figure 4.6; note that data from Nyarling River were very variable pre-1990 and so these data were not included). Typical group size was related to numbers of bison ($F_{1,39} = 60.2$, $p < 0.001$), but this relationship did not vary among populations ($F_{2,39} = 1.34$, $p = 0.27$). Based on this relationship, I predict that typical group sizes in the Delta population during 1950-1970 was in excess of 100 bison, 3-5 times that seen during the 1997-99 surveys. Forty-four percent of bison tested in the Delta population from 1950-1970 were seropositive, significantly more than the 19% seroprevalence in the Hay Camp population over the same period ($\chi^2 = 120.78$, df = 1, $p < 0.001$; data from Carbyn et al. 1993: p. 30). In contrast, my data show that seroprevalence in the Hay Camp population is presently greater than in the Delta population (1997-99, Figure 4.4). Thus, a decline in *Brucella* seroprevalence may be associated with a decline in typical group size.

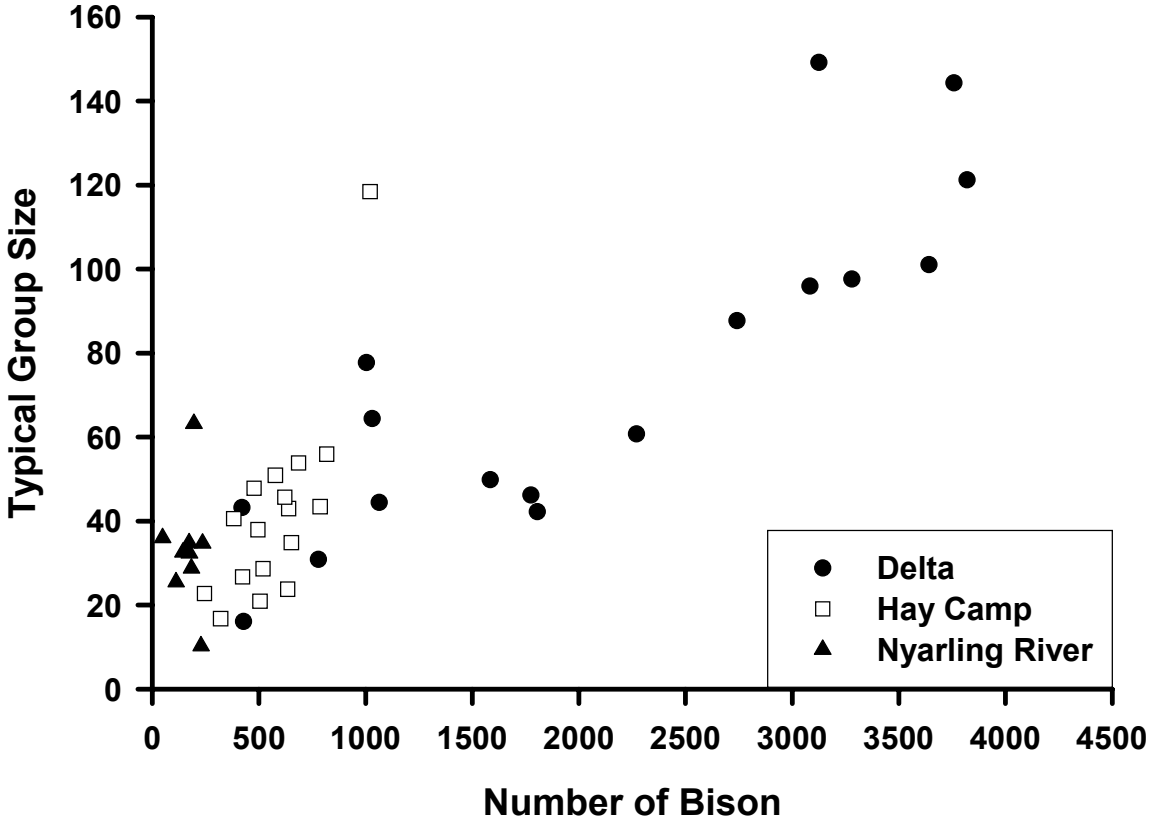

Figure 4.6. Typical group sizes seen on bison total counts in the Delta, Hay Camp and Nyarling River populations. Typical group size is related to population density ($F_{1,39} = 60.2$, $p < 0.001$) and this relationship did not vary among populations ($F_{2,39} = 1.3$, $p = 0.27$).

However, it is important to note that these results may be a function of different age and sex compositions in the 1950-70 samples. Further, the maximum number of bison counted in the Nyarling River population since 1990 is 236 bison, not biologically different from the minimum threshold for brucellosis persistence of 200 bison suggested by Dobson and Meagher (1996). I was unable to detect a difference in brucellosis seroprevalence between Nyarling River and Delta bison. Movement of seropositive bison from the Hay Camp and Delta to the Nyarling River population is likely too low (see section 3) to account for seroprevalence in the latter population, and so I conclude that brucellosis is self-sustaining in this population. Clearly, the minimum number of bison required for brucellosis to persist is very low, likely significantly lower than that proposed by Dobson and Meagher (1996). I conclude that brucellosis will persist indefinitely in the WBNP bison metapopulation in the absence of management intervention.