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ANTHRAX IN THE MACKENZIE WOOD BISON (*BISON BISON ATHABASCAE*) POPULATION: 2012 ANTHRAX OUTBREAK AND HISTORICAL EXPOSURE IN NONOUTBREAK YEARS

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ABSTRACT: Anthrax, caused by the spore-forming bacterium *Bacillus anthracis*, poses a threat to wood bison (*Bison bison athabascae*) conservation. We used descriptive epidemiology to characterize a large outbreak of anthrax in the Mackenzie bison population in the Northwest Territories, Canada, in 2012 and investigated historical serologic exposure of the bison to the bacterium in nonoutbreak years. Between late June and early August 2012, 451 bison carcasses were detected; mortality peaked from 13–19 July. A substantial number of calves, yearlings, and adult females died in the 2012 outbreak, unlike in two previous anthrax outbreaks in this population that killed mostly mature males. On the basis of the difference in estimates of population size prior to the outbreak (2012) and after the outbreak (2013), it is possible that not all dead bison were found during the outbreak. We assessed serologic history of exposure to *B. anthracis* by using samples from the Mackenzie wood bison population collected between 1986 and 2009. Overall, 87 of 278 samples were positive (31%). Seroprevalence was lower in females (18%, 10/55) than males (36%, 72/203). The highest proportion of positive submissions (90%) was from 1994, the year following the only anthrax outbreak within the historical data set. Both adult males and females had a higher likelihood of being seropositive than the younger age categories. There was a trend toward declining antibody levels between the 1993 and 2012 outbreak years.

Key words: Anthrax, *Bacillus anthracis*, conservation, Northern Canada, wood bison.

INTRODUCTION

Prior to 2012, the Mackenzie population was the largest wood bison *(Bison bison athabascae)* population in the world free of both bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*; Government of the Northwest Territories [GNWT] 2010). This population, established in 1963 by the translocation of 18 wood bison that were released near Fort Providence, Northwest Territories, Canada, has been the focus of conservation and disease control efforts over the years (Gates et al. 1995). Anthrax was first detected in this population in 1993 when 172 bison perished (Gates et al. 1995). Although anthrax outbreaks had occurred in other bison populations in northern Canada before 1993, none were within 150 km of this population.

A routine anthrax carcass detection surveillance flight conducted by the GNWT disclosed 128 bison carcasses on 3 July 2012 (Miltenberger 2012). The GNWT activated its Anthrax Emergency Response Plan, which included large-scale control measures, specifically enhanced surveillance, and carcass disposal by burning (Nishi et al. 2002; Elkin et al. 2013). These efforts were instituted to prevent seeding of the environment with anthrax spores, as it is believed that the frequency and severity of future outbreaks can be reduced if the spore load within the environment is kept low (Nishi et al. 2007). Research on the level of spores in the environment in and around fresh and burned carcass sites suggests control efforts are important undertakings (Dragon et al. 2001, 2005).

Much of our understanding of anthrax outbreaks in wildlife in northern Canadian was derived from opportunistic mortality data rather than antemortem serologic surveillance, which contributed to the hypothesis that anthrax is generally rapidly fatal in wood bison (Gates et al. 1995; Bagamian et al. 2013). However, some wood bison in the Mackenzie population had antibody titers against *Bacillus anthracis* in years both before and after the first outbreak in 1993, suggesting that some individuals had been exposed to the bacterium and survived (Rijks 1999).

We provide a detailed description of the 2012 outbreak from field-based mortality information that we compare to previous anthrax mortality events in the population. We also describe the serologic epidemiology of anthrax in the Mackenzie bison population in the context of known mortality events.

MATERIALS AND METHODS

The 2012 anthrax outbreak

As detailed in the Anthrax Emergency Response Plan (Elkin et al. 2013), emergency response teams were dispatched throughout the Mackenzie bison population region in the Northwest Territories, covering over 11,000 km^2 west of Great Slave Lake (61°30′N, 117°000′W), to dispose of carcasses beginning in early July. Each team was provided with carcass disposal forms to document each time a carcass was visited (i.e., date found, date of sampling for anthrax, date control was initiated, and date of confirmation of control completion). Data forms were maintained in the outbreak headquarters for postoutbreak entry into a database.

Information collected on the carcass collection forms included date, reason for visit, geographic coordinates (latitude and longitude), weather conditions, number of other bison present, gender, age class, and estimated age of animal in years. The carcass was described by estimated length of time dead, condition of the carcass (i.e., good, fair, poor, mummified, or disarticulated), and evidence of scavenging. The environment was described by a site description (i.e., clearing, wooded area, burned area, muskeg, hillside, in or near water, and other). If the visit was for carcass disposal, the type of control method was recorded and whether it was the initial treatment or a follow-up disposal visit. A reevaluation of the carcass was made at each subsequent visit. Due to the extent of the outbreak and the number of response teams involved, most of the forms collected were for the initial visit only; therefore, the analysis was done using this information only.

TABLE 1. Observer-estimated length of time (to the nearest half day) wood bison (*Bison bison athabascae*) had been dead when found, sampled, or treated during an anthrax (*Bacillus anthracis*) outbreak in the Mackenzie bison population, Northwest Territories, Canada, in 2012.

Recorded time since death (days)	Categorized time since death (days)[a]	No. of bison
0–6.5	Same as recorded	29
7–10.5	7	39
11–17.5	14	95
18–24.5	21	21
25–31.5	28	14
32–59	42	10
≥60	60	53

[a] On the basis of expert opinion.

An epidemic curve demonstrating date of death in 2012 was constructed by subtracting the estimated length of time dead in days from the date the carcass was first visited (Sergeant and Perkins 2015). We suspected that the accuracy of estimating time dead for a carcass decreased as the carcass aged; as well, the use of multiple observers to record this information likely also contributed to variation in the data. Therefore, we categorized the length of time dead as suggested by a wildlife veterinarian (B.E.) with experience with bison mortalities and used those values in creating the epidemic curve (Table 1).

Both age in years and age class were estimated and recorded on outbreak investigation forms by multiple observers. We followed a standardized classification system for age class in bison (Komers et al. 1994). We refer to both subadult and adult females as cows and subadult and adult males as bulls.

Historic serologic data

Opportunistic serologic samples (n=238) were collected from 1990 to 2009 during bison research and monitoring programs. We tested the samples by using the Immunetics QuickELISA Anthrax-PA Kit (Immunetics, Inc., Boston, Massachusetts, USA), which detects immunoglobulin G antibodies against protective antigen (PA) in serum. Testing was done by the Canadian Food Inspection Agency (Lethbridge, Alberta) in collaboration with the GNWT and Parks Canada. The Canadian Food Inspection Agency categorized samples as negative, "low positive," or positive; in this study,

we considered low positives and negatives to be negative.

Other serologic data (n=40), collected from 1986 to 1994, were also available (Rijks 1999). An enzyme-linked immunosorbent assay was used to test sera for immunoglobulin G and immunoglobulin M antibodies against all three toxin factors (PA, edema, and lethal) but with an emphasis on PA due to its use in previous studies. Rijks (1999) categorized results as negative, "grey,", "intermediate," or "high" on the basis of defined titer values. For this study, negative and grey were considered negative, while intermediate and high were considered positive (Bagamian et al. 2013). As comparable testing methods were used, the values from each serologic data set were categorized accordingly to combine the data for analysis. Rolling averages of the proportion of positive samples each year were calculated by averaging the values of three adjacent years, when 3 yr of consecutive data were available.

Combined mortality and serologic data

We used population size and composition data of the Mackenzie bison population for 10 different years spanning 1983–2013 to compare cow to bull ratios for population, seropositivity, and mortality. Age and sex composition ratios in 2011 (the exception was to use average bull composition across all years) were used to represent the herd in 2012. Population estimates and SEs were calculated for 2012 before the outbreak and again for 2013 after the outbreak. This was used to estimate the 2012 outbreak mortality proportions.

Statistics

Descriptive and comparative statistics were calculated by using EpiTools (AusVet Animal Health Services 2017). The 95% confidence intervals (95% CI) for proportions were calculated with the Wilson Score interval (Brown et al. 2001). Differences between proportions were tested by using a two-sample z-test or chi-squared test, with Fisher's exact test when expected cell counts were less than five. The trend in the proportion of seropositive samples over time was calculated by using ptrend in STATA 13 (StataCorp. MP, College Station, Texas, USA).

RESULTS

The 2012 anthrax outbreak

In 2012, 451 bison deaths were recorded. Carcasses were found from 3 July to 25 August (n=143) and sampled or treated from 5 July to 9 August. The estimated length of time dead for 261 bison ranged from 0.5 to 150 d, emphasizing the need to use the categorization of estimated length of time dead instead of the time itself (Table 1). The first estimated date of death was in late June, with peak mortality occurring from 13 to 19 July, tapering off by the beginning of August (Fig. 1).

Carcass condition (n=401) was mostly classified as either mummified (n=213) or poor (n=102). Scavenging was reported in 54% of carcasses for which this was recorded (131/242). Suspected scavenger species included wolves (*Canis lupis*), black bears (*Ursus americanus*), birds, and multiple other species. Because more than one site characteristic option could be selected for each carcass, 425 descriptions were recorded for 388 bison carcasses. Bison were most often found in clearings (n=282), followed by wooded areas (n=71) and water (n=24). Geographic coordinates were recorded for 395 carcass locations of which several sites represented multiple carcasses (Fig. 2).

Estimated ages of the dead bison (n=307) ranged from 0.3 to 15 yr (median 6 yr). As ages were estimated by multiple field observers of varying expertise, age class was considered more reliable. We recorded carcasses of 41 calves, 27 yearlings, 39 subadults, and 293 adults; 178 (53%) were male, and 158 (47%) were female. Of the 178 males, 150 were adult, 13 were subadult, five were yearlings, and one was a calf, and for the 158 females, 114 were adults, 19 were subadults, three were yearlings, and one was a calf.

Historical serologic data

Serum sample results (n=278) were available from 19 different years between 1986 and 2009, and 31% (87/278; 95% CI: 26–37%) tested positive for antibodies against PA from *B. anthracis* (Table 2). The highest annual proportion positive occurred in 1994, and the highest rolling average was in 1998 (Table 2 and Fig. 3). The overall average 3-yr rolling proportion positive (0.28) was slightly lower than that of the average annual proportion

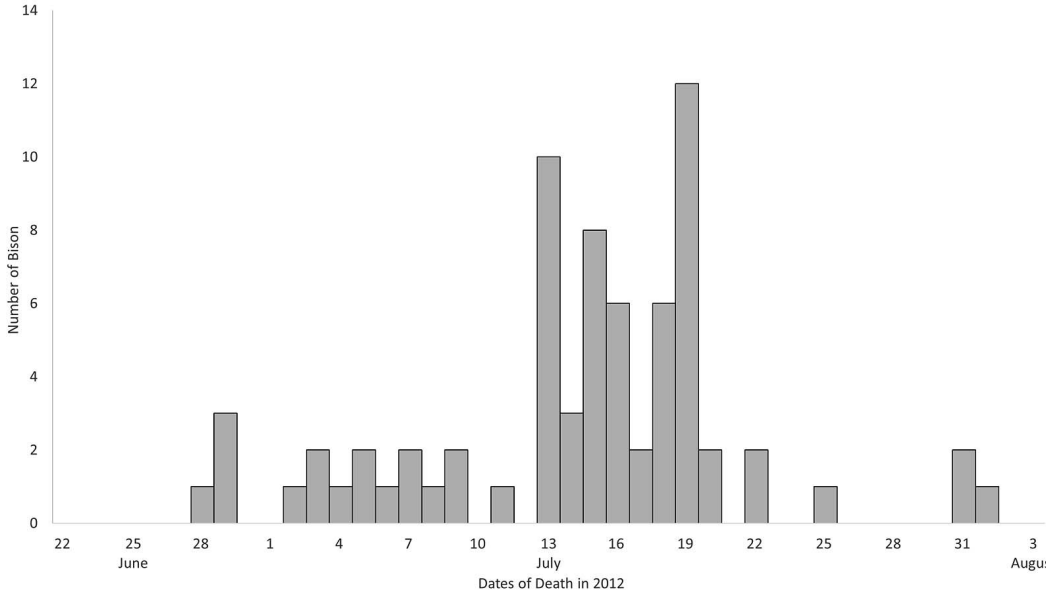

FIGURE 1. Estimated dates of deaths due to anthrax (*Bacillus anthracis*) in the Mackenzie wood bison population (*Bison bison athabascae*), Northwest Territories, Canada, in 2012. In the calculation of date of death, values for estimated length of time dead were categorized on the basis of expert opinion, as described in Table 1.

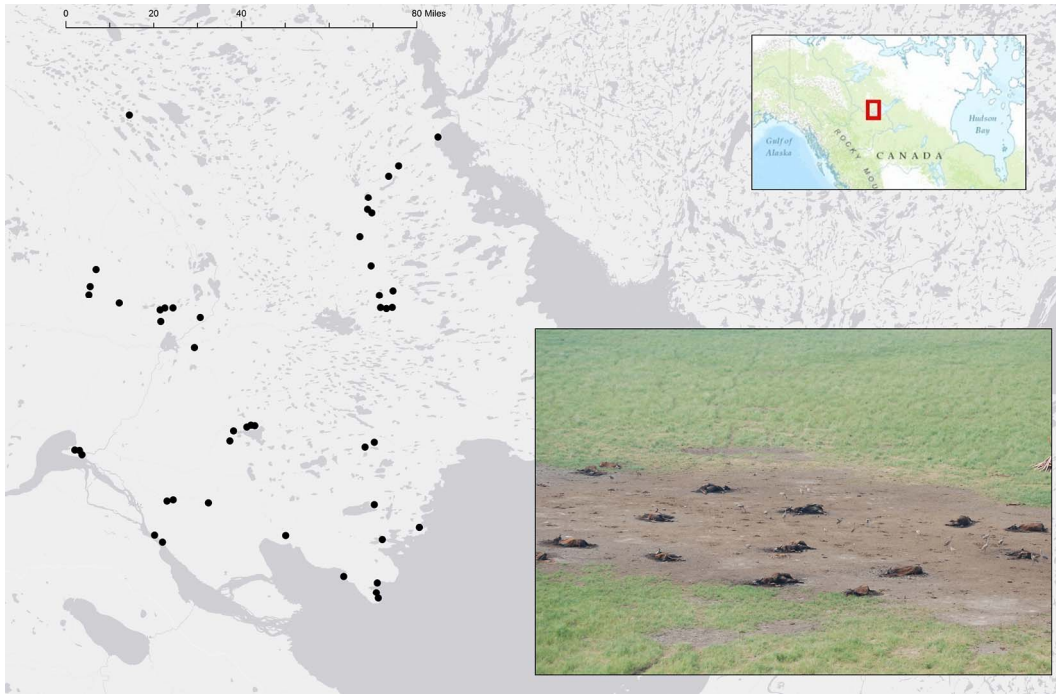

FIGURE 2. Geographic locations of 395 dead Mackenzie wood bison (*Bison bison athabascae*) found during the 2012 anthrax (*Bacillus anthracis*) outbreak, Northwest Territories, Canada. Each dot represents a geographic coordinate of at least one dead bison, although many sites had multiple dead bison, as shown in the picture inset.

TABLE 2. The proportion of serum samples collected each year from the Mackenzie wood bison (*Bison bison athabascae*) population, Northwest Territories, Canada, that tested seropositive by enzyme-linked immunosorbent assay for protective antigen against *Bacillus anthracis*. Rolling averages were calculated by averaging values from three consecutive years. Includes data sourced from Rijks (1999).

Year	No. tested	Proportion positive[a]	95% Confidence interval	Rolling average of proportion positive[b]
1986	4	0.25	0.05–0.7	—
1987	11	0.54	0.28–0.79	0.38
1988	3	0.33	0.06–0.79	—
1990	3	0.67	0.21–0.94	—
1994	20	0.9	0.7–0.97	—
1996	8	0.5	0.22–0.78	—
1997	8	0.12	0.02–0.47	0.38
1998	17	0.53	0.31–0.74	0.36
1999	19	0.42	0.23–0.64	0.52
2000	13	0.62	0.36–0.82	0.46
2001	20	0.35	0.18-0.57	0.39
2002	25	0.2	0.09–0.39	0.26
2003	23	0.22	0.1–0.42	0.18
2004	18	0.11	0.03–0.33	0.20
2005	19	0.26	0.12–0.49	0.15
2006	13	0.08	0.01–0.33	0.13
2007	20	0.05	0.01–0.24	0.1
2008	12	0.17	0.05–0.45	0.09
2009	15	0.07	0.01–0.3	—
Averages (SD)	13.9 (6.7)	0.32 (0.24)		0.28 (0.15)

[a] Proportion of positive submissions by year (n=271); not every year contains samples from both genders nor all ages. No samples were tested from 1989, 1991–93, and 1995.

[b] — = no samples were taken in at least one of the three consecutive years; thus, the rolling average could not be calculated.

positive (0.32) due to the exclusion of years 1990 and 1994 due to insufficient data in adjacent years. There was a statistically significant decreasing trend in proportion positive between 1994 and 2009 (slope=–0.03, $P<0.001$, model fit: P=0.046).

Females had a lower proportion positive (18%, 10/55; 95% CI: 10–30%) than did males (36%, 72/203; 95% CI: 29–42%; P=0.01). In years with samples, the female proportion positive ranged from 0% to 50%, and males ranged from 0% to 100%; overall, 25% (5/20) of samples of unidentified gender were positive (Fig. 4). Older bison were more likely to have positive titers than younger animals. Estimated animal age ranged from 1 to 20 yr (n=128), with the proportion positive highest in ages 9–20 yr (87%, 32/37; 95% CI: 72–94%) compared with 1–8 yr (12%, 11/91; 95% CI: 7–20%). Male proportion positive by age class (n=191) was 13% for yearlings, 18% for subadults, and 46% for adults ($P<0.001$). For females (n=43), only adults tested positive, and 29% (8/28) had a positive titer.

Combined mortality and serologic data

All three documented outbreaks of anthrax in the Mackenzie bison population were based on positive cultures for *B. anthracis* from samples collected from bison found dead (Gates et al. 1995; Elkin et al. 2013). In 1993, only two male calves were discovered, and no dead yearlings were found, with the remaining males consisting of 13 subadult and 135 adult animals. In 2010, a second outbreak (13–21 August) involved nine adult male bison found dead. Finally in 2012, the largest anthrax outbreak (Fig. 1), anthrax carcasses were found in new and repeated locations

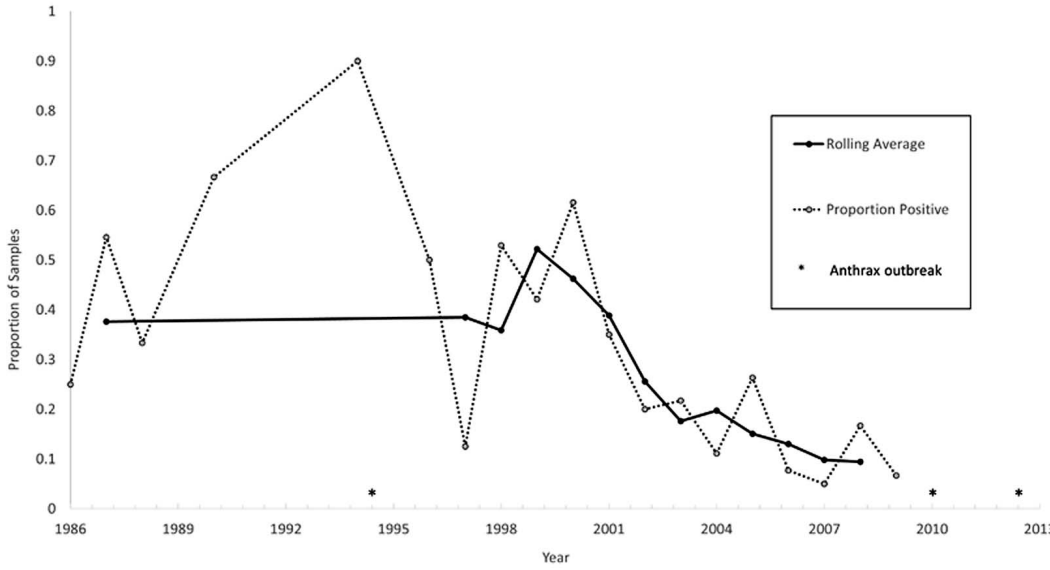

FIGURE 3. The proportion positive of serologic samples collected from the Mackenzie wood bison (*Bison bison athabascae*) population, Northwest Territories, Canada, and tested by an enzyme-linked immunosorbent assay for protective antigen against *Bacillus anthracis* available by year between 1986 and 2009. Included are data sourced from Rijks (1999). Rolling average calculated by averaging three consecutive years, as noted in Table 3.

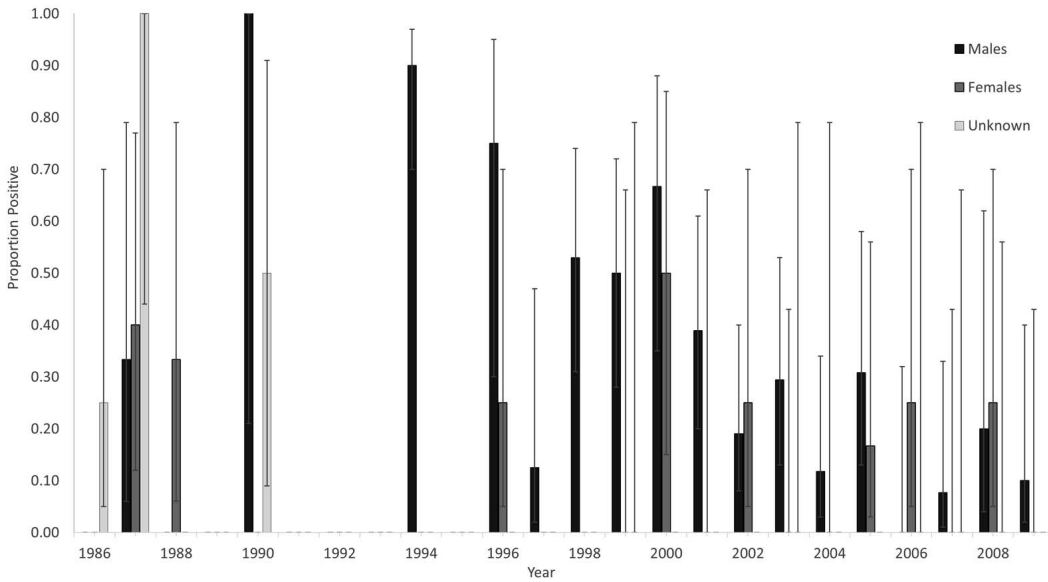

FIGURE 4. The proportion positive of serum samples collected from the Mackenzie wood bison (*Bison bison athabascae*) population, Northwest Territories, Canada, and tested by an enzyme-linked immunosorbent assay for protective antigen against *Bacillus anthracis* available by year and by gender (n=271). No samples were available from 1989, 1991–93, or 1995 (includes data sourced from Rijks 1999). Error bars represent 95% confidence intervals. Seven samples are excluded, as year of collection was not recorded.

TABLE 3. Annual population estimate (SE) and herd composition of the Mackenzie wood bison population (*Bison bison athabascae*), Northwest Territories, Canada, from 1989–2013.[a]

Year	Annual composition[b]			Population estimate (95% confidence interval; SE)[c]
	Calves	Yearlings	Bulls	
1989	44	22	83	2,431 (1,985–2,877)
1992	—[c]	—	—	2,026 (1,793–2,259)
1996	—	—	—	1,868 (1,160–1,466)
1998	—	—	—	1,908 (1,707–2,109)
1999	42.9	30.7	94.3	—
2000	28.9	18.3	75.1	1,998 (1,552–2,444; 208)
2001	37.3	21.8	76.9	—
2002	19	17	90.6	—
2003	41.6	7.7	88.9	—
2004	31.1	15.5	65.8	—
2005	—	—	—	—
2006	36.9	16.8	78.0	—
2007	47	16	91.7	—
2008	32.2	24.4	105.2	1,555 (1,268–1,842; 121)
2009	42.1	24.3	88.0	—
2010	—	—	—	—
2011	37.6	27.5	88.1	—
2012	—	—	—	1,525 (1,118–2,079; 241)
2013	11	8.5	86.8	706 (453–1,100; 160)
Mean (SD)	34.7 (10.3)	19.3 (6.8)	85.6 (10)	—

[a] — = no herd composition or population estimate was available for these years.

[b] Herd composition is expressed as the number of each age and sex class observed per 100 cows.

[c] Gates et al. (1991); Larter et al. (2000); Armstrong and Boulanger (2016).

from previous outbreak years and involved males and females of varying age classes.

We assumed that all 451 carcasses detected were an unbiased sample of the bison that died in the 2012 outbreak. Prior to the 2012 outbreak, population size was estimated at 1,525 (SE 241) bison, but an estimated 706 (SE 160) remained in 2013. The difference (819 animals; 95% CI: 797–837) represents an important decline of 54% (95% CI: 51–57%). We calculated that 18% (41/229; 95% CI: 13–23%) of all calves, 16% (27/167; 95% CI: 11–23%) of the yearlings, and 26% (296/1,128; 95% CI: 24–29%) of the adult bison in the Mackenzie bison population died in the 2012 outbreak. Approximately 31% (163/520; 95% CI: 28–35%) of the adult male bison died, compared with 22% (133/608; 95% CI: 19–25%) of the adult females ($P<0.001$).

Population size estimates for the Mackenzie bison region ranged from 706 to 2,431 animals (mean: 1,751; SD: 509) in the years 1989–2013; herd composition estimates were also available (Table 3). We averaged the bull composition numbers because it was suspected that most of the variation between cow and bull numbers in different years was due to sampling error rather than true population changes. The average cow:bull ratio for the population from 1989 to 2013 was 1.2:1 (Table 4). Even though there tended to be more cows in the herd than bulls, more bulls were seropositive for anti-PA; although more bulls than cows died in all outbreaks, the ratio between bull and cow deaths varied dramatically between outbreak years (Table 4).

There were differences in mortality by age and gender seen between all three outbreaks; these same trends are evident in the serologic samples as well. Overall, 29% (8/28) of samples from adult females were seropositive; however, no female bison were sampled in

TABLE 4. Ratio of cows to bulls for average herd composition (see Table 3), overall mean proportion positive of serum samples (see Table 2), and bison found dead (mortality) in outbreak years for the Mackenzie wood bison population (*Bison bison athabascae*), Northwest Territories, Canada. Includes data sourced from Rijks 1999.

Parameter	Year	Ratio
Herd composition	1989–2013	1.2:1
Prevalence of seropositivity	1996–2009	1:1.9
Mortality[a]	1993	1:6.8
	2012	1:1.1

[a] Only bulls were found dead in the 2010 outbreak; no ratio was calculated for that year.

1994. In the 1993 outbreak, an estimated 2% of the adult cows in the herd died from anthrax. No female bison were found dead in the 2010 outbreak, and in 2012, approximately 19% of the adult cows died from the disease. In comparison, overall, 46% (57/123) of the samples from adult males were seropositive; the proportion of males that were positive was 90% in 1994 samples. The 1993 outbreak killed 23% of the adult males, and 35% of the adult males died from anthrax in 2012. No population estimate was available in 2010 to calculate what proportion of the adult males in the herd died then, but the number would be small, as only nine bison were found dead. The serologic data set included only one calf, which was seronegative. None of the female yearlings were seropositive, while 13% (1/8) of the male yearlings had titers against PA. In the 1993 and 2010 outbreaks, calves or yearlings were largely spared from anthrax (only two calves reported dead). However, there was an estimated 18% loss of calves and 16% loss of yearlings in the 2012 outbreak not specifically related to anthrax.

DISCUSSION

Wood bison were designated as an endangered subspecies in 1978 and were downlisted to threatened in 1988, following active national conservation efforts. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) reassessed wood bison as a species of special concern in November 2013, but they are still listed as threatened under the federal Species at Risk Act (Government of Canada 2011; COSEWIC 2016). Anthrax, with over 2,000 confirmed bison deaths between 1963 and 2000, is recognized as a threat to wood bison recovery (Nishi et al. 2002; Elkin et al. 2013; COSEWIC 2016). The 2012 Mackenzie outbreak had a significant impact on the total population size, and the high number of cows killed may affect the population's productivity for some time. Management actions have already been taken on the basis of the impacts of this outbreak, and ongoing population and anthrax mortality monitoring will be important to inform future decisions.

The difference between the 2013 and 2012 population estimates (819 animals) was larger than the documented number of anthrax cases. There are several possible explanations for this discrepancy (Wobeser 2006). First, estimates of wildlife population size can often have large SEs; the 95% CI of the 2012 estimate was 1,118–2,079 bison and 453–1,100 for the 2013 estimate. Second, there is a range of other causes of mortality in wild bison populations, including starvation, drowning, predation, and motor vehicle collisions. Finally, an unknown number of carcasses may have been missed during the outbreak. Of the 163 carcasses in the 1993 Mackenzie bison population outbreak for which vegetation cover was recorded, only 45% were found in clearings, and most of the carcasses located in wooded areas were detected by using infrared imaging (Gates et al. 1995). During the 2012 outbreak, 73% of carcasses were in clearings, and thermal imaging was not available for surveillance, which may have prevented detection of animals otherwise unobservable from an aircraft. We did not assess the impact of not finding all anthrax carcasses on the potential environmental spore load and future outbreaks.

To adequately describe a disease outbreak, both location and time should be considered (Sergeant and Perkins 2015). The geographic extent of this outbreak encompassed locations

that were similar to those of both previous outbreaks but also extended further than had been previously recorded. Because no information exists on the specific exposure route or travel patterns of infected bison, we cannot evaluate if this is an expansion of the environmental reservoir for the pathogen or not. This outbreak was generally contained within the month of July, with a few outliers at the end of June and into August. Estimating the length of time that a bison has been dead becomes quite difficult as decomposition progresses; however, combining both the date of sampling or treatment and categorized length of time dead provided a way to estimate a date of death. Unfortunately, estimated lengths of time dead were unavailable for both the 1993 and 2010 outbreaks in the Mackenzie bison population. Therefore, outbreak start and end dates could only be compared on the basis of dates the carcasses were found. Dead bison in the 1993 epidemic were found between 29 July and 26 August (Gates et al. 1995), while in 2010, carcasses were discovered in mid-August. In 2012, carcasses were found from 3 July until 25 August, which suggested that this outbreak probably started earlier than either of the previous outbreaks. Overall, better and more consistent methods of estimating length of time dead would benefit wildlife outbreak description.

Early detection of carcasses in a wildlife population presents many challenges. In this outbreak, most bison carcasses were recorded as mummified, which described an advanced state of decomposition wherein the remaining flesh was desiccated, or poor, which described carcasses in an advanced stage of decomposition but that not yet desiccated. Both suggested that there was a significant delay between time of death and carcass detection. Carcass condition can be affected by factors such as weather and scavenging in addition to time since death. About half of the carcasses were scavenged in the 2012 outbreak compared with the 1993 outbreak, when most of the carcasses had signs of scavenging or the presence of scavengers, and the 2010 outbreak, when there was little evidence of scavenging (Gates et al. 1995). Wolves, birds, and bears were the suspected scavengers in 2012, which is similar to 1993 (Gates et al. 1995). Reasons for decreased scavenging in the 2012 outbreak could include an abundant availability of other food sources (including carcasses) or a decrease in the populations of scavenging species (Selva et al. 2005).

The GNWT responds to suspected and confirmed anthrax-caused wildlife deaths by increasing surveillance for dead animals and implementing measures to minimize environmental contamination by spores released from carcasses (Elkin et al. 2013). Carcasses detected during outbreaks are burned on site, and soil contaminated by leaked body fluids is treated with a chemical sporicide (Hugh-Jones and Vos 2002; Elkin et al. 2013). Control measures have been shown to effectively reduce spores at carcass sites (Dragon et al. 2001, 2005); however, carcass decomposition and scavenging can hinder this effort.

Although there was a wide range in estimated ages of bison affected in this outbreak, 73% of the bison carcasses found were considered to be adults. Previous outbreaks in bison in northern Canada have had a similar age distribution (Gates et al. 1995; Salb et al. 2014). However, the number of dead calves and yearlings in the 2012 outbreak was noticeably higher than in other outbreak years, but some of that loss may have been secondary to the high losses of dams. In contrast to this outbreak, the 1993 outbreak had a lower overall mortality but a higher male to female mortality ratio (Gates et al. 1995). The possible reasons for this difference include changes in pathogen virulence across all age categories or for males in particular, decreased immunity due to nutritional or other environmental stressors, or increased environmental exposure to anthrax from a large number of infected carcasses.

On the basis of mortality data alone, the Mackenzie bison population has only experienced three anthrax outbreaks. However, serologic data suggested that these bison have been exposed to *B. anthracis* much more frequently than previously thought (Rijks 1999). Positive titers against PA can indicate

several different situations. The bison may have had clinical disease caused by *B. anthracis* and recovered (Dragon et al. 1999), they may have experienced subclinical (inapparent) disease after exposure and then developed immunity against the pathogen, or they may have been actively infected at the time of sampling. We cannot differentiate between these scenarios on the basis of the available data.

There was always at least one seropositive sample detected in every year from which samples were submitted. The year with the highest proportion of positives was 1994, the year following the only known anthrax outbreak within the time frame of the serologic data set, suggesting that not all bison exposed to the bacteria died during the 1993 anthrax outbreak, with some having either recovered from clinical disease or subclinical infection (Rijks 1999). However, there appeared to be a declining trend in seropositivity up until the more recent outbreaks. It is unknown what level of exposure to *B. anthracis* occurred within the population immediately leading up to or after the 2012 outbreak, as there was no serosurveillance conducted after 2009. The proportion of seropositive samples matched the trends in proportion of fatalities in all three outbreaks, with respect to both gender and age class. Low seropositivity in juveniles suggested that the relatively small numbers of calf and yearling deaths in outbreaks may not be due to the resistance afforded from maternal antibodies against PA but rather because fewer young animals may be exposed to *B. anthracis* than are the older animals in the herd.

One of our limitations was a lack of available information about the half-life of anti-PA antibodies in wood bison after natural exposure to *B. anthracis*. Antibody levels will decline once the animal is no longer exposed to the antigen, at a rate dependent on the half-life of the antibody. Although some work has been done to test the duration of vaccine-induced titers against PA in livestock (Shakya et al. 2007; Ndumnego et al. 2013), few data exist of the half-life of antibodies created by natural exposure (Turnbull et al. 1992; Bagamian et al. 2013). In one study, zebras (*Equus quagga*) had a mean time (SD) to negative seroconversion of 6.3 (1.3) mo, while elephants (*Loxodonta africana*) were 17.5 (4.6) mo (Cizauskas et al. 2014). It is possible that positive titers in our data set reflected exposure to the pathogen within the previous year, but it is also feasible that some bison retained immunity for longer. This created uncertainty in knowing if the proportion of seropositive animals each year reflected the true incidence, rather than prevalence, of exposure. Furthermore, it is also unknown what minimum level of anti-PA antibodies would provide protection against *B. anthracis* in wood bison (Little et al. 1997). Susceptibility to infection by *B. anthracis* is very species dependent (Bagamian et al. 2013), hence extrapolating protective values from another species, such as cattle (*Bos taurus*), is likely not a valid estimate. It is possible that bison with antibodies above a certain titer are immune to the disease and hence would no longer be considered part of the susceptible population. It is currently not possible to determine if there is protective immunity after natural exposure in bison because it is still unknown by which route of exposure they are infected or the infective dose required.

It is unknown how many bison in a population are exposed to *B. anthracis* during an outbreak, how many develop clinical disease, or how many clinically infected bison actually die from disease, all parameters required to calculate case fatality risk. This is largely because surveillance is focused on mortalities and not identifying clinical cases (i.e., depressed, off feed, drop in milk production) in wildlife. Case fatality has been suggested to be close to 100% for herbivores, even though studies have identified examples of subclinical or sublethal infection (Turnbull et al. 1992; Cizauskas et al. 2014). Instead, if a case of anthrax in wood bison is defined as an animal having a positive titer against PA, the "exposure" fatality could be calculated, but only if serosurveillance was conducted in outbreak years before and after deaths occurred. It also assumes that the annual seropositive estimate is representative of the

population. Because most wild bison sera were obtained by convenience sampling, this assumption may not hold true in our study. The true fatality risk of anthrax in wood bison in the Mackenzie population remains unknown.

The 2012 anthrax outbreak killed at least 451 bison in a herd of approximately 1,500 animals. Managing a disease in a wildlife population that is maintained as spores in the environment is a challenging task, and additional research on the ecology and epidemiology of anthrax is needed. It is likely that at least some of the Mackenzie bison population is exposed to *B. anthracis* in years when no mortalities are detected. It is still unknown why deaths occur in some years but not in others. Further research investigating indicators of stress in wood bison may help to uncover if a modification of host resistance plays a role in anthrax mortalities. On the basis of the available evidence, it is likely that anthrax outbreaks in this bison population are multifactorial in nature, influenced by the likelihood of exposure to spores in the environment, the dose and route of exposure to spores, and the status of individual host resistance.

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