EPIDEMIOLOGY OF ANTHRAX OUTBREAKS IN WOOD BISON (*BISON BISON ATHABASCAE*) OF THE MACKENZIE BISON POPULATION

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ABSTRACT

Wood bison (*Bison bison athabascae*) conservation in Northern Canada is negatively affected by diseases that kill these animals, such as anthrax caused by the bacterium *Bacillus anthracis*. Although this disease is considered ancient and was identified more than 2000 years ago in Egypt, little is still known about this disease in wild bison, such as why adult males are often predominantly affected and if the reason there are mortalities in some years and not in others is due to environmental, pathogen, or host factors. The overall objective of this thesis was to use descriptive and serological epidemiology to provide evidence needed to enhance our understanding of anthrax in wild wood bison.

The first chapter explored the 2012 anthrax outbreak in bison of the Mackenzie bison population using descriptive epidemiology. Field crews discovered 451 bison carcasses during the outbreak. The carcasses were found between late June and early August, and it was estimated that the epidemic peaked between July 13-19 based on the date carcasses were found and the estimated length of time the animal had been deceased. A unique feature of this outbreak compared with the two previous outbreaks in the same population, as well as outbreaks in other wild wood bison herds, is that numerous calves, yearlings and adult females died rather than mostly adult males. Three separate geographic regions were identified by a field wildlife veterinarian, and examined for differences in outbreak characteristics. One region had proportionally more male carcasses than the others, and one had more calf deaths. Lack of complete data made it difficult to ascertain if the outbreak truly started in one of these regions before the others, or if it began simultaneously in all three.

The second component of this project used serological epidemiology of anthrax in the Mackenzie bison population to gain an understanding of wood bison exposure to the bacterium. Serological samples were collected through various sources between 1986 and 2009, and later tested for anti-PA antibodies. Of the 278 samples tested, 191 (69%) were positive, indicating previous exposure to *B. anthracis*. Of the samples with a recorded gender, approximately 18.2% of those from females and 35.5% from males tested positive. The dataset spanned only one anthrax outbreak year in this population of animals, and the year with the highest proportion of

positive samples was the year following this known epidemic (1994, 90% positive submissions). Adults had a higher prevalence of being seropositive than any of the other age categories, for both sexes.

This research has revealed that in some outbreak years, all age classes and both genders of bison are affected by anthrax unlike in most outbreaks where predominantly adult males succumb to disease. Furthermore, bison are likely exposed to *B. anthracis* in non-outbreak years, indicating that they either experience subclinical disease or recover from clinical disease.

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B. anthracis	*Bacillus anthracis*
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
EF	Edema Factor
GNWT	Government of the Northwest Territories
LF	Lethal Factor
MBS	Mackenzie Bison Sanctuary
NWT	Northwest Territories
PA	Protective Antigen
SRL	Slave River Lowlands
WBNP	Wood Buffalo National Park

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Humans have a long history with anthrax. Although more recent media attention has pertained to terrorist use of the agent, it is suspected that early outbreaks in livestock and people recorded in the Bible as the fifth and sixth plagues of Egypt were in fact caused by anthrax (Exodus, New International Version). Each of the names “Black bane”, “Charbon” and “Woolsorters’” have been used to label its disease in people, while “Murrain” was a term previously used to describe the disease in livestock (Hart and Beeching, 2002).

Anthrax also played a role in the careers of both Robert Koch and Louis Pasteur (Hart and Beeching, 2002; Moynihan, 1963). Koch was able to culture the causative agent, *Bacillus anthracis*, which he inoculated into animals thereby causing disease. Louis Pasteur was later able to create a vaccine against the disease, demonstrating for the first time that this could be done using an attenuated culture of the causative agent.

Anthrax is distributed across the globe, from tropical places to frigid polar areas (Moynihan, 1963). In most continents, the bacteria is considered endemic (Dragon, Rennie et al., 2001). Outbreaks of anthrax in animals used to be much more common in Canada than at present. A significant decrease in the number of outbreaks evident in Canadian livestock can be attributed to the availability of an effective vaccine, in combination with regulations put in place to decrease the amount of spores transported into the country and disinfection of high risk materials (Moynihan, 1963). The ability to control anthrax in livestock through the use of these measures has probably led to a reduced interest from some stakeholders in evaluating the epidemiology behind the disease (Hugh-Jones and De Vos, 2002; Sterne, 1982). However, anthrax outbreaks are detrimental to the recovery efforts of the threatened wood bison (*Bison bison athabascae*) who continue to experience losses from the disease (Dragon and Elkin, 2001). Furthermore, the potential exists for the disease to affect domesticated livestock in regions surrounding the outbreak areas. This review aims to provide background information about wood bison, describe

anthrax disease and transmission hypotheses, highlight outbreaks within the Mackenzie bison population and provide an overview of management techniques employed during outbreaks in wildlife.

1.2 WOOD BISON CONSERVATION IN CANADA

1.2.1 BISON HISTORY

Two subspecies of American bison (*Bison bison*) exist: the plains bison (*Bison bison bison*) and the wood bison (*B.b. athabascae*) (Gates, Freese et al., 2010). Original habitat of the estimated population of 168,000 wood bison included parts of British Columbia, Alberta, the Northwest Territories (NWT), Yukon, and Alaska (Aune, Berger et al., 2010; Gates and Aune, 2008). Overhunting within the past 200 years caused a tremendous population decline in these animals, to an estimated 300 wood bison (Gates and Aune, 2008).

In an effort to assist in the recovery efforts of bison populations, more than 6000 plains bison were moved from Buffalo National Park near Wainwright, AB to Wood Buffalo National Park (WBNP) between 1925 and 1928 (Van Camp, 1989). This decision, made by the Government of Canada, proved to have two major consequences. First, it allowed for the potential hybridization of the wood bison subspecies with plains bison. Prior to 1925, all bison in WBNP were wood bison. Second, the imported bison were infected with tuberculosis (*Mycobacterium bovis*) (Gates, Freese et al., 2010; Wobeser, 2009). As such, the disease was able to spread within the park to indigenous bison which were previously free of the disease. The identification of hybrid bison, versus pure wood bison, became a critical issue with respect to management of the once-endangered subspecies (Van Camp, 1989). For example, a population of bison residing near Hook Lake was once assumed to be hybrid bison, which resulted in them not being classified as “endangered” or protected by law. Before improved laboratory diagnostic tools were made available, taxonomic classification relied strongly on visual inspection of the animal.

1.2.2 CREATION OF THE MACKENZIE BISON SANCTUARY

Bovine tuberculosis and brucellosis are endemic in many bison herds located in and around WBNP (Gates, Freese et al., 2010). These diseases were recorded in WBNP bison herds as early as the 1930's (Tessaro, Gates et al., 1993). It is generally accepted that both of these diseases were introduced to Northern Canadian bison when infected plains bison were transported to the area in the 1920's, although the evidence is less clear for brucellosis than tuberculosis (Ferguson and Laviolette, 1992; Wobeser, 2009). In contrast, commercial Canadian cattle are now declared both tuberculosis and brucellosis-free (Argue and Koller-Jones, 2009; Gainer and Saunders, 1989). The presence of tuberculosis and brucellosis in free-roaming wildlife presents a risk of transmission into captive cattle herds.

In an effort to produce a herd of wood bison free of both tuberculosis and brucellosis, disease-free bison were captured in 1963 from WBNP, transported down the Slave River and across Great Slave Lake to corrals near Fort Providence, NWT (Gates, Elkin et al., 1995; Tessaro, Gates et al., 1993). Eighteen animals were released 25km northeast of Fort Providence, into what was called the Mackenzie Bison Sanctuary (MBS). Within 37 years, this small population of bison had increased to over 2000 animals and their range expanded well beyond the original boundaries of the MBS. This herd of bison will hereafter be called the Mackenzie bison population to highlight the fact that many bison reside outside the actual MBS perimeter.

1.2.3 CURRENT STATUS OF WOOD BISON IN CANADA

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) classified wood bison as "endangered" in 1978, then upgraded the status to "threatened" in 1988 (COSEWIC, 2002). Their status was once again changed to "special concern" in November 2013, yet they remain listed as threatened in the federal Species at Risk Act ("Species Profile (Wood Bison)," 2011). There are currently 5,000-7000 adult wild wood bison in Canada found among 9-11 subpopulations, including the Mackenzie bison population, the Slave River Lowlands (SRL), and WBNP (COSEWIC, 2002; Gates and Aune, 2008; Nishi, Dragon et al., 2002). Only three of these herds have populations greater than 1,000 animals. Although total bison population

numbers in North America have increased significantly, approximately 97% of bison in the continent are found in commercial production herds (Gates and Aune, 2008). In Canada, bison are listed simultaneously as wildlife and as livestock in four provinces and two territories. Wild bison are still classified as “near threatened” by the International Union for Conservation of Nature (Gates and Aune, 2008).

1.3 ANTHRAX

Anthrax is caused by the Gram-positive bacterium *Bacillus anthracis*. This non-motile, non-hemolytic, facultatively anaerobic rod can exist in a vegetative state, or as an endospore. *Bacillus anthracis* is of the same species as less pathogenic *B. cereus* and *B. thuringiensis*, but has acquired virulence determinants that the other two lack (Hart and Beeching, 2002). These virulence factors include its polypeptide capsule which resists phagocytosis, as well as its tripartite toxin composed of lethal factor, edema factor, and protective antigen (Watson and Keir, 1994).

Under certain conditions, vegetative *B. anthracis* can sporulate. The metabolically-dormant endospores can persist for many years in water, in bone, on hides, in animal feed or in soil (Minett, 1950; Moynihan, 1963; Van Ness, 1971). The spores are resistant to many disinfectants, drying, heat and low temperatures (Dragon, Rennie et al., 2001; Moynihan, 1963). As the persistence of spores plays a critical role in the transmission of disease, further detail about the spores will be provided later.

1.3.1 PATHOLOGY AND CLINICAL SIGNS

When *B. anthracis* organisms enter the body, the spores germinate in lymphoid tissue and the bacteria rapidly multiply in the bloodstream (Moynihan, 1963; Welkos, Kenner et al., 1986; Widdicombe, Hughes et al., 1956). Bacteremia ensues, and the production of toxins by the bacteria can lead to death. Under experimental conditions, goats had detectable bacteremia for 5-8 hours before the onset of death (Sen and Minett, 1944). Death occurs in animals when the number of bacilli in the blood reaches a certain threshold, depending on the host species (Hugh-

Jones and De Vos, 2002; Lincoln, Walker et al., 1967). The number of organisms present in the blood is directly related to the amount of circulating toxin, and it is the combined effects of the 3 toxin components that eventually kill susceptible hosts (Cizauskas, Bellan et al., 2014).

Herbivores such as cattle, sheep, mice and guinea pigs are much more susceptible to anthrax than carnivorous and omnivorous species, such as rats, dogs and pigs (Watson and Keir, 1994). For comparison, the median lethal dose (LD_{50}) for mice via subcutaneous inoculation can be as low as 5 spores, while the same value for the domestic dog can be as high as 5×10^{10}. Some animals are better able to resist infection by *B. anthracis* but are very susceptible to effects of the toxin. In contrast, some are very susceptible to infection by the bacteria but are resistant to the toxin. In one experiment, mice were very susceptible to spore challenge, requiring as few as 5 spores to cause infection (Lincoln, Walker et al., 1967; Watson and Keir, 1994). However, 1000 units/kg of intravenous (IV) toxin (specific toxin not identified in the study) were required to cause death in mice. In dogs, 5×10^7 spores were required to establish anthrax infection, yet only 60 units/kg of toxin IV were needed to cause death. Although different susceptibilities among various species have been identified, the specific mechanisms affording resistance remain unclear (Welkos, Kenner et al., 1986).

Anthrax has traditionally been considered to be generally rapidly fatal in bison (Gates, Elkin et al., 1995; Novakowski, Cousineau et al., 1963). Morbid animals become depressed and unresponsive to stimuli (Cousineau and McClenaghan, 1965; Dragon, Elkin et al., 1999; Moynihan, 1963). They lie down, and stagger when attempting to walk (Cousineau and McClenaghan, 1965; Novakowski, Cousineau et al., 1963). Edematous swellings occur in preputial or umbilical areas, as well as elsewhere on the body. Although some bison feed fervently, the animals often appear gaunt (Cousineau and McClenaghan, 1965). In most outbreaks, cape hair is sloughed from carcasses when they are discovered (Dragon, Elkin et al., 1999).

When death occurs, it can be very sudden as animals have been observed to literally fall over onto the ground. In the 1993 Mackenzie bison population outbreak, the soil surrounding carcasses was noted to not have any indications of flailing or distress by the animal (Gates, Elkin

et al., 1995). Severe bloating, rapid decomposition, a “saw-horse” appearance, and subcutaneous edema have been described in many of the bison found dead in outbreaks (Cousineau and McClenaghan, 1965; Gates, Elkin et al., 1995). One freshly-dead animal in the 1993 Mackenzie bison population outbreak had a frothy white nasal exudate. In many, but not all outbreaks, carcasses had bloody exudate from the mouth and anus (Cousineau and McClenaghan, 1965; Dragon, Elkin et al., 1999; Moynihan, 1963).

In some of the earliest outbreaks in wood bison of Northern Canada, necropsies were conducted (Cousineau and McClenaghan, 1965; Moynihan, 1963). *Bacillus anthracis* was identified in cadavers and in sick bison, which were shot. Findings common to most of these bison included congestion of the spleen, urinary bladder, kidneys, liver and pancreas. Male bison had preputial edema. Lymph nodes adjacent to areas of swelling were enlarged and hemorrhagic in most shot animals, and the colour was described as “brick red” by the veterinarian who conducted a single post mortem during the 1963 outbreak (Moynihan, 1963). Some cadavers had a significantly enlarged spleen, while this was not seen in any of the shot animals. Splenic hemorrhage was noted in some cadavers, while petechiae were evident on the spleen of some shot animals. Blood from cadavers was also noted to be darker than usual. The authors concluded that the difference in gross pathological signs between the carcasses and the shot animals was due to the stage of disease at the time of death and post-mortem.

Two adult male bison, suspected to have anthrax, were shot during the Mackenzie bison population outbreak of 1993 (Gates, Elkin et al., 1995). These animals were nonresponsive to a nearby helicopter, and one had severe lameness while moving. Post-mortem examinations revealed subcutaneous edema and localized alopecia in the scrotal, inguinal and inner thigh regions of both animals. A blood sample, an ear and a subcutaneous swab were submitted from both animals, but *B. anthracis* was not demonstrated in any of the samples. The authors concluded that, although clinical signs were highly suggestive of anthrax, other differentials such as malignant edema (*Clostridium septicum*) infection and blackleg (*Clostridium chauvoei*) infection could not be excluded. Alternatively, it was suggested that the concentration of *B. anthracis* may have been too low in the peripheral circulation at the time of death by shooting as suggested by Krishna et al., 1958 (as cited in Gates, Elkin et al., 1995). In goats, for example, the

animals may live for only 5 to 8 hours while bacilli are detectable in the blood (Sen and Minett, 1944).

1.3.2 ANTHRAX IN OTHER CANADIAN WILDLIFE

In the largest outbreaks in Northern Canadian bison, including in 1963, 1964, 1993 and 2000, as well as in some reports dating to the 1800's, dead moose (*Alces alces*) were recorded (Dragon, Elkin et al., 1999; Dragon, Rennie et al., 2001; Ferguson and Laviolette, 1992). *Bacillus anthracis* was cultured from a moose calf found dead in the 1993 Mackenzie bison population outbreak, confirming the disease. As moose typically have minimal habitat overlap with wood bison, it is suspected that they may contract the disease due to either significant contamination of the moose habitat with *B. anthracis* spores from infected bison carcasses, or due to direct contact between the moose and the infected bison carcasses (Dragon, Elkin et al., 1999).

Anthrax has never been confirmed in any omnivore or carnivore in Northern Canada (Dragon, Elkin et al., 1999). Many carcasses discovered during outbreaks have evidence of significant scavenging, and wolves (*Canis lupus*) can scavenge so extensively that their abdomens become visibly distended. Scavengers also include black bears (*Ursus americanus)*, foxes (*Vulpes vulpes*) and birds (Gates, Elkin et al., 1995). Herring gulls (*Larus argentatus*) and ravens (*Corvus corax*) have been found scavenging on bison carcasses, and occasionally eyes appear to have been plucked out of carcass skulls by these birds (Gates, Elkin et al., 1995). However, scavengers appear to be resistant to the bacterium. Four healthy-appearing ravens, found near bison carcasses, were shot by researchers in the 1993 Mackenzie bison population outbreak (Gates, Elkin et al., 1995). *Bacillus anthracis* was isolated from a pooled sample of their crops, gizzards and intestines, and has also been cultured from wolf and black bear feces in WBNP (Coker, 2002).

1.3.3 ROLE OF SPORES

Vegetative cells released from an infected carcass can transform into resistant endospores. This process is not stimulated by the presence of oxygen; instead, vegetative cells released into the environment are spared destruction by competitive anaerobic bacteria present during putrefaction of the host (Dragon and Rennie, 1995). The nutrient-depleted environment outside of the host initiates sporulation, if other factors are also favorable.

Minett (1950) demonstrated that ambient temperature is one of the main factors in determining if vegetative bacilli released from an infected carcass will sporulate. At 32˚C, sporulation of vegetative cells from blood will occur in 24 hours. In temperatures below 21˚C, the vegetative bacilli will not sporulate and will be overgrown by contaminant bacteria. Work by many other researchers has confirmed that time until sporulation is temperature-dependent, with high temperatures favouring the process.

It was recognized that soil was associated with anthrax before the bacteria was identified as the causative pathogen (Van Ness, 1971). Anthrax tends to occur in areas where soil has a pH>6.0, or that are located downstream from alkaline or neutral soil (Van Ness and Stein, 1956). Soil samples (composed of mostly sand), collected during one survey in WBNP, were found to have fewer anthrax spores than soil from other areas sampled (Dragon, Rennie et al., 2001). It was hypothesized that the low pH of the sandy soil may have decreased the viability of the spores.

Some of what we understand about *B. anthracis* spores has been derived from research conducted for the use of anthrax as a weapon. In 1942 and 1943, experiments were conducted on Gruinard Island, off the coast of Scotland, to evaluate the use of *B. anthracis* spores as a biological weapon (Manchee, Broster et al., 1981; Manchee, Broster et al., 1994). Small bombs filled with anthrax spores in solution were detonated. Movement of spores through the air was tested by observing sheep tethered downwind from the explosion sites for clinical signs of disease, and by sampling air near the sheep to quantify spore numbers, presumed to have been inhaled. Within a few days of detonation, several sheep were found dead, confirming that spores could potentially travel by air.

Annual soil sampling began in the late 1940's to assess spore concentration on the island, to help determine the amount of time spores may persist in soil (Manchee, Broster et al., 1981; Manchee, Broster et al., 1994). Viable spores were detected in samples until 1972, but spore numbers were not quantified (Manchee, Broster et al., 1981). In 1979, more extensive soil sampling was conducted across the entire island, to assess distribution of contamination. Only 2.6 hectares of the 211-hectare island still contained spores at a detection level of 3 spores per gram of soil (Manchee, Broster et al., 1981; Manchee, Broster et al., 1994). No spores were detectable in paddocks that had contained infected sheep carcasses.

Anecdotal evidence suggests that spores can persist for decades in Canadian soil. For example, cattle died of anthrax on a farm in British Columbia in 1962 (Moynihan, 1963). Anthrax had been reported at that location more than four decades earlier, though the farm had not housed cattle in the interim.

Various environmental samples were collected between 1992 and 1997 in Northern Canada in an effort to quantify the level of spore contamination near anthrax carcass sites and further understand the movement of spores (Dragon, Rennie et al., 2001). Sites sampled included mounds where bison had been buried from the Hook lake area 14-30 years earlier, 2-year-old burial sites without mounds in WBNP, carcass incineration sites in the Falaise Lake region less than 2 years old, and finally bison wallows and meadows with no known carcasses. *Bacillus anthracis* was not found in any of the Hook Lake samples. Approximately 2% of the WBNP samples were positive, as well as 4% of the Falaise samples. The highest levels of spores were detected in scavenger scat and bone remains. None of the wallow and meadow samples were positive. It was hypothesized that spores in the Hook Lake samples had been inactivated over time, or dispersed into the surrounding environment.

Results of soil sampling conducted in WBNP suggested that spore concentrations were highest next to carcasses where the soil had been saturated with body fluids (Dragon, Bader et al., 2005). Most positive samples were collected within 2m of a carcass, and the authors believed that positive samples beyond 2m were more likely due to scavengers dragging contaminated tissue

rather than from water run-off. At one site, spores were found at some distance from the carcass along the trail that personnel had previously followed to necropsy the animal.

1.3.4 TRANSMISSION

Anthrax is not directly transmissible; instead, animals become infected from environmental exposure to *B. anthracis* spores (Moynihan, 1963).

It is not known whether bison are infected with *B. anthracis* via oral, inhalational, or percutaneous exposure (Dragon, Rennie et al., 2001). It is generally assumed that other herbivores become infected by ingesting the bacterium (Watson and Keir, 1994). Clinical disease caused by *B. anthracis* spores, given orally, in cattle is dose dependent (Schlingman, Devlin et al., 1956). Peracute death sometimes required administration of up to 10^9 spores; lower doses caused either subacute or inapparent infections.

Although sheep are considered highly susceptible to anthrax, as many as 2×10^5 aerosolized spores were required to cause disease in the animals, whereas resistant animals such as pigs required 2.7×10^7, and dogs required 1.8×10^7 spores (Lincoln, Walker et al., 1967). Although only a few spores are probably required to reach the alveoli in order to establish disease in susceptible animals, high inhalational LD_{50}'s may be due to the size of the particles and the spores themselves (Watson and Keir, 1994). Only particles < 5µm reach the alveoli, and larger spore sizes may be unable to access these areas (Druett, Henderson et al., 1953). Median lethal doses were found to increase in relation to particle size in both rhesus monkeys and guinea-pigs, suggesting that the site presenting the highest risk for serious infection is the lung rather than the upper respiratory tract (Druett, Henderson et al., 1953). In guinea-pigs, 5×10^4 spores were required for infection when aerosolized as individual spores; 8.6×10^5 were required when the aerosolized particles reached 12µm.

As summarized by Watson and Keir,

"… the ability of the anthrax spore to produce disease via the respiratory route is not high, even in a species regarded as very susceptible such as the guinea-pig or sheep" (Watson and Keir, 1994, p. 488).

Animals do not generally experience the cutaneous form of disease (Beyer and Turnbull, 2009; Watson and Keir, 1994). Animals that received experimental subcutaneous and intramuscular injections developed systemic disease rather than cutaneous lesions; however, it was noted that since the injections were beneath the skin, they may not be representative of true cutaneous exposure. Cattle administered anthrax intracutaneously and subcutaneously developed local reactions that resolved (Schlingman, Devlin et al., 1956). Spore administration via intracutaneous, subcutaneous and conjunctival routes all failed to cause death in the cows.

1.4 ANTHRAX OUTBREAK THEORIES

1.4.1 THREE HYPOTHESES

Three general hypotheses have been formulated to explain the occurrence of anthrax outbreaks in animals. These include the "incubator area hypothesis", the "spore concentration hypothesis", and the "modified host resistance hypothesis" (Dragon and Rennie, 1995; Gainer and Saunders, 1989; Van Ness, 1971). The "incubator area hypothesis" and the "spore concentration hypothesis", assume that outbreaks reflect a dose-dependent relationship between number of spores ingested and clinical signs of disease. The "modified host resistance hypothesis" suggests that it is unlikely that animals are exposed to spore levels shown experimentally to induce clinical infection, but rather become much more susceptible to disease at lower doses. None of the hypotheses may be mutually exclusive.

a) Incubator Area

The incubator area hypothesis speculates that *B. anthracis* vegetative cells can survive in some environments outside of a host organism, multiply, and sporulate (Gainer and Saunders, 1989; Van Ness, 1971). It was hypothesized that release of contaminated fluids from a dead animal onto the soil, followed by a period of warm weather, would be sufficient to cause the spores to germinate, multiply, and finally sporulate once again (Minett, 1950). It was postulated that alkaline soils, rich in organic matter, allowed these cycles of propagation to occur.

The hypothesis that vegetative *B. anthracis* can survive outside a host and multiply has been disputed, due to very specific physiological and nutritional requirements as well as competition from other bacteria (Davies, 1960; Dragon and Rennie, 1995). In support of this, sampling of Gruinard Island in 1979 strongly suggested that *B. anthracis* had not multiplied in the soil since its release there (Manchee, Broster et al., 1994; Sterne, 1982), as high numbers would have been anticipated and were not evident. However, recent soil sampling in Etosha National Park near anthrax carcass sites revealed slight increases in spore density within a 1m radius around some carcasses over the length of 1 year (Bellan, Turnbull et al., 2013). Vegetative reproduction of the bacilli in the soil was not excluded as an explanation. Furthermore, it has been shown that *B. anthracis* both germinates and multiplies in amoeba (*Acanthamoeba castellanii*) under certain simulated environmental conditions (Dey, Hoffman et al., 2012).

b) Spore Concentration

Another theory to explain outbreaks of anthrax in wildlife is that spores become concentrated in certain years, leading to a much higher dose exposure than in other years (Dragon and Rennie, 1995; Hugh-Jones and Hussaini, 1974). If *B. anthracis* spores are carried by water, they may become deposited in depressions where the water tends to pool. Wallows are depressions formed in the ground by bison bulls, due to pawing and rolling as part of behavioural displays (Dragon, Elkin et al., 1999). Wallows are utilized more frequently near the rutting season, and are revisited each year. In years with flooding, excess water could potentially transport anthrax spores. The last area where the standing water will clear may be wallows, causing concentration

of spores. Bulls could aerosolize spores when using the wallows, as they produce dust clouds which can drift up to 75m before settling. During the 1993 outbreak in the Mackenzie bison population, most carcasses were found in a geographical depression, where standing water would have accumulated (Dragon, Elkin et al., 1999).

Gainer and Saunders (1989) collected 75 soil samples from low-lying areas near Raup Lake and Hook Lake-Grand Detour, which had experienced anthrax outbreaks within the past 3 years. The samples were from areas where surface water tended to accumulate, and a few samples contained soil with parts of a carcass from previously buried anthrax-positive animals, which had been exhumed by wolves. None of 75 samples was culture positive for *B. anthracis*. This surprised the authors, who expected to find a relatively high number of spores in these areas, under the assumption that 1×10^7 spores may be required to illicit peracute clinical disease in bison, as was experimentally shown in cattle (Schlingman, Devlin et al., 1956). Upon further reflection, Gainer and Saunders (1989) suggested that typical anthrax outbreaks did not exhibit dose-dependent behavior with respect to exposure to spore numbers. They felt that observed outbreaks involved primarily peracute infections resulting in sudden death, rather than a range of clinical signs in herds as might be expected, if disease in the animals was solely dose-dependent.

c) Modified Host Resistance

Conflicting evidence about a dose-dependent relationship between spores and disease, such as extreme difficulty in isolating spores from soil in areas which have recently experienced an outbreak, led to a third theory. This hypothesis suggests that, rather than being exposed to an abnormally high number of anthrax spores, a modification of host-resistance results in peracute disease in animals exposed to spore numbers much lower than may be required experimentally to produce clinical signs (Gainer and Saunders, 1989). The original authors of this hypothesis explain that some clostridial diseases, such as malignant edema, blackleg and bacillary hemoglobinuria, exhibit a similar pattern within animal populations. Animals may be exposed to the clostridial pathogens much earlier than when they exhibit disease and are asymptomatic carriers for a period of time. The avirulent strain that they carry may develop virulence if host resistance is modified or lost. It is hypothesized that rather than being exposed to large numbers

of spores, asymptomatic carriers of anthrax experience a modification of host resistance which then causes them to undergo peracute infection and sudden death (Gainer and Saunders, 1989).

The proponents of the modified host resistance hypothesis point out that conditions such as excessive heat, mating, a high burden of insects, and reduced availability of grazing forage are often evident in anthrax outbreaks (Gainer and Saunders, 1989). It has been noted that outbreaks tend to occur in years after a wet spring, followed by a summer drought (Dragon and Rennie, 1995). This can result in high temperatures, high numbers of biting insects, a decreased availability of food and water, and a crowding of animals around remaining resources (Dragon, Elkin et al., 1999). It was noted that horses used for transportation during early anthrax outbreaks in Northern Canada lost significant amounts of weight possibly due to environmental stressors such as a high insect burden (Gainer and Saunders, 1989). It has been observed that in many outbreaks, adult male bison are more predisposed to mortality than cows or juveniles (Moynihan, 1963; Salb, 2010). Breeding bulls can lose weight during the rut, and this may predispose them to peracute infection compared to cows and young bison in some outbreaks (Dragon, Elkin et al., 1999; Moynihan, 1963). However, anthrax outbreaks in Northern Canada usually precede the bison rut, hence, bulls are at their best body condition (Dragon, Elkin et al., 1999). It has been recognized in other species that testosterone is immunosuppressive (Folstad, Nilssen et al., 1989; Nelson and Demas, 1996), and testosterone is at increased levels in the pre-rut period in bison which may help to explain this phenomenon (Dragon, Elkin et al., 1999; Folstad, Nilssen et al., 1989; Nelson and Demas, 1996).

Bacillus anthracis has been identified in retropharyngeal lymph nodes in cattle free of clinical signs (Provost and Trouette, 1957). Humans may be subclinical carriers of *B. anthracis*, as was demonstrated when 100 healthy workers from goat hair mills were tested for the bacteria (Carr and Rew, 1957). Nose and throat swabs from 14 of these unvaccinated individuals were culture positive for *B. anthracis*. It was noted by the authors, however, that the length of time the bacteria had been present was unknown, as the individuals were exposed daily. Anecdotal evidence also suggests that many humans handled *B. anthracis*-contaminated materials before the adoption of vaccination or disinfection procedures, yet incidence of inhalational or cutaneous anthrax was very low (Watson and Keir, 1994). Many textile mill workers are presumed to have

inhaled hundreds of spores every day when working, and yet did not develop inhalational anthrax. As such, it has been questioned whether spore dose is sufficient alone to cause disease in humans, or if other precipitating factors are required (Watson and Keir, 1994). Specifically, that the minimum lethal dose required is probably largely influenced by both health of the host as well as the strain virulence.

It has been observed that variation in photoperiod affects the susceptibility of mice to pneumococcal infection, and of humans to influenza (Dowell, 2001). There is growing evidence that immune functions change in animals and humans based on photoperiod length or melatonin administration (Dowell, 2001; Nelson and Demas, 1996). Although field studies have yielded conflicting results, a review by Nelson and Demas notes:

"In every laboratory study in which only day length is manipulated, immune function is enhanced in short days as compared to animals maintained in long-day-length conditions" (Nelson and Demas, 1996, p. 513).

The authors speculate that many animal species evolved an increased immune function during the winter in an effort to compensate for stresses such as low temperatures and decreased food availability. This may partially explain the seasonality of anthrax outbreaks in bison.

An intriguing statement by Dowell with regards to the modified host resistance theory may be valuable in the research of anthrax in bison:

"Epidemiologists have long puzzled over why seasonal infectious disease outbreaks occur when they do. Perhaps the more important question is why they do not occur when they do not." (Dowell, 2001, p. 372)

1.4.2 CLUES FROM SEROLOGY

While carnivores often have naturally-acquired antibodies to anthrax, the occurrence is much less common in herbivores which may have led to the assumption that anthrax infections were

always fatal in these animals (Hugh-Jones and De Vos, 2002; Turnbull, Doganay et al., 1992). Turnbull et al. (1992) detected antibodies against anthrax protective antigen (PA) in clinically-normal domestic cattle and wild herbivores. Serum was collected from cattle on two farms which experienced anthrax outbreaks. On the first farm, a single dairy cow died of anthrax. Serum was collected from the herd 1 year after the mortality, and 1/20 was seropositive. On the second farm, ten cows died of which two were confirmed to be due to anthrax. Of 22 serum samples collected from asymptomatic animals 1-2 months after the outbreak, six were seropositive. Turnbull et al. (1992) also collected serum from elephant (*Loxodonta africana*), giraffe (*Giraffa camelopardalis*), hyaena (*Crocuta crocuta*), black-backed jackal (*Canis mesomelas*), black rhino (*Diceros bicornis*), springbok (*Antidorcas marsupialis*), a wildebeest (*Connochaetes taurinus*), and zebra (*Equus burchelli*) in Etosha National Park, Namibia, where anthrax outbreaks in wildlife species are recurrent. Most of the sera from carnivores were seropositive. Of the herbivores tested, one giraffe and three springbok had anti-PA antibodies. Seropositive asymptomatic herbivores provide evidence that some highly-susceptible species may experience sub-acute or mild anthrax. In a separate study, three species of vultures were shown to possess antibodies against PA. It was noted, however, that the antibodies may have been developed due to the translocation of protective antigen components through the gastrointestinal tract after consumption of contaminated meat, rather than due to infection (Turnbull, Diekmann et al., 2008).

Humans who have recovered from naturally-acquired cutaneous anthrax gain immunity against the bacteria (Ingram, Metan et al., 2010). It is possible that human subclinical carriers of the disease may also become immune (Watson and Keir, 1994). It remains unclear which factors determine the persistence of antibodies to anthrax toxins in animals (Turnbull, Doganay et al., 1992). As well, it is unknown the extent to which antibodies against PA prevent the development of clinical disease.

There are also some clues which point to the possibility that wood bison in Northern Canada may not always experience acute clinical disease leading to death. Observations from the first outbreaks in Northern Canada suggested that some animals experienced the acute fatal form of

disease, while others appeared to recover (Cousineau and McClenaghan, 1965; Moynihan, 1963; Novakowski, Cousineau et al., 1963).

Research conducted by Rijks (1999) showed that 8 months after the 1993 anthrax epidemic in the Mackenzie bison population, 70% of adult male bison in the area had a high titre to PA. Interestingly, several bison sampled in the years before the 1993 epidemic had intermediate titres against PA, and one had anti-PA titres equivalent to post-epidemic levels. This supports the theory that bison can be exposed to *B. anthracis*, and survive. Bison calves housed in areas considered to be "anthrax-free" had intermediate or high titres of anti-PA antibody, which was hypothesized to be from maternal antibodies delivered via colostrum. The detection of high levels of IgG and IgM antibodies in calves may help to explain why juveniles are often spared in anthrax outbreaks in bison.

Based on her findings, Rijks concluded that:

"*B. anthracis* was in the Mackenzie Bison Sanctuary for at least a decade before the 1993 outbreak occurred" (Rijks, 1999, p. 53).

It is evident based on serological evidence both from Northern Canadian bison, as well as other herbivores such as African Buffalo and zebras, that susceptibility to infection in herbivores by anthrax may be variable (Bagamian, Alexander et al., 2013).

1.5 POSSIBLE RISK FACTORS

1.5.1 STRAIN VIRULENCE

Median lethal doses of *B. anthracis* by subcutaneous inoculation in mice vary from 5 spores to 10^8 spores, depending on the virulence of the organism used (Welkos, Kenner et al., 1986). Other experimental data for lethal infectious doses in mice and other species has confirmed that the virulence of the strain used significantly influences the LD_{50} (Watson and Keir, 1994; Welkos, Kenner et al., 1986).

The two main virulence factors, the capsule and the toxin, are found within two different plasmids (Watson and Keir, 1994). *Bacillus anthracis* which has lost either plasmid will have reduced virulence, and may not cause the same levels of mortality or morbidity in exposed animals (Turnbull, Hutson et al., 1992). There are currently no data about the strain virulence of the bacterium causing anthrax epidemics in the Mackenzie bison population during any outbreak years.

1.5.2 SCAVENGERS

Scavenging of dead bison that have succumbed to anthrax releases blood into the environment. Vegetative *B. anthracis* cells, which otherwise may have been destroyed by the process of putrefaction, are generally thought to be released and permitted to undergo sporulation (Dragon, Elkin et al., 1999). Carcasses scavenged by mammals may contaminate soil extensively, whereas those scavenged by birds may release less bodily fluid (Dragon, Elkin et al., 1999). In contrast to the commonly-held belief about scavenging and soil contamination, research by Bellan et al. (2013) demonstrated no statistical difference in soil spore concentration between carcass sites in Etosha National Park that were protected from scavengers, and those that were not(Bellan, Turnbull et al., 2013). Similarly, soil sampling in WBNP detected no statistical difference in spore concentration between carcass sites with different degrees of scavenging (Dragon, Bader et al., 2005).

In the 1993 Mackenzie bison population outbreak, 143 carcass sites had evidence of the presence of ravens either by direct observation of ravens on the carcasses, or the presence of raven feces (Gates, Elkin et al., 1995). *Bacillus anthracis* spores were cultured from the gastrointestinal tracts of four ravens shot during this outbreak, and it was suspected that birds may have played a role in the dissemination of spores (Gates, Elkin et al., 1995). As there were also anthrax outbreaks in domestic cattle of Alberta in the same year, it was hypothesized that the birds may have spread the spores by carrying them either externally on limbs and feathers, or internally (Dragon, Elkin et al., 1999). Other scavenging animals, which do not succumb to the disease, could potentially transport the bacterium. Many carcass sites showed evidence of scavenging by

wolves, black bears, and red foxes (*Vulpes vulpes*) (Gates, Elkin et al., 1995). High levels of anthrax spores have been detected in fox scats from WBNP (Dragon, Rennie et al., 2001).

It was hypothesized that carrion eaters may have played a role in introducing anthrax to the Grand Detour area in 1963, after an outbreak east of the Slave River at Hook Lake in 1962 (Cousineau and McClenaghan, 1965). However, it has also been noted that although bison herds on the east and west side of the Slave River remain isolated in the summer months, the river freezes in the winter and the animals can mix (Moynihan, 1963). Sub-clinically infected bison may have transported the disease from the Hook Lake area to the Grand Detour area. It has been shown that although vultures in Africa can carry the bacterium in their gastrointestinal tract, anthrax has not been reported in some of the areas that the birds are known to visit (Turnbull, Diekmann et al., 2008). As such, scavengers may play only a minimal role in the spread of the disease to new geographic areas.

1.5.3 INSECTS

Insects have been hypothesized to be involved with transmission of anthrax in animals, both within outbreak areas and between geographical regions. In many outbreaks of anthrax in Canada, it has been noted that flies appeared to be abundant (Moynihan, 1963). These flies may potentially play a role transmitting disease between animals, or their presence may cause stress in the animals which modifies their resistance to subclinical disease. Necrophilic and hematophagous flies have both been suspected to contribute to the transmission of disease (Blackburn, Curtis et al., 2010).

Necrophilic flies feed on contaminated animal carcasses. They may ingest *B. anthracis* vegetative cells or spores, or carry them on their body (Blackburn, Curtis et al., 2010). These flies then defecate or regurgitate on vegetation near the carcass, potentially contaminating the area with *B. anthracis* which could be ingested by a ruminant (Braack and De Vos, 1990). As well, they can mechanically transmit the organism through direct contact with open wounds on susceptible animals (Sen and Minett, 1944). Many studies have evaluated the role of necrophilic

insects in relation to their ability to transmit anthrax. A few pertinent studies are highlighted below.

One study demonstrated the ability of both blow-flies (*Calliphora erythrocephela*) and houseflies (*Musca domestica*) to infect goats via exposure to open wounds (Sen and Minett, 1944). The insects fed upon open wounds of anthrax-infected carcasses, and then were permitted to feed on cauterized skin of live susceptible goats. The flies were unable, however, to infect susceptible goats via exposure to eyes.

A more recent study examined the feces and vomit of 200 house flies that had fed on anthrax-infected rabbit carcass or blood for periods of time varying between 2-8 hours (Fasanella, Scasciamacchia et al., 2010). *Bacillus anthracis* was detectable in feces or vomit from all groups for at least 2 hours after feeding, and organisms were absent from most flies after 24 hours. There was no association between the length of feeding time and the number of *B. anthracis* organisms detected in vomit or feces. Vegetative cells were detected in the gut contents of the flies, and the authors speculated that germination and replication of *B. anthracis* spores may occur after feeding on blood.

Bacillus anthracis was successfully isolated from necrophilic flies collected near dead deer in West Texas during the summer months (Blackburn, Curtis et al., 2010). However, only a single colony of the bacterium was isolated from eight separate pools of flies, which resulted in a wide confidence interval including zero (95% CI, 0-52%). The authors concluded that since they were searching only for spores, they may have missed a significant number of vegetative cells in the flies. Furthermore, a study design with a larger sample of flies may have yielded better results.

Braack and De Vos (1990) administered a radioactive phosphorus solution to an impala (*Aepyceros melampus*) in Kruger National Park, South Africa, then euthanized the animal and made a deep incision to simulate hemorrhage after death by anthrax. Using a Geiger-Counter, 4 days later, they were able to determine that blood-contaminated fluids from flies were largely deposited between 1-3 meters high on vegetation, near the carcass. This was significant, as kudus (*Tragelaphus strepsiceros*) primarily browse at this height and are the predominantly affected

species in some outbreaks in the area. Fly traps captured radioactive flies at distances ≥37.5 km from the impala. However, the authors noted that most contamination would be near the carcass, because of the flies' behavior of resting near the carcass for several hours after feeding, wherein most of the regurgitation and defecation would occur. It was concluded that feeding habits of wild animals may be a strong determinant of mortality during anthrax outbreaks as a result of varied contamination of vegetation by insects. The authors noted that burning the area surrounding a carcass would effectively eliminate the contamination caused by the insects and was a management technique being employed in Kruger National Park. While these data suggest that necrophilic flies may only play a limited role in transmitting anthrax over vast geographic distances, they may contribute to what is termed the "Case-Multiplier Hypothesis", wherein anthrax infection in one single animal contributes to infection in many animals via contamination of the environment by flies (Blackburn, Curtis et al., 2010).

Hematophagic insects, such as horseflies (*Tabanidae*), stable flies (*Stomoxy calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*) may also play a role in the transmission of anthrax. Of 49 field investigations reported by the Center for Disease Control and Prevention (CDC) for anthrax between 1950 and 2001, 12 mentioned the presence of horseflies (Bales, Dannenberg et al., 2002). Although cutaneous cases of anthrax in humans have been anecdotally associated with insect bites (Fasanella, Scasciamacchia et al., 2010), there is a lack of supporting evidence demonstrating their ability to transmit *B. anthracis* from animals to humans (Bales, Dannenberg et al., 2002). Biting flies have tested positive for *B. anthracis*, which led to the hypothesis that they could infect mammalian hosts as mechanical vectors via intradermal inoculation (Bales, Dannenberg et al., 2002). Furthermore, it has also been speculated that biting insects could be important vectors in transmitting the disease to new geographic locations (Moynihan, 1963).

One early study evaluated the ability of stable flies to transmit anthrax to goats either through bites or defecation on open wounds (Sen and Minett, 1944). Transmission was not successful by either method of exposure. *Bacillus anthracis* was undetectable by culturing the mouth-parts of almost all tested flies. *Bacillus anthracis* could be cultured both from within fly bodies and fly feces for a maximum of 72 hours after feeding (Sen and Minett, 1944).

Stable flies and two mosquito species (*Aedes aegypti* and *Aedes taeniorhynchus)* were able to infect neighbouring guinea pigs in an experimental study (Turell and Knudson, 1987). Guinea pigs were inoculated with *B. anthracis* spores via intramuscular injections. When they became bacteremic, stable flies or mosquitoes were given the opportunity to feed on them for 1 minute or less. The insects were then permitted to feed on susceptible guinea pigs after varying time intervals. Stable flies and mosquitoes had transmission rates of 17% and 12%, respectively. No flies held for 24 hours after feeding on an infected animal infected a susceptible guinea pig. The authors concluded that these insects may play a role in transmission of the disease even in light of their low transmission rates, as biting insect populations are often very high during outbreaks.

Field observations from an outbreak of anthrax in horses in Quebec during 1960 suggested that horseflies may have played a key role in transmission (Moynihan, 1963). Disease did not spread sequentially between neighbouring pastures; instead, the disease spread from one premise to another over long distances. Tabanids are considered aquatic or semi-aquatic insects, which may also explain why the spread of the outbreak was observed to follow a water pathway.

In cattle and elephants (*Loxodonta africana)*, parenteral administration of *B. anthracis* spores has been shown to produce only localized reactions rather than peracute disease (Schlingman, Devlin et al., 1956; Sen and Minett, 1944). If this occurs in wild ruminants, it may potentially explain why some bison of Northern Canada appear to recover from anthrax infections, rather than succumb to the disease (Cousineau and McClenaghan, 1965). These bison may also become immune to the disease via inoculation through biting flies. Mosquitoes and deerflies (*Chrysops*) were collected at the end of the 1962 outbreak in Northern Canada, but none of the insects tested positive for *B. anthracis* (Dragon and Elkin, 2001). Although tabanids were the insect most suspected to be involved in transmission of the disease, the insects had disappeared near the end of the outbreak with the arrival of cooler temperatures.

1.5.4 WEATHER AND TIME OF YEAR

Anthrax outbreaks in Northern Canada usually occur in late summer, particularly when a wet spring is followed by a hot, dry summer (Dragon and Elkin, 2001; Dragon, Elkin et al., 1999).

Dowell (2001) described three different potential explanations for seasonality of infectious diseases: pathogen appearance and disappearance, environmental changes, and host-behaviour changes. The first explanation would suggest that an organism such as *B. anthracis* is not present during periods of the year that are free of outbreaks. The second identifies that weather is sometimes correlated with disease outbreaks, although biological explanation is often lacking. Finally, behavior of the host may change during different seasons. For example, crowding may occur during anthrax seasons.

Salb (2010) failed to demonstrate a statistically significant correlation between any specific type of weather event and anthrax outbreaks in Northern Canada. It was noted that anthrax outbreaks in the bison occurred in years following both wet and dry periods, as well as during seasonably warm or cool summers. While the inability to find a consistent correlation between weather variables and outbreaks does not preclude the possibility that weather may still play a role in the transmission of disease, it may limit the usefulness of using weather as a means to predict future outbreaks.

It has also been hypothesized that weather conditions, such as flooding, may bring spores residing in the soil to the surface, which are then able to be ingested or inhaled by animals if drought ensues (Moynihan, 1963). Conversely, precipitation may disperse spores and end an anthrax outbreak. Anthrax spores can remain viable in sterile water for more than 2 years (Minett, 1950). Four water samples collected in WBNP near anthrax-confirmed carcasses were all positive for *B. anthracis* (Coker, 2002), suggesting that movement by water may play a role in transmission.

Anthrax is considered quite rare in Australia, particularly outside of the area deemed the “anthrax belt” (Durrheim, Freeman et al., 2009). A significant outbreak of the disease occurred in late 2007 in an area 350 km from this anthrax zone. It was hypothesized that a 1-in-100 year rain event, earlier that year, may have unearthed anthrax spores that had been dormant in the soil. Moving water was not suspected to play a major role in disease transmission, as downstream areas were affected before their upstream counterparts, and some properties had no creeks. As well, scavenging was very minimal and no biting flies were seen on any carcasses.

The first recorded outbreak of anthrax in bison of Northern Canada in 1962 followed a period of flooding earlier that summer. The end of the 1964 anthrax outbreak in Northern Canada coincided with the arrival of heavy rainfall in all regions where carcasses had been found (Dragon and Elkin, 2001). As no anthrax cases were discovered in the following years of 1965 and 1966, it has been hypothesized that the heavy rainfall which caused significant flooding may have transported anthrax spores away from the sites. Springs and summers between 1988 and 1992 also saw higher than normal levels of precipitation immediately west of Great Slave Lake, near the Mackenzie bison population, which caused flooding of meadows near some lake beds (Gates, Elkin et al., 1995). In the outbreak year of 1993, dry conditions caused the water in these flooded meadows to recede. Environmental sampling in WBNP found that fewer *B. anthracis* spores were able to be cultured from samples taken from sandy soil, in comparison to other areas (Dragon, Rennie et al., 2001). Among other hypotheses, the authors suggested that water may have played a role in removing spores from sandy areas.

1.6 ANTHRAX IN BISON OF NORTHERN CANADA

1.6.1 SLAVE RIVER LOWLANDS AND WOOD BUFFALO NATIONAL PARK

The first recorded anthrax outbreak in free-roaming Northern Canadian bison occurred in 1962, killing at least one fifth of the estimated herd of 1300 (Cousineau and McClenaghan, 1965; Novakowski, Cousineau et al., 1963). This was also the first recorded anthrax outbreak in any Canadian wildlife species. The epidemic occurred in the SRL of the NWT, near Hook Lake (Dragon and Elkin, 2001; Dragon, Elkin et al., 1999). Since that time, anthrax outbreaks in wood bison have been recorded seven times in the SRL, and ten times in WBNP (Wohlberg, 2013) (see Figures 1-1 and 1-2).

1.6.2 MACKENZIE BISON POPULATION

In July 1993, the Mackenzie bison population experienced an outbreak (Gates, Elkin et al., 1995). The disease had never before been reported in the area, and the wood bison herd had been moved to the region 30 years prior. One hundred and sixty nine dead wood bison were discovered via helicopter aerial surveillance, utilizing an infrared camera mounted on the aircraft to locate carcasses in forested or otherwise covered areas. This was the first use of a thermal imaging camera for bison detection in the NWT, and approximately half of the carcasses discovered were located in areas that would not have otherwise been visible from an aircraft. In early July, preceding the outbreak, a herd composition survey had been conducted via air surveillance and ground-based observers, during which 473 bison were observed and classified. Cows >2 years old represented 44.6% of the sample, while adult (7-11 years old) and mature (12 years and older) male bison comprised 12.5% and 12.1% of the sample, respectively. Utilizing this survey data in combination with carcass data collected during the outbreak, it was estimated that adult and mature male wood bison were predominantly affected in the outbreak. Approximately 25% of the adult males and 21% of the mature males in the herd succumbed to the disease. In contrast, only 2% of the cows were found dead from the disease.

In 2010, nine bison were found dead due to anthrax (Brett Elkin, unpublished data). In 2012, approximately 450 bison from the same herd died from the disease (GNWT, 2014). Figure 1-1 gives an overview of outbreaks within bison of Northern Canada.

Anthrax Outbreaks in the NWT

Mackenzie Bison Range	Slave River Lowlands	Wood Buffalo National Park
1993 - 172	1962 - 281	1963 – 47
2010 - 9	1963 - 257	1964 - 60
2012 - 440	1964 - 303	1967 - 120
	1971 - 33	1968 - 1
	1978 - 39	1978 - 47
	2001 - 12	1991 - 32
	2006 - 26	2000 - 106
	2010 - 46	2001 - 91
		2007 - 64
		2010 - 7

2

Figure 1-1 Number of dead bison found in anthrax outbreaks in Northern Canada by year. Original reports indicated 440 bison deaths in the Mackenzie bison population in 2012, but further review of field data revealed there were 451 (Wohlberg, 2013).

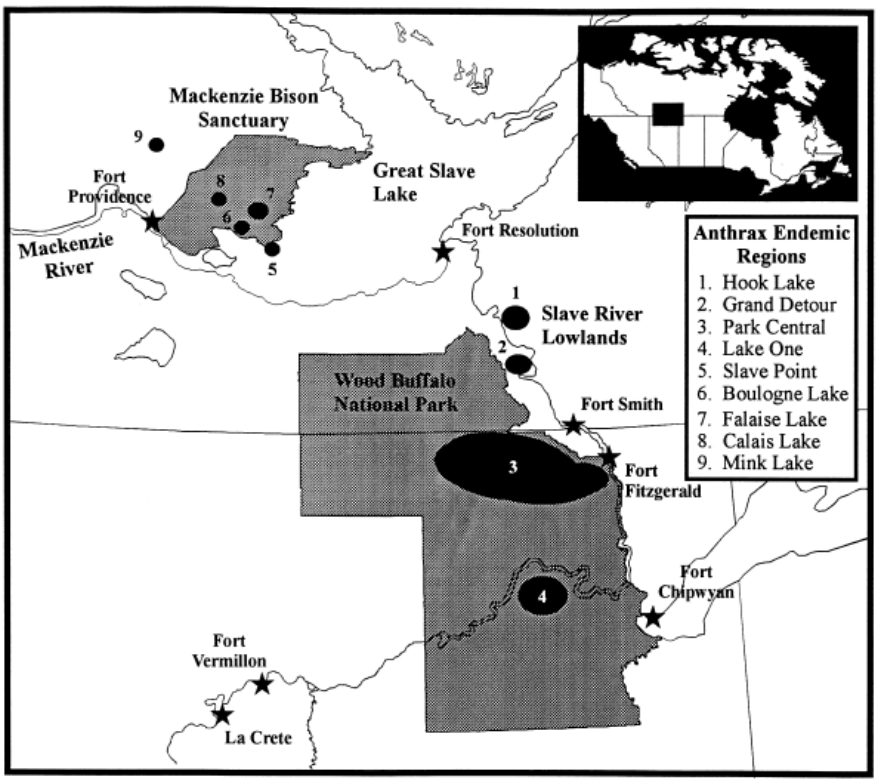


Figure 1-2 Anthrax endemic regions in Northern Canada (Dragon, Elkin et al., 1999).

1.7 OUTBREAK MANAGEMENT TECHNIQUES

Many different techniques have been utilized to manage anthrax in wood bison. Depopulation and vaccination have been attempted in the past, while surveillance, testing, and carcass disposal are methods still being used. Current management protocols used in the Mackenzie bison population are outlined in a document entitled “Anthrax Emergency Response Plan” developed by the Government of the Northwest Territories (Division, 2001). The main objectives of this plan are:

“…to identify time periods that are favourable for the development of anthrax, detect any dead or noticeably sick bison should an outbreak occur, reduce the rate of carcass scavenging and dispose of the carcasses quickly to limit the extent of the outbreak” (Division, 2001).

1.7.1 DEPOPULATION

In an effort to decrease the spread of anthrax among various bison herds in Northern Canada, depopulation programs have been attempted (Dragon and Elkin, 2001). A total of 554 bison were culled in the Grand Detour region in 1964 and 1965. The efforts were largely unsuccessful, as the area was quickly repopulated with bison. Bison (44) were shot during the 1971 outbreak in the Hook Lake area, in another attempt to decrease spread of the disease.

1.7.2 VACCINATION

A vaccine against anthrax in animals has been available since 1881, as developed by Louis Pasteur (Baillie, 2001). The development of the Sterne attenuated spore vaccine has provided a more effective and safe vaccination alternative for animals than the heat-attenuated vaccine produced by Pasteur.

In order to effectively use a vaccine in any animal species, both the dose required and the duration of immunity provided should be determined. As the Sterne strain vaccine is a live vaccine, it is important that an appropriate dose be calculated for various species and ages of

animals in order to prevent adverse reactions. As an example, three young llamas developed disease after being inoculated with the Sterne vaccine at a dose approved for bovine calves (Cartwright, McChesney et al., 1987). Two of the animals died.

Sera collected from one rhinoceros (*Diceros bicornis*) and four elephants (*Loxodonta africana*) in Etosha National Park known to have been vaccinated against anthrax were negative for anti-PA antibodies (Turnbull, Doganay et al., 1992). All of these animals were vaccinated more than 12 months prior to sample collection, which may suggest that immunity provided by the live vaccine in these species is of short duration. Serum collected in the same area from captive horses, previously vaccinated with the Sterne strain live spore vaccine, revealed that horses vaccinated only once, 10 months prior to testing, had no detectable antibodies. Horses that had received multiple annual doses (2-9 doses) had detectable antibodies when the sample was collected 10 months after their most recent vaccination. These findings emphasize the need to know the duration of immunity provided by a vaccine in any species when evaluating its potential effectiveness in creating herd immunity.

An effort to vaccinate herds of Northern wild bison against anthrax was scheduled for the summer of 1964, but the process never ensued (Dragon and Elkin, 2001). A large anthrax outbreak occurred that year which competed for available resources, and flooding of the areas after the outbreak made the herds inaccessible. The vaccination program was successfully initiated in WBNP in 1965 (Dragon and Elkin, 2001). The bison were herded into corrals, and then through a chute system. As data for the dose required and duration of immunity provided were unavailable for wood bison, cattle doses and immunization schedules for the Sterne-type live spore vaccine were extrapolated to the species (Dragon and Elkin, 2001). Vaccinated animals were also branded for identification. Approximately 27,000 bison were vaccinated between 1965 and 1977. The vaccination program had an estimated 2.0% mortality of those that were herded. Exhaustion due to travelling over long distances to arrive at the chutes, over-crowding, trampling, separation of calves from dams, and exertional myopathy contributed to the fatalities. No data have been published regarding the number of animals revaccinated each year. Anecdotal evidence suggests that in some years as few as 1% may have been revaccinates, with the highest revaccination rates reaching close to 50%.

Only one case of anthrax was recorded during the years of the vaccination program within the areas being targeted (Dragon and Elkin, 2001). It is unknown whether the vaccination program contributed to the absence of anthrax disease. In 1977, the bison vaccination program was ended due to public outcry over the 800 animals that died during the process, and concern by stakeholders that the strategy was not effective.

1.7.3 SURVEILLANCE FLIGHTS

Routine aerial surveillance was initiated after the 1993 outbreak in the Mackenzie bison population (B. Elkin, personal communication, April 11 2014). Flights are now conducted over known bison habitat every 2-3 weeks beginning in late June until mid-August each year by a fixed wing aircraft. These flights detected bison mortalities in both the 2010 and 2012 outbreak years. Surveillance flights in all years since 1993 have been comparable with respect to methodology, and it is not expected that bias in detecting carcasses was present due to differences between flights.

Once anthrax-positive carcasses are detected, flights are conducted more frequently both with fixed-wing aircraft and helicopters. In 1993, infrared imaging was used from a helicopter in order to detect carcasses hidden by vegetation (Gates, Elkin et al., 1995). It should be noted that groups of dead bison and large carcasses are easier to detect than single carcasses and small animals such as calves. As such, some selection bias may have been introduced into the data in outbreak years, since some genders or age classes of bison may have been systematically missed by aerial surveillance.

1.7.4 TESTING

There are various ways to test a carcass for anthrax. Historically, ears were considered the best tissue to be sent to laboratories for confirmation. In comparison to other submitted samples such as the spleen, they did not require opening the carcass and potentially releasing contaminated body fluids, and were less likely to undergo putrefaction as they contained a relatively small amount of soft tissue (Hagan, 1920). It had been noted that vegetative cells often did not

sporulate in tissue being sent for pathogen confirmation, and that lengthy transit times between the sampling site and the lab often meant that few organisms survived the shipment. Because of this, ears were often submitted to labs in the hope that vegetative cells would not be destroyed by putrefactive processes. In a 2008 outbreak among plains bison in Prince Albert National Park, SK, ears were submitted to the lab, since the discovered carcasses were already in advanced stages of decomposition (Shury, Frandsen et al., 2009). As well, bones, hair and hide from the four animals were also sent. All four of the bison were confirmed positive by culture.

As an historical aside, if tissues delivered to a lab were decomposed and had undergone putrefaction, previous convention was to analyze them by inoculating guinea pigs with submitted samples in an attempt to elicit clinical signs of anthrax (Hagan, 1920). However, the guinea pigs often died because of infection by other bacteria present in the tissue before *B. anthracis* had time to develop. As such, this technique had limited usefulness.

Blood and fluid samples from carcasses can also be submitted for analysis. Recommendations for submissions in the early 20th century included submitting a dry string soaked in blood. It had been hypothesized that vegetative cells died in submitted tissues due to a lack of available oxygen, and that blood on a string exposed to air would allow the vegetative cells to sporulate (Hagan, 1920). In the sporulated form, the sample was able to be examined for many months from the date of arrival. In a similar fashion, blood swabs taken from carcasses in the NWT are air dried for 5-10 minutes before being packaged, in order to provide the oxygen required for sporulation (Division, 2001). As well, air drying the sample destroys many contaminant microbes on the swab. Samples are sent to the Animal Disease Research Institute in Lethbridge, Alberta for testing, as soon as possible after collection.

In live animals and humans, an enzyme immunoassay has been successfully used to detect antibodies against PA (Turnbull, Doganay et al., 1992). This method could be used to confirm suspected cases as well as identify subclinical infections, although its applicability in a large wild species may be minimal, as capture and restraint are difficult.

1.7.5 CARCASS DISPOSAL

Vegetative *B. anthracis* are much more susceptible to destruction than their sporulated counterparts (Nishi, Dragon et al., 2002). As such, carcass disposal of animals confirmed to be anthrax-positive is critical in order to decrease the number of vegetative cells that may produce highly resistant spores. Carcass disposal is one of the main management techniques that has been utilized by both Parks Canada and the GNWT in an effort to break the transmission cycle of the bacterium (Nishi, Dragon et al., 2002). It is hoped that by preventing contamination of the environment with spores from a carcass, future outbreaks may be less likely to occur. Carcass site sampling has shown that incineration and burial significantly reduce *B. anthracis* colony forming units when compared to carcasses left untreated (Coker, 2002). The number of bacteria found after incineration, burial and burial with lime were not statistically different from one another. However, it was postulated that burial may simply disguise most of the contamination from the surface although the spores could persist.

Techniques that have been utilized in wildlife anthrax outbreaks either alone or in combination include burial, treatment with lime, incineration, treatment with formaldehyde, and covering the carcass with a tarp to promote putrefaction.

a) Burial

In outbreaks in Northern Canada prior to 1967, carcasses were buried and then covered with a large mound of earth (Cousineau and McClenaghan, 1965; Dragon, Elkin et al., 1999; Dragon, Rennie et al., 2001). These mounds were created due to difficult terrain making digging deep holes difficult, and are still clearly visible today. Alternatively, in outbreaks such as the 1991 epizootic in WBNP, some carcasses were buried without placing mounds of earth on top (Broughton, 1992; Dragon, Rennie et al., 2001).

Burial is no longer a favoured technique in disposing of bison carcasses during outbreak situations (Dragon, Elkin et al., 1999). Carcasses that are buried beneath soil may release spores that could contaminate groundwater (Nishi, Dragon et al., 2002). Furthermore, spores may be spread by animals that burrow into the ground. Vegetation preferred by bison, such as particular

grasses and sedges, tend to grow on the burial mounds which encourages grazing of the bison near the burial site (Dragon, Elkin et al., 1999). Raised mounds can be ideal sites for wallowing by bison bulls, which could potentially aerosolize spores found within the soil or create deep depressions where water will accumulate and concentrate spores.

b) Lime

Burial, in combination with lime, was used to dispose of the majority of carcasses in the 1962 outbreak (Cousineau and McClenaghan, 1965). Carcasses discovered in wet areas in the 1991 WBNP outbreak were covered with lime, presumably because burial or incineration were considered too difficult in those locations (Broughton, 1992). Treatment with lime has been shown to provide an alkaline-rich environment in the soil surrounding the carcass, which is now understood to support the maintenance of anthrax spores in the soil (Dragon and Rennie, 1995; Nishi, Dragon et al., 2002). Furthermore, it is ineffective at eliminating scavenging by other animals. For these reasons, treatment with lime is no longer recommended in most management protocols.

c) Incineration

In contrast to burial, incineration destroys anthrax spores, and does not leave any remaining tissue for scavengers (Dragon, Elkin et al., 1999; Nishi, Dragon et al., 2002). Bison wallowing has not been observed at previous cremation sites, so it is suspected that the ash or charcoal make these sites less appealing to the bulls (Dragon, Elkin et al., 1999). Vegetation that grows on these sites tends to be broad-leafed species, which typically are not grazed by the bison.

Soil sampled from carcass incineration sites in Mexico did not produce *B. anthracis* isolates, while similar samples from Nevada did (Coker, 2002). It was hypothesized that the time between death and incineration may be responsible for this difference, as carcasses were burned within hours of death in Mexico compared to a couple of days following death in Nevada. It may be prudent to begin carcass incineration as quickly as possible.

Between 1967 and 1991, bison carcasses in Northern outbreaks were primarily disposed of via incineration in a pit, which was then filled with dirt (Dragon, Elkin et al., 1999). In the 1993

Mackenzie bison population outbreak in the NWT, all but 12 carcasses were incinerated on the ground surface (Gates, Elkin et al., 1995). Fuels used included turbo Jet-B fuel in combination with timber, or Jet-B fuel and coal (Dragon, Elkin et al., 1999). The fuels were placed on top of, and adjacent to, carcasses (Dragon, Rennie et al., 2001). It was observed that coal provided a higher temperature than wood, which allowed for more complete cremation. A few cremation sites revisited 1 year later were found to be covered in ash from the burnt logs (Dragon, Rennie et al., 2001). The majority of these sites contained remnants of charred bones, as well as intact cape hair. Most of the *B. anthracis*-positive environmental samples taken at Falaise Lake were from these bone beds. It was hypothesized that the large carcass of a bison may have insulated the ground from the heat of the fire, limiting the sporicidal effect of the incineration. The authors concluded that in future outbreaks, disposal sites should be revisited in order to burn any bone remains or cape hair.

d) Sporicides

In response to the Gruinard Island contamination with *B. anthracis*, laboratory tests were conducted to test the efficacy of various sporicides (Manchee, Broster et al., 1983). Chemicals tested included potassium permanganate, formaldehyde, glutaraldehyde, dodecylamine, and "activated" glutaraldehyde. Based on laboratory results, the scientists chose to field test formaldehyde, glutaraldehyde, peracetic acid and dodecylamine on Gruinard island. Soil samples were taken on experimental plots prior to, and 10 days after, treatment. The soil cores were categorized by soil depth, and were examined 21 days after collection. After testing the efficacy of the various sporicides on small experimental plots, 5% formaldehyde was selected for use in larger-scale field trials. This decision was based on its effectiveness, in combination with its relative low cost (Manchee, Broster et al., 1983).

Plots of 3m by 3m were sprayed with 5% formaldehyde solution, at either 20L per meter^2 or 50L per meter^2 (Manchee, Broster et al., 1994). Mowing or burning was used to remove vegetation from some of the plots, whereas some vegetation was left undisturbed. A week after application, soil cores revealed residual levels of formaldehyde in the samples (Manchee, Broster et al., 1994). However, no formaldehyde was detectable in samples taken at 52 or 96 weeks. Application of 50L per meter^2 was considered to be more effective than 20L per meter^2 at killing

spores (Manchee, Broster et al., 1994). Removal of vegetation seemed to be prudent, particularly when using 20L per meter^2. It was concluded that formaldehyde is an effective sporicide, as long as it is used in high enough concentrations, on areas with limited vegetation. Decontamination of Gruinard island was conducted in 1986 (Manchee, Broster et al., 1983; Manchee, Broster et al., 1994). Vegetation was sprayed with an herbicide and later burned. Irrigation tubing was laid, which released the biocide as a fine spray intended to decontaminate the top 15cm of soil. Seawater was used to dilute formaldehyde to 5%, and 1 km^2 plots received 50L per meter^2 of solution. Of 58 soil samples taken from known-contaminated areas, 2 months after the formaldehyde treatment, 3 were still contaminated (Manchee, Broster et al., 1994). All contaminated sites were irrigated with 37% formaldehyde to the layer of the bedrock. A flock of sheep were then grazed on the island for 5 months; none exhibited any symptoms of anthrax. This was considered a testament to the success of the decontamination procedure.

In the 1993 Mackenzie bison population outbreak, ten carcasses were treated with 400L of 3-5% formaldehyde (Gates, Elkin et al., 1995). Formaldehyde has not only been shown to decrease spore concentrations in areas where it is used, but also decreases scavenging by wildlife (Nishi, Dragon et al., 2002). Extensive scavenging had been noted on many of the carcasses upon discovery. Carcasses treated with formaldehyde were left unscavenged from that point forward, until incineration could be conducted.

e) Putrefaction

It was recognized almost a hundred years ago that it was very difficult to identify *B. anthracis* organisms in tissues submitted for laboratory confirmation of disease, if they had undergone significant putrefaction (Hagan, 1920). Gases produced during the process of putrefaction may destroy vegetative bacillus in a carcass if left intact (Andrjevski, 1928; Nishi, Dragon et al., 2002). Minett (1950) demonstrated that anthrax-positive goat carcasses stored at temperatures between 18-23 degrees Celsius contained viable anthrax bacilli in bone marrow for about a week(Minett, 1950). Goats stored at 10-15 degrees Celsius had bacilli in the skin for about 2 weeks. Unfortunately, scavenging releases vegetative cells into an aerobic environment, away from competitive putrefactive bacteria in the gut. This nutrient-depleted environment gives the vegetative cells the opportunity to transform into much more resistant endospores if exposed to

the correct temperature and humidity (Dragon and Rennie, 1995). Although covering a carcass with a tarp can deter scavenging and prevent the release of some bodily fluids (Turnbull, Diekmann et al., 2008), contaminated fluids may still be released into the soil due to ruptured skin caused by bloating or maggot activity (Bellan, Turnbull et al., 2013).

1.7.6 RISKS TO HUMANS

Management of anthrax outbreaks in wildlife is influenced by the potential zoonotic transmission of the disease. Humans can be infected in three different ways. The most common manifestation of disease is cutaneous anthrax, which may be seen as a papule that later develops into a vesicle (Watson and Keir, 1994). The vesicle will rupture and be replaced with a black eschar. The cutaneous form is treatable, and mortality rates are low if treatment is pursued. “Woolsorter’s Disease” is a historical term used to describe inhalational anthrax which, in contrast to the cutaneous form, has a very high mortality rate despite treatment, unless diagnosis is made very early. Individuals become infected by inhaling spore-laden dusts which reach the alveoli in the lungs. A severe bacteremia ensues, which overwhelms the immune system.Gastrointestinal anthrax can also affect humans. This disease is often limited to under-developed countries, where individuals may consume undercooked meat from anthrax-infected animals. This disease is also often fatal, as the time until diagnosis can be lengthy.

There are inadequate data to confidently establish what dose of spores would cause disease in humans for any of the types of exposures (Watson and Keir, 1994). However, humans are considered fairly resistant to anthrax, particularly in comparison to herbivorous animals. Both the virulence of the bacterial strain and the health of the human are expected to play a key role in determining the minimal infectious dose. A very conservative estimated dose for cutaneous infection in humans, based on research in mice and guinea-pigs with subcutaneous and intramuscular injections, is ten spores, but this estimate is probably very inaccurate due to the difference in susceptibilities between species and inoculation beneath the skin versus intradermal exposure. Although monkeys are considered more susceptible to anthrax than humans and body weight may play a role in the infectious dose, extrapolating inhalational doses from these animals would suggest a very conservative inhalational dose of 6000 spores.

For much of the public, anthrax is associated with bioterrorism. However, the risk of contracting the disease has predominantly been an occupational risk in North America, particularly for butchers, textile workers, and veterinarians. Field investigations of anthrax outbreaks between 1950-2001 by the CDC revealed most investigations were in agricultural settings or associated with textile mills.(Bales, Dannenberg et al., 2002). Exposure of agricultural employees to *B. anthracis* spores was suspected to be from direct contact during animal slaughter, butchering meat, or disposal of carcasses. Textile mill workers were exposed primarily through contact with goat hair. Six veterinarians were documented with cutaneous anthrax lesions, each after completing necropsies on infected animals.

Infection with anthrax can be treated, with success dependent on the route of exposure and time until treatment. Current recommendations for humans exposed to anthrax via a bioterrorism event include both vaccination and antibiotic treatment (Bales, Dannenberg et al., 2002). Penicillin, tetracycline, erythromycin and chloramphenicol are effective against most strains of anthrax (Watson and Keir, 1994).

The zoonotic potential of anthrax has played a role in the management of outbreaks and during research in Northern Canada. In the 1962 outbreak of anthrax in wood bison, two personnel involved in the disposal and decontamination processes contracted the disease (Moynihan, 1963). One was the Canadian Wildlife Service biologist who discovered the first bison carcasses of the outbreak (Dragon and Elkin, 2001). As anthrax had not been reported in the area previously, he had not worn appropriate protective equipment, such as gloves, when collecting samples from the carcasses. He developed cutaneous anthrax, and recovered with antibiotic therapy.

Recommendations regarding precautionary measures have been set forth in outbreaks due to concerns regarding human health. When a carcass is confirmed to be anthrax-positive, access to the area should be restricted from the public (Nishi, Dragon et al., 2002). Veterinarians are advised not to conduct post-mortem examinations on animals suspected to be infected with *B. anthracis*, and to instead collect only diagnostic specimens to be sent for confirmation (Bales, Dannenberg et al., 2002). The soil surrounding a carcass should always be considered highly

contaminated and a threat to human health (Dragon, Bader et al., 2005). During environmental surveys of bison carcass sites conducted between 1992-1997, personnel involved with sample collection were required to undergo prophylactic vaccination with the US anthrax vaccine (Dragon, Rennie et al., 2001). Furthermore, personal protective equipment such as double-layered gloves, disposable coveralls, rubber boots, and a HEPA-filtered respirator were worn.

Individuals involved in bison carcass-disposal wear personal protective equipment, with specific requirements determined by the governing body overseeing the outbreak management. The GNWT "Anthrax Emergency Response Plan" outlines precautionary measures to be taken by individuals involved in carcass disposal (Division, 2001). Crews in the 1991 WBNP outbreak were outfitted with respirators and protective clothing, and were vaccinated (Broughton, 1992). Although humans are generally considered resistant to anthrax, there have been several recorded incidents of inhalational anthrax associated with minimal exposure to spores (Watson and Keir, 1994). As such, precautionary measures such as personal protective equipment are critical during carcass disposal and research.

1.8 RESEARCH OBJECTIVES

1. To describe the 2012 anthrax outbreak in the Mackenzie Bison Population of wood bison, in order to compare and contrast it with other known outbreaks.
2. To evaluate the serological epidemiology of anthrax in the Mackenzie Bison Population, to provide a better understanding of wood bison exposure to *B. anthracis* both in outbreak, and non-outbreak, years.

CHAPTER 2 ANTHRAX IN WOOD BISON OF THE MACKENZIE BISON POPULATION 2012 – AN OUTBREAK OVERVIEW

This chapter outlines the investigation of an anthrax (*Bacillus anthracis*) outbreak in the Mackenzie bison population of wood bison (*Bison bison athabascae*) in 2012. The main purpose of the chapter is to describe the epidemiology of the outbreak, and to compare and contrast it with previous outbreaks of the same disease in the herd. In addition to completing the descriptive epidemiology, the author of this thesis was dispatched into the field to assist with carcass disposal and data collection during the epidemic.

2.1 ABSTRACT

Anthrax, caused by the spore-forming bacterium *Bacillus anthracis*, poses a threat to wood bison (*Bison bison athabascae*) conservation. This paper details a large outbreak of anthrax in the Mackenzie bison population, NWT in 2012 using descriptive epidemiology. Datasheets were completed in the field by Emergency Response Team personnel, then entered into an excel database. Between late June and early August, 451 bison were found dead, with peak mortality from July 13-19. Unlike previous anthrax outbreaks in the herd, a substantial number of calves and yearlings died, as well as adult females. Three geographic regions (A, B, C) were examined for differences in outbreak characteristics: Location C had a greater percentage of male carcasses than the other sites and Location A had more calf deaths. Based on the herd population estimate in 2013, it is possible that not all dead bison were found during the outbreak. Further research is required to understand the reasons why bison die from this disease in some years, but not others, in order to implement control measures.

2.2 INTRODUCTION

The Mackenzie bison population was once the largest wild herd of wood bison in the world free of both bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortis*), before an anthrax outbreak in 2012 killed a significant number of the animals (Northwest Territories Environment and Natural Resources, 2010). The herd was established in 1963 from 18 wood bison, captured in WBNP, which were transported across Great Slave Lake and released near Fort Providence (Gates, Elkin et al., 1995). The herd size increased to an estimated 2000 animals by 1992. In 1993, an anthrax outbreak swept through the herd and killed 172 bison (Gates, Elkin et al., 1995). Although anthrax outbreaks had previously been observed southeast of Great Slave Lake in the SRL and WBNP, this was the first time anthrax had been detected in the Mackenzie herd since their arrival 30 years earlier. The herd experienced another outbreak in 2010 in which 9 animals were found dead (GNWT unpublished data).

A routine surveillance flight conducted by the GNWT discovered 128 bison carcasses on July 3, 2012 (Miltenberg, 2012). The carcasses were located at Mills Lake within the bison herd range. At this time, the Anthrax Emergency Response Plan was activated which included enhanced surveillance and carcass disposal in order to decrease environmental contamination with anthrax spores. This report provides a detailed summary of the outbreak based on information recorded from the field, and compares and contrasts this outbreak with previous outbreaks.

2.3 MATERIALS AND METHODS

Emergency Response teams were dispatched throughout the Mackenzie bison population to dispose of bison carcasses beginning in early July. Each team was given carcass disposal reports to complete by hand for each carcass. Data sheets were collected and maintained in the outbreak headquarters for post-outbreak entry into a database.

Data were entered into an Excel database; the database was separated into “Visit 1”, “Visit 2”, and “Visit 3” (V1, V2, V3) sheets. The front of the carcass disposal report (see Figure 5-1 in Appendix), which usually represented a record of the first visit, was entered as either V1 or V3

data, depending on the date when the sheet was filled. If there was only one front side report for an animal, it was entered as V1, unless it described the effects of the disposal method used. In this case, it was entered as V3, even if no earlier reports were available. If there were two front side reports, the earlier visit was entered as V1 and the later visit as V3. The backside of the carcass disposal report, which was not always recorded on the same day as the front, was entered as V2 data (Figure 5-2 in Appendix).

The majority of any analysis was done using V1 information. If information was missing for an animal from V1, but provided in V2 or V3, that information was then used. If there were discrepancies between V1, V2 or V3 sheets, V1 data were used; the only exception to this was an obvious mistake (such as an incorrect month).

Herd composition surveys of the Mackenzie bison population were conducted by the GNWT in 2011 and 2013. We used the 2011 composition numbers to represent the 2012 herd. As the 2012 outbreak was quite large and didn't necessarily affect all ages within the group equally, we were concerned that averaging the age composition numbers of 2011 and 2013 may have created an inaccurate representation of the 2012 herd. A herd population estimate and accompanying standard error was calculated by the GNWT for the Mackenzie bison population in 2012 before the outbreak, and again in 2013.

Descriptive statistics were completed using Ausvet epitools (Sergeant 2013) and SPSS Version 22 (IBM, 2012). Confidence limits for a single proportion were calculated using the Wilson Score technique as recommended by Brown, Cai et al. (2001).

Three separate geographic areas with bison deaths were identified by an experienced field wildlife veterinarian (Brett Elkin, personal communication, October 5 2013). These are described as "Location A", "Location B", and "Location C".

2.4 RESULTS

During the outbreak, 451 bison deaths were recorded and entered into the database. Only V1 data were available for 65 bison, no animals had only V2 data, and 2 animals had only a V3 sheet; 369 had both V1 and V2 data, and 9 had each of V1, V2 and V3. Data were also available for three moose, which were not included in the analysis.

The date when the carcass was initially found was recorded for 143 of the animals. Dates ranged from July 4-August 25 2012. The date that sampling/treatment was performed on a carcass was also recorded for 143 animals, with dates ranging from July 5 – August 9 2012. Of the 69 records with both the date found and date of sampling/treatment recorded, 54 had different dates for the two fields while 15 had the same date. Differences between the two dates ranged from 1 to 6 days. Estimated length of time dead was noted for 261 bison, and ranged from 0.5-150 days.

Two epidemic curves demonstrating date of death were constructed by subtracting the estimated length of time dead in days from the date of sampling/treatment of the carcass (Figure 2-1 and 2-2). It was assumed that the estimate of time dead would have been made on the same day recorded as the date of sampling/treatment. For example, if a carcass was treated or sampled on July 10 and estimated to have been dead for 5 days, the animal died on July 5. Both the estimated length of time dead and the date of sampling/treatment needed to be recorded in order for the animal to be included in the analysis. Estimated lengths of time dead were truncated to their integer value (example “>14 days” recorded as 14 days).

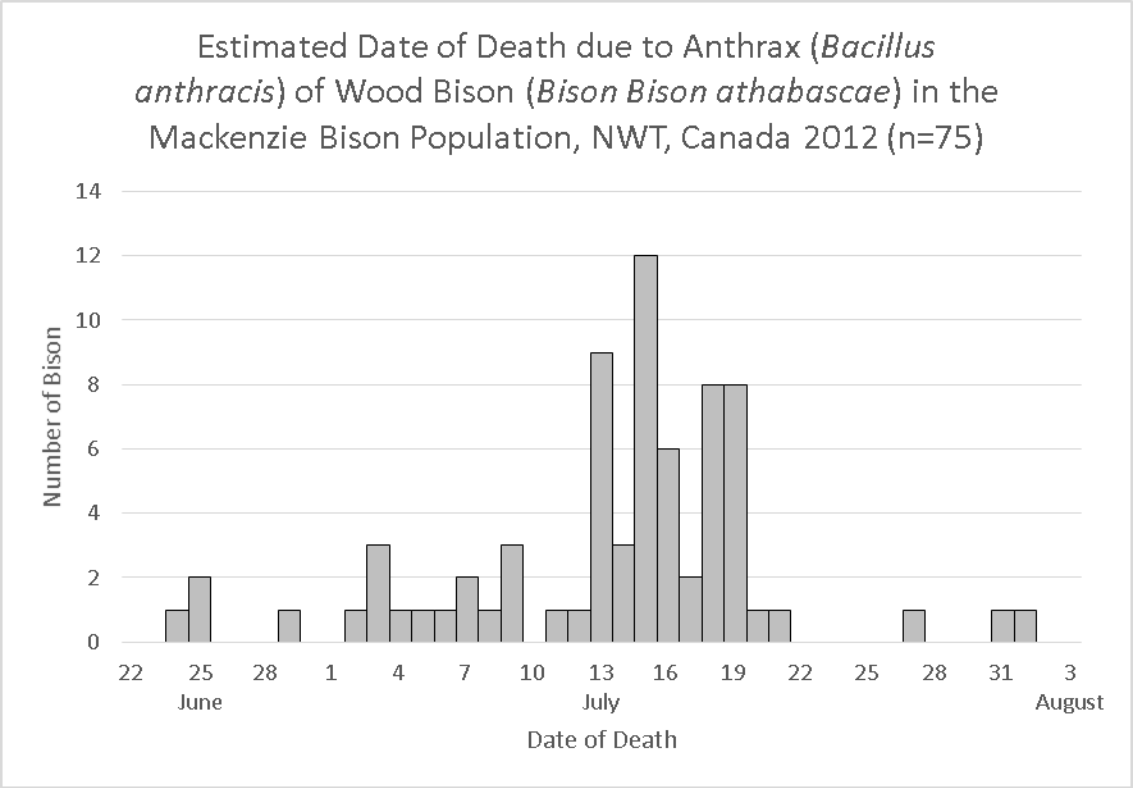


Figure 2-1 Estimated date of bison death calculated as (date of sampling/treatment – estimated length of time dead). No values were categorized in this chart. Records with dates over one month before all other records were considered incorrect data and excluded (April 29, n=3).

We suspect that the accuracy of estimating time dead for a carcass decreases as the carcass ages. In order to account for this variable accuracy, estimated lengths of time dead were categorized as suggested by an expert wildlife veterinarian (Brett Elkin, personal communication, October 17 2013) (Table 2-1, Figure 2-2). In both Figures 2-1 and 2-2, the deaths began in late June 2012, with peak deaths July 13 - July 19 and the outbreak tapering off by the beginning of August.

Table 2-1 Categorization of estimated length of time dead.

Recorded Length of Time Dead on Sheet (days)	Categorized Length of Time Dead (days)	Number of bison
0-6.5	Same as recorded on sheet	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

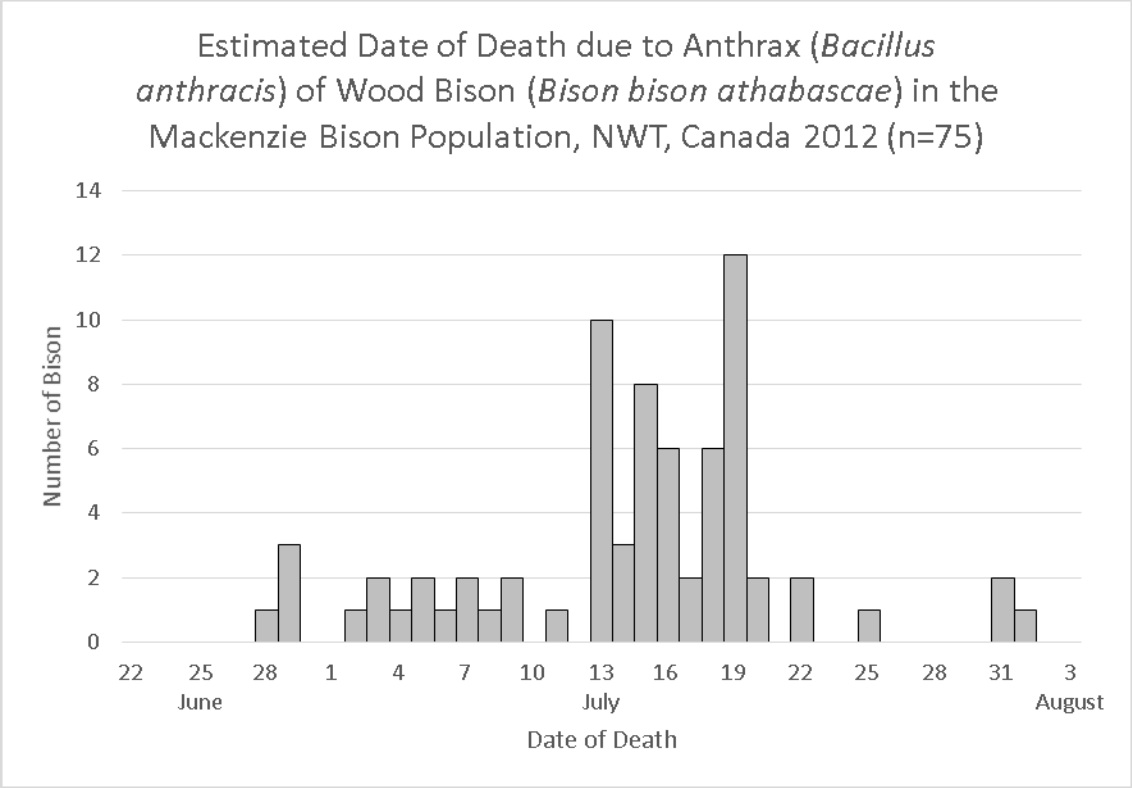


Figure 2-2 Estimated date of bison death calculated as (date of sampling/treatment - estimated length of time dead). Values for estimated length of time dead were categorized as described in Table 3-1. Records with dates over 28 days before all other records were considered incorrect data and excluded (May 29, n=3).

The carcass condition was recorded for 401 bison. In descending order, 213 were classified as “mummified”, 102 as “poor”, 36 as “disarticulated”, 29 as “good” and 21 as “fair”. The percentage of carcass mass remaining was recorded for 391 animals (Figure 2-3).

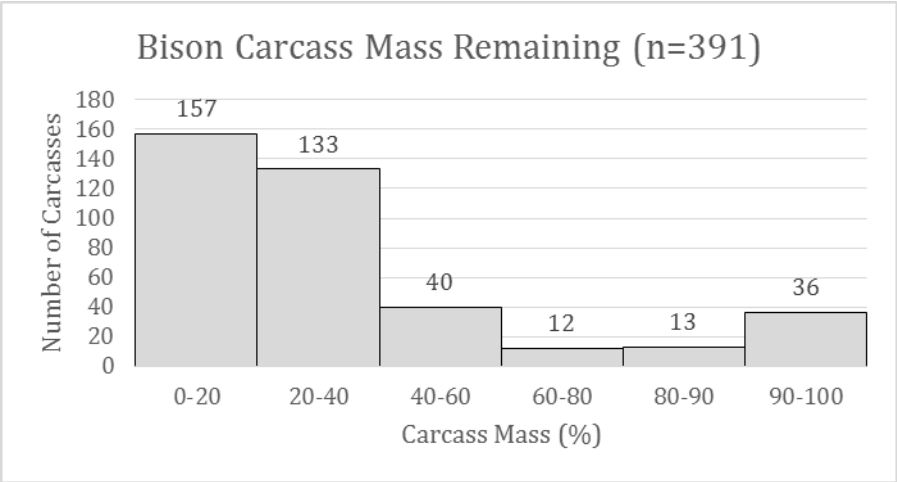


Figure 2-3 Percentage of carcass mass remaining at time datasheet was recorded. Excluded missing records (n=60). Carcasses with higher masses (ex 90-100%) would be considered in better condition than those with lower values.

Datasheets from 242 bison carcasses noted the presence or absence of carcass scavenging. One hundred and thirty one of these recorded scavenging; 111 reported an undisturbed carcass. Only 23 sheets described the suspected scavenger species. Seven were thought to be scavenged by wolves, 5 by birds, 7 by bears, and 3 had more than one scavenger species recorded.

There was a statistically significant correlation between carcass condition and evidence of scavenging (Spearman’s rho -0.380, $p<0.001$), carcass condition and percentage carcass remaining (Spearman’s rho -0.692, $p<0.001$), and evidence of scavenging and percentage carcass remaining (Spearman’s rho 0.508, $p<0.001$).

An estimated age in years was recorded for 307 of the bison, which excludes 22 animals where only a range was given (for example, >10 years).

Table 2-2 Age distribution of 307 wood bison carcasses in the 2012 Mackenzie bison population anthrax outbreak.

	Age (Years)
Minimum	0.3
Maximum	15
Median	6
Mean	6.4
Mode	10

Age classes were recorded for 400 bison. Age class distribution is shown in Figure 3-4.

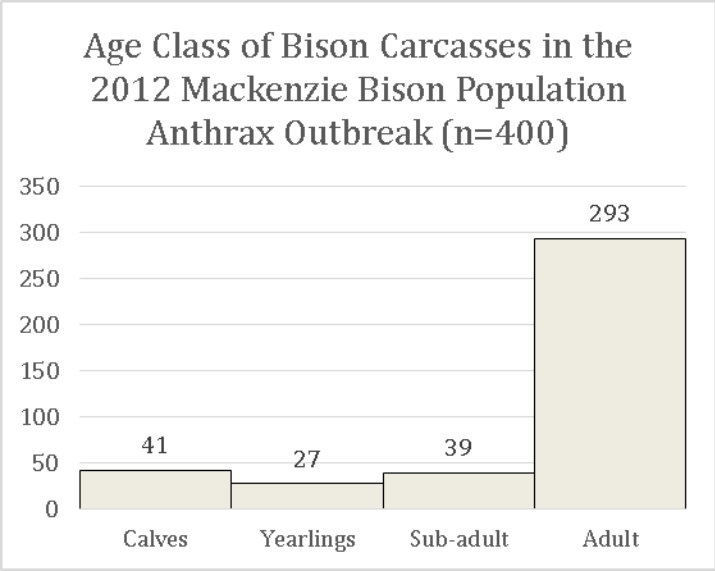


Figure 2-4 Age class distribution of wood bison in the 2012 Mackenzie bison population anthrax outbreak (n=400).

Gender was recorded for 336 animals, of which 178 (53%) were male and 158 (47%) were female. Seventy six of the sheets recorded gender as “unknown”, and 39 sheets had no data entered in this field.

Age class was recorded for 169 males, of which 150 were adult, 13 were sub-adult, 5 were yearlings, and 1 was a calf. Of the bison known to be female, 114 were adult, 19 were sub-adult, 3 were yearlings, and 1 was a calf.

The type of geographic site in which a carcass was located was recorded for 388 of the animals. Options included clearing, wooded area, burned site, muskeg, hillside, water, or other. More than one option could be selected. If no options were circled but a written description was given that did not fit any of the options, "Other" was recorded in the database and the written description was captured in the "Comments" field. The results are displayed in Figure 2-5.

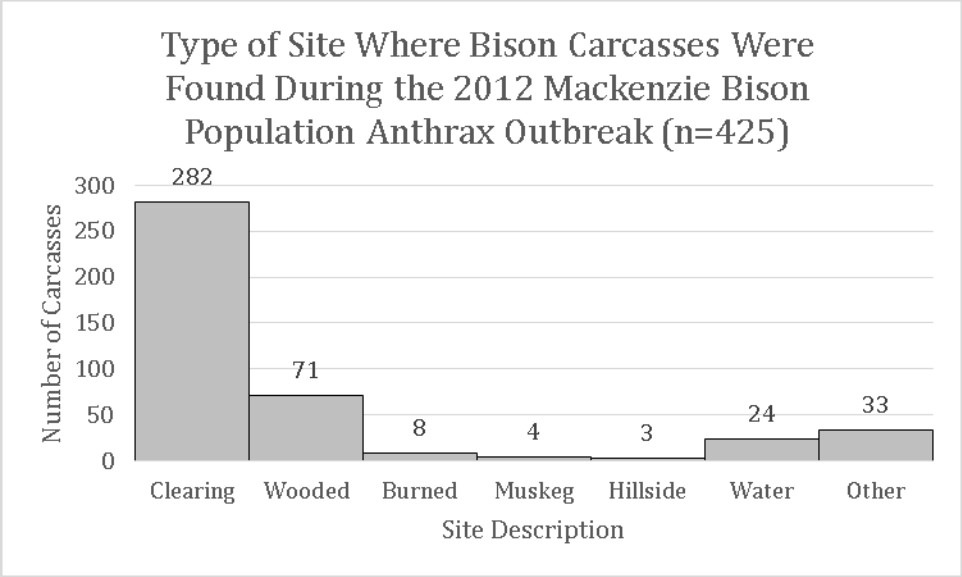


Figure 2-5 Description of the geographic site where bison carcasses were discovered during the 2012 Mackenzie bison population anthrax outbreak. More than one option could be selected for each carcass (n=425 selected site descriptions, recorded for n=388 bison).

Three hundred and ninety five bison carcasses had GPS coordinates recorded (Figure 2-6).

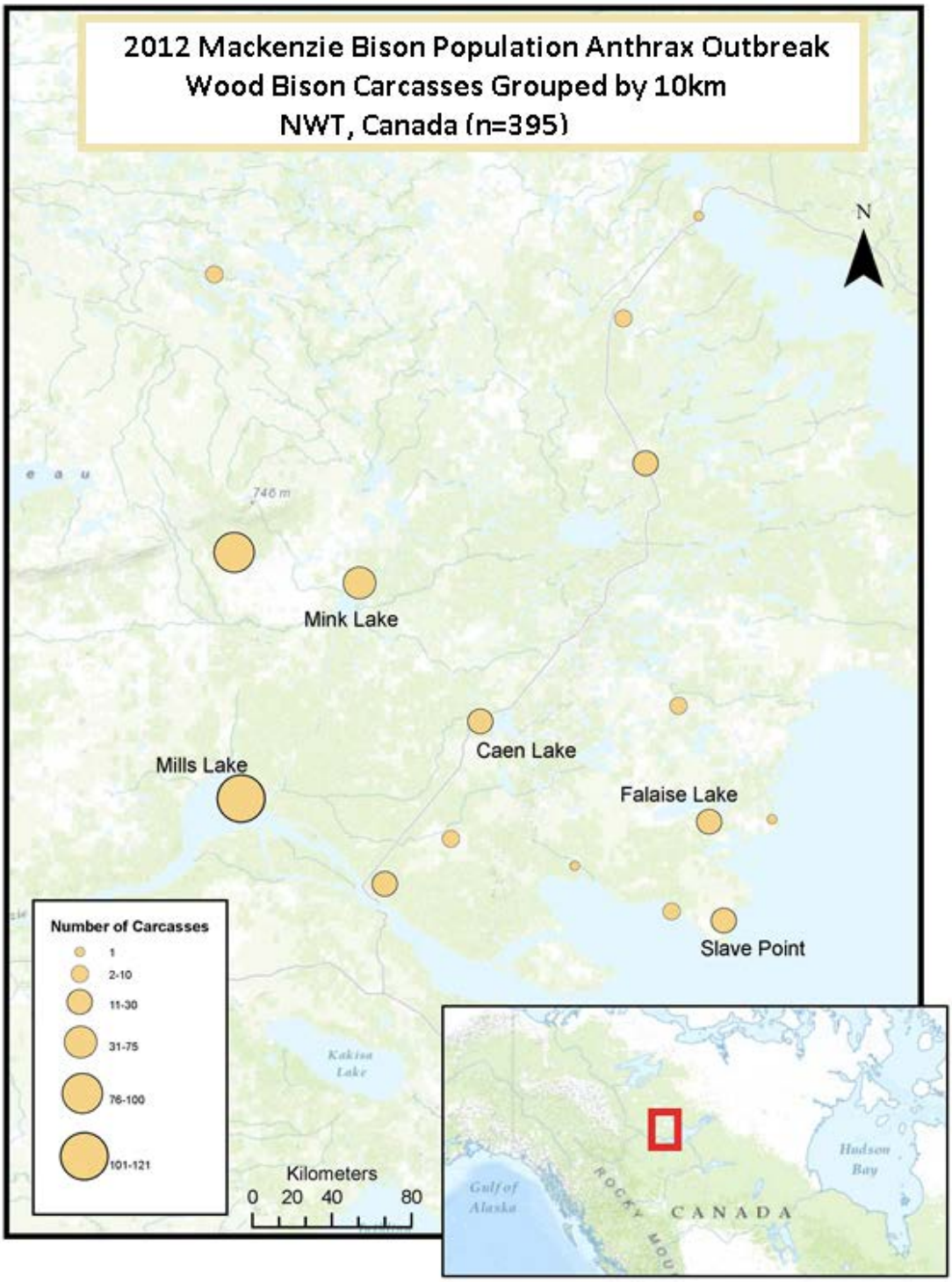


Figure 2-6 Carcass locations grouped by 10km radius. NWT, Canada (n=395).

The 2012 epidemic had anthrax cases both in new and repeat locations from other outbreak years. Figure 2-7 illustrates the carcass locations from 1993, 2010 and 2012 grouped by a 10km radius.

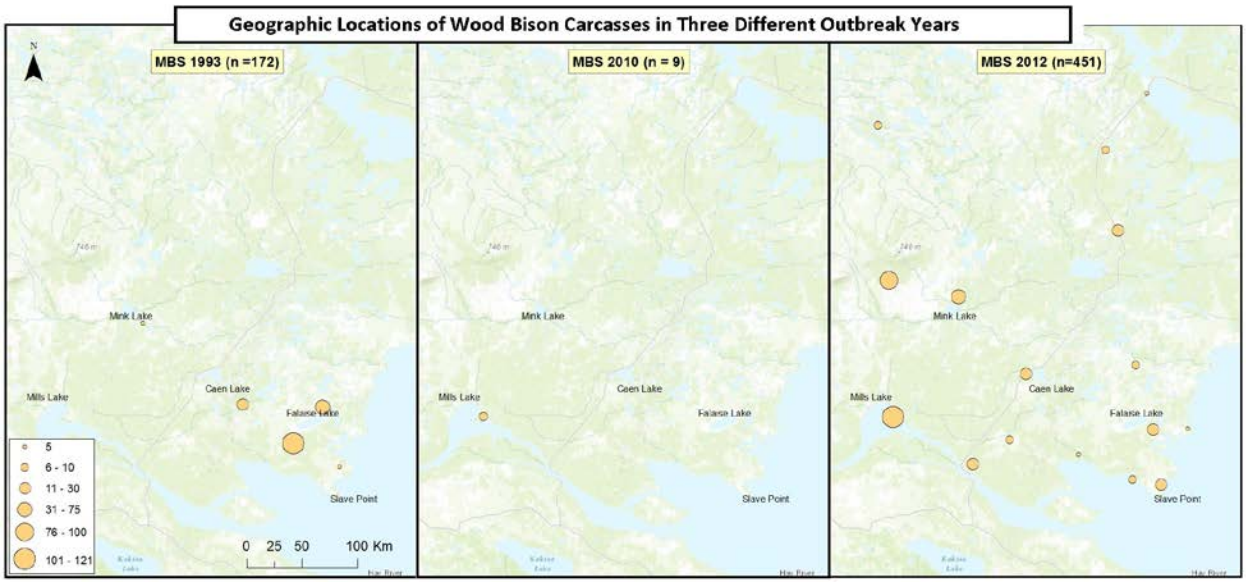


Figure 2-7 Carcass locations grouped by 10km radius from three different outbreak years. Total number of discovered carcasses shown. NWT, Canada.

Three geographic areas from the 2012 outbreak (Figure 2-8) were compared with respect to age and sex composition of the dead bison, the date of first deaths and the duration of the outbreak in order to detect any significant differences between the locations.

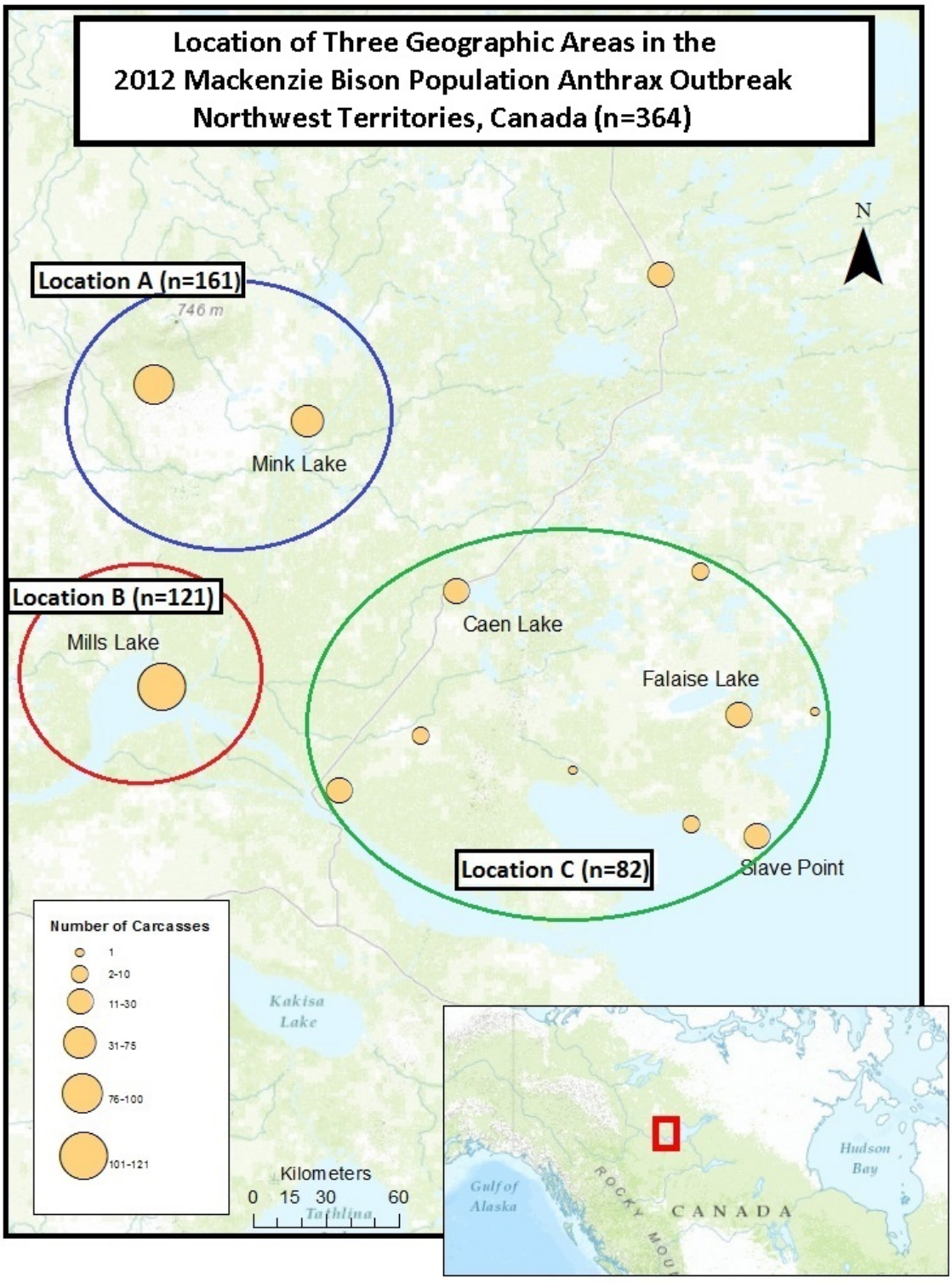


Figure 2-8 Three geographic areas, NWT, Canada (n=364).

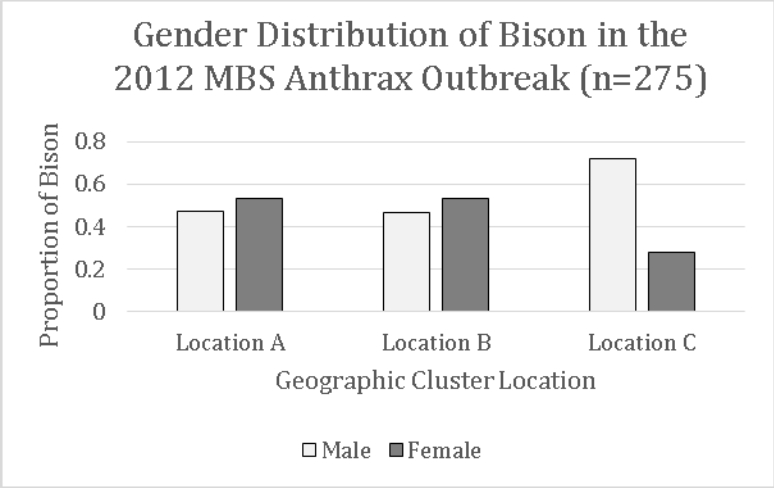


Figure 2-9 Comparison of gender distribution across three geographic regions, NWT, Canada (n=275). Excluded records missing a gender value (n=89).

There was a significant association between the geographic area location and the proportion of males and females found (X^2 = 13.811, p=0.001). Gender proportions at Locations A and B were not significantly different. Location C had a greater percentage of male carcasses than the other areas, and fewer females.

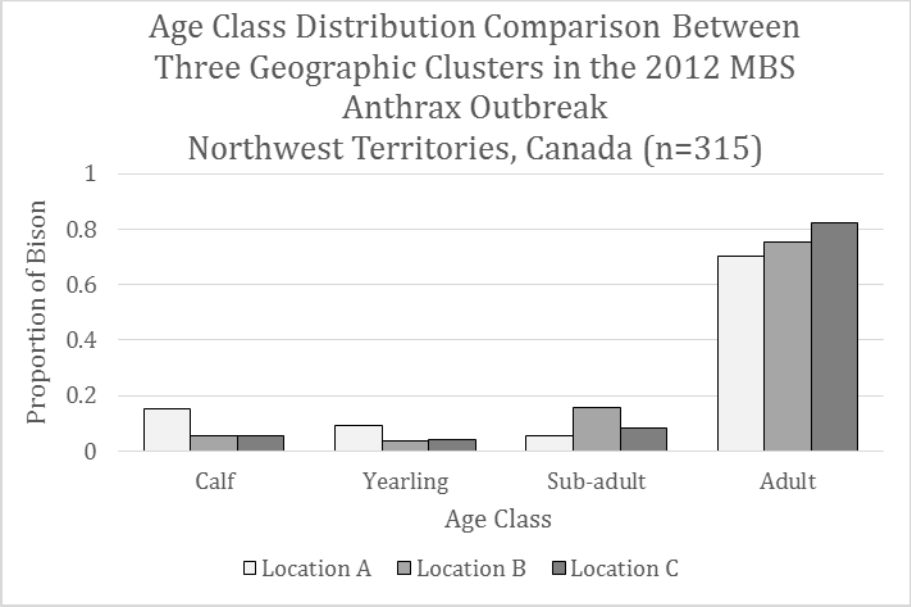
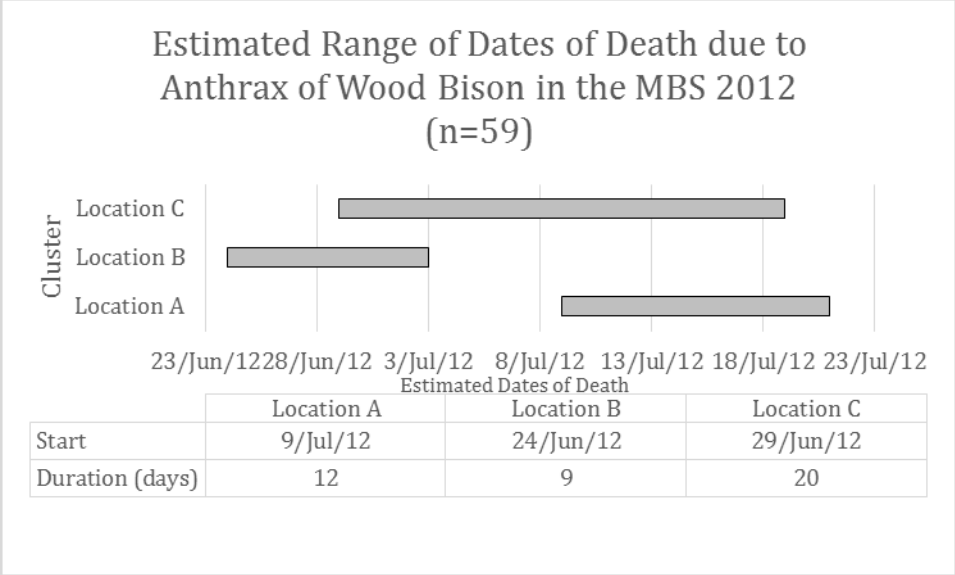


Figure 2-10 Comparison of age class distribution between three geographic areas. NWT, Canada (n=315). Excluded records with missing values or “unknown” age class (n=49).

Three hundred and fifteen animals within the geographic regions had an age class recorded. There was a significant association between the region location and the proportion of different age classes of bison (X^2=18.630, p=0.005), but the strength of the association was weak (Cramer's V=0.172, p=0.005). Location A had a higher proportion of calves than the other locations, but the proportions of yearlings, sub-adults and adults were not significantly different between the three locations at the 0.05 level.

The anthrax outbreak began earliest in Location B, followed by Location C and finally Location A (Figure 2-11). Location B had the shortest outbreak duration, while Location C had the longest.



	Location A	Location B	Location C
Start	9/Jul/12	24/Jun/12	29/Jun/12
Duration (days)	12	9	20

Figure 2-11 Duration of anthrax outbreak in three geographic regions in Mackenzie bison population, NWT, Canada 2012. Records missing either the Date of Treatment or the Estimated Length of Time Dead were excluded (n=302). Also excluded outliers (April 29, n=3).

Assuming that all carcasses were found, all animals found died from anthrax, datasheets missing recorded values did not bias the overall picture, and that the 2011 herd composition survey was an accurate depiction of the 2012 herd before the outbreak, the following observations can be noted.

The 2013 wood bison herd population estimate was significantly different than the 2012 estimate (2 sample t-test, $p<0.001$). The 2012 herd contained 1531 (SE 257) bison before the outbreak; the 2013 herd had only 714 (SE 156). The difference (817 animals; 95% CI 796.6-837.4) equals a 53.4% (95% CI 0.509-0.559) reduction in overall herd size. As well, 18% (95% CI 0.136-0.236) of all calves in the Mackenzie bison population died in the outbreak. Sixteen percent (95% CI 0.114-0.226) of the yearlings died, and 28% (95% CI 0.256-0.31) of the adult bison died. Finally, a larger proportion of adult males were killed in the herd than adult females ($p<0.0001$, $z=5.7$, one tailed-test). Approximately 35% (95% CI 0.303-0.392) of the adult male bison died in the herd, whereas about 19% (95% CI 0.159-0.222) of the adult females died.

2.5 DISCUSSION

Not all bison carcasses found had the same fields of data recorded. Some bison had only one visit; others had multiple visits with more than one disposal record. There were many people filling in the datasheets during the outbreak, so there was often a lack of consistency in the way answers were reported. Sheets were filled in by disposal crew personnel, who usually had a long list of bison to incinerate each day. As such, data management might not have always been done consistently by crews working on the ground. Numerous data fields were left blank for many of the carcasses, which is a major limitation of this analysis. Since there was no field to record who filled in each sheet, we could not identify if missing values bias the overall results (for example, if a certain person consistently left the same values blank). Adding this field to future surveys might be an asset for data analysis.

Furthermore, we could not assess discrepancies between different recorder's subjective opinions. For example, some recorders may have had lots of experience determining the age of bison in years, while others may have been very new to the procedure. Individuals uncertain about an answer to a field were sometimes only able to choose to estimate an answer, or leave a field blank. There is no way to differentiate between these two scenarios on any sheet, which may affect the accuracy of the results.

As records were written by hand on paper, duplicates were sometimes made for the same carcass if a sheet was temporarily misplaced. Finding a way to record consistent data for all carcasses during every outbreak would be beneficial for epidemiological analysis of anthrax outbreak information. As well, developing an electronic format to enter data in the field could save time by removing the need to input the handwritten data onto a computer, decrease data entry errors, and might prevent duplicates. Past outbreaks have had only one or two people recording data for the bison carcasses. The reports were not developed to be used by many different individuals with diverse backgrounds and experience. Future large anthrax outbreaks may warrant designated personnel to be specifically trained in the methods of recording datasheets, rather than sharing this responsibility among disposal crew members.

One hundred and twenty eight bison were found by air surveillance on July 3, 2012. None of the datasheets had this date recorded as “date found”, since it took at least one day for personnel to be mobilized to the sites. Similarly, many bison were found by air surveillance throughout the outbreak, and teams were dispatched the following day to begin the disposal process. In some of these cases, the “datefound” recorded on the record sheets may have been the date the crew reached the carcass by ground, not when it was found by aircraft. This slightly biases the “datefound” results towards a later date. A more effective method of data collection may be an electronic format, so that carcass information is recorded as soon as it is found in the aircraft and is accessible to ground crew who later visit the site.

Estimating the length of time that a bison has been dead probably becomes quite difficult past a certain time. This is why two epidemic curves were created, in order to see if categorizing lengths of time dead would provide a seemingly more accurate curve. Surprisingly, both of the charts show a similar trend. In order for a carcass to have been included in this analysis, the record sheets needed to have contained both a date of sampling/treatment, and an estimated length of time dead. Only 75 of the sheets had both of these values. None of the estimated lengths of time dead that were suspected to be very inaccurate (such as 150 days) also contained a date of sampling/treatment, so were not included in either curve. This explains why both curves show a similar trend. As was mentioned previously, we assumed that the estimation of time dead was made on the same day as the date of sampling/treatment. It was unclear whether the

"datefound" field was the day the carcass was found by air or the day it was reached by ground crew, which is why it was not used in the calculations.

Combining both the "date of sampling/treatment" and "time dead" provided a way to estimate a date of death. This outbreak was generally contained within the month of July, with a few outliers at the end of June and the beginning of August. 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 based on dates the carcasses were found. Dead bison in the 1993 epidemic were found between July 29 and August 26 (Gates, Elkin et al., 1995), and carcasses in 2010 were discovered between August 13-21 (Brett Elkin unpublished data). Carcasses were found from July 3 until August 25 in 2012, suggesting that this outbreak probably started earlier than either of the other two. However, it is difficult to compare estimated outbreak dates without an understanding of the state of decomposition of each carcass found in the 1993 and 2010 outbreaks.

Carcass condition and mass can be affected by many different factors, including length of time dead, weather, ante-mortem health of the bison, and scavenging. The majority of bison carcasses were recorded as "mummified", which describes an advanced state of decomposition where the remaining flesh is desiccated. Another 138 were described as either "poor" or "disarticulated", either of which may describe a carcass in advanced decomposition or releasing fluid into the surrounding environment. Only 29 were listed as "good" condition. In a similar trend, 290 of the bison had less than 40% of their carcass mass remaining. In contrast, only 36 had almost completely preserved carcass masses (90-100%). It is likely that many of the carcasses were not found soon after death, which could account for both poor carcass conditions and decreases in mass. Hot summer temperatures may have also accelerated decomposition rates. Ideally, the bison should be found before advanced decomposition has occurred which can release bodily fluid, and spores, into the environment. Detecting mortalities in a wildlife population presents many challenges, sometimes including a lengthened period of time before a carcass is discovered.

There was a wide range in ages of bison affected in this outbreak; however, 73.2% of the bison carcasses found were adults. Previous outbreaks in bison in Northern Canada have had a similar age distribution (Gates, Elkin et al., 1995; Salb, 2010). However, the number of calves and yearlings also affected by the disease was noticeably higher than in other outbreaks, particularly of those in the Mackenzie bison population. In 1993, only 2 calves were found and no yearling bison (Gates, Elkin et al., 1995). Based on a pre-outbreak herd composition survey, the authors estimated that essentially 0% of the calves or yearlings in the herd died from anthrax. Similarly, none of the 9 carcasses discovered in the Mackenzie bison population in 2010 were calves or yearlings (GNWT, unpublished data). In contrast, 18 percent of the Mackenzie bison population calves died in the 2012 outbreak as well as 16 percent of the yearlings. The reasons for this are unknown. Possibilities could include an acquired virulence factor in *Bacillus anthracis* which made it more pathogenic across all age categories, decreased immunity in the young bison due to nutritional stress or other environmental stressors, or increased environmental exposure to anthrax from the large number of infected carcasses.

The proportion of male and female carcasses found was not strikingly different, although the proportion of adult male bison in the herd which died was larger than adult females. In the 1993 outbreak, approximately 2% of the adult cows in the herd died, while an estimated 23% of the adult and mature males were lost (Gates, Elkin et al., 1995). In 2010 only male bison were found, but a herd composition survey was unavailable to compare the total number of adult males in the herd. Regardless, only a few animals were found hence the proportion of adult males in the herd which died would be very low. As such, the 2012 outbreak more similarly resembled the 1993 outbreak than the 2010. However, 35% of the adult males and 19% of the adult cows died in this outbreak, both of which are higher than the 1993 epidemic. Again, reasons for this are unknown but are likely related to the same mechanisms by which more calves and yearlings were affected than in previous years.

There was no significant difference between the proportion of scavenged carcasses and those left intact (95% CI 0.009-0.169, p=0.078). In other words, about half of the carcasses were scavenged and half were not. In the 1993 outbreak, the vast majority of carcasses had signs of scavenging or the presence of scavengers such as birds (Gates, Elkin et al., 1995). Wolves, birds

and bears were the suspected scavengers 2012, which is similar to 1993. Minimal scavenging data was recorded in the 2010 outbreak. Reasons for decreased scavenging in the 2012 outbreak could include an abundant availability of other food sources that year for scavengers, or a decrease in population of scavenging species.

Most of the carcasses (n=282) were found in clearings, with the next most common site description being wooded (n=71). One of the often debated questions in anthrax management of wood bison is the number of dead bison being missed by surveillance. Of the 163 carcasses in the 1993 Mackenzie bison population outbreak that had vegetation cover recorded, only 45% were found in clearings (Gates, Elkin et al., 1995). If that is where most of the bison reside, then it is unlikely that many dead bison are being missed since carcasses are relatively easy to locate by aircraft when in clearings. However, if the majority of carcasses are being detected in clearings because they are difficult to locate in other geographic areas, then carcasses may have been missed in 2012 and the geographic location results would be skewed. In the 1993 outbreak, most of the carcasses located in wooded areas were detected using infrared imaging (Gates, Elkin et al., 1995). Since an infrared camera was not available for use during the 2012 outbreak, it is possible that many bison carcasses in wooded areas were not discovered by surveillance. The impact of missing numerous anthrax carcasses on environmental spore load and future outbreaks is difficult to assess and has never been quantified.

Three different geographic regions were analyzed in an attempt to detect any differences between the locations, in case deaths grouped by geographic region could provide unique clues about the epidemic. One of the locations (Location C) had more dead male carcasses than the other regions, although there is no data to compare the herd gender ratios between the three areas. It is possible that there were simply more male bison inhabiting this area, which caused a greater number to be found dead. Location A had more dead calves than the other locations, but the outbreak seemed to affect most age classes equally across all three regions.

One of the major limitations in detecting where this outbreak may have first originated across the regions is the lack of data. Only five animals in Location B had sufficient data to predict a date of death, therefore the estimated start date or duration of the outbreak in this area may be

inaccurate. Based on available data, it appears the outbreak started near Mills Lake, then proceeded first east then north.

Four hundred and fifty one bison were found dead in this outbreak. However, the difference between the 2013 and 2012 herd survey estimates was 817 animals; nevertheless, there are several possible explanations for this discrepancy. First, herd population estimates of wildlife have large standard errors. The 2012 herd estimate was actually 1274-1788 bison, while the 2013 estimate was 558-870. Using these ranges, it is possible that a significant portion of all dead bison were found. Conversely, there may have been an even larger discrepancy than predicted. Second, wild bison die for reasons other than anthrax. For example, deaths would have occurred between the 2012 and 2013 surveys due to natural mortality, predation and highway mortalities. Furthermore, starvation and drowning may have also affected the herd. Finally, an unknown number of carcasses may have been missed during the outbreak. As was mentioned previously, thermal imaging was unavailable for surveillance which may have prevented detection of animals otherwise unobservable from an aircraft.

Wood bison were once an endangered subspecies who have gained “special concern” status by COSEWIC through strong conservation efforts, but are still listed as “threatened” under the federal Species at Risk Act (COSEWIC, 2002; "Species Profile (Wood Bison)," 2011). Some of the conservation herds have struggled with endemic diseases such as bovine tuberculosis and brucellosis, but the Mackenzie bison population has thus far remained clear of these threats (Gates, Elkin et al., 1995; Northwest Territories Environment and Natural Resources, 2010). However, this herd has been significantly affected by anthrax, and all hunting was suspended beginning in 2012 (Miltenberg, 2012). The high number of cows affected in this outbreak may significantly affect calving rates in subsequent years, and the herd will need to be closely monitored to make appropriate management decisions.

2.6 CONCLUSIONS

The 2012 anthrax outbreak killed at least 451 bison in a herd of approximately 1500 animals. It is clear that this disease presents a significant challenge to the wood bison conservation effort, particularly in this Northern Canadian population of animals. Managing a disease with an environmental reservoir in a wildlife population is an incredibly challenging task, with ongoing research needed to provide the best evidence for decision makers.

CHAPTER 3 SEROLOGICAL EPIDEMIOLOGY OF ANTHRAX OUTBREAKS IN THE MACKENZIE BISON POPULATION

The following chapter investigates serological epidemiology of wood bison (*Bison bison athabascae*) in the Mackenzie bison population with respect to anthrax (*Bacillus anthracis*). This herd has experienced anthrax outbreaks 3 times, and serological data help to provide evidence about exposure to the bacterium in non-outbreak years. The author of this thesis retrieved and collated data from several different sources in order to create the most complete database possible.

3.1 ABSTRACT

Serum samples from 278 wood bison (*Bison bison athabascae*) in the Mackenzie bison population were collected from various sources between 1986 and 2009, then tested for antibodies against protective antigen (PA) as an indication of exposure to *Bacillus anthracis*. These results were made available for use in this study. Overall, there were 191 positive samples. With respect to proportion positive by gender, 18.2% of the submissions from females and 35.5% from males were seropositive. The year with the highest proportion of positive submissions was in 1994 (90%), the year following the only anthrax outbreak within the dataset. In both males and females, adults had a higher prevalence of being seropositive than any of the younger age categories.

3.2 INTRODUCTION

The Mackenzie bison population, located northwest and adjacent to Great Slave Lake in the NWT, has had three known anthrax (*Bacillus anthracis*) outbreaks since the wood bison (*Bison bison athabascae*) were introduced in 1963 (Gates, Elkin et al., 1995; GNWT, 2014). Much of our understanding of anthrax outbreaks in Northern Canadian wildlife is derived from opportunistic mortality data rather than antemortem serologic surveillance, which has

contributed to the hypothesis that anthrax is generally rapidly fatal in wood bison (Bagamian, Alexander et al., 2013). Microbiological investigation by Jolianne Rijks (1999) revealed that some wood bison in the Mackenzie bison population had antibody titres against *B. anthracis* both before and after the first outbreak in 1993, suggesting that the animals were exposed to the bacterium in non-outbreak years and that some animals survive exposure(Rijks, 1999)(Rijks, 1999)(Rijks, 1999)(Rijks, 1999). The objective of this study is to describe the serological epidemiology of anthrax in the Mackenzie bison population and to compare those results with known outbreaks.

3.3 MATERIALS AND METHODS

Opportunistic serological samples were collected from 1990 to 2009 as part of a collaborative effort between GNWT, Parks Canada and CFIA (GNWT ENR, personal communication). The samples were tested with the "Immunetics QuickELISA Anthrax-PA Kit", which detects IgG antibodies against protective antigen (PA) in serum (Immunetics, Inc, Boston, MA 02210-2377 USA). Samples were categorized as "negative", "low positive" or "positive". The resulting data was provided for use in this analysis. We chose to combine "low positives" and "negatives" for this study.

Other serological data from 1986-1994 collected by Jolianne Rijks (1999) as part of a Master of Science thesis was also made available for use. Specific information about the laboratory tests used to calculate antibody titres can be found in the thesis entitled "A serological study of bison (*Bison bison*) in an area of northern Canada experiencing sporadic and epizootic anthrax". In summary, an ELISA was used to test sera for IgG and IgM antibodies against all three toxin factors, but with an emphasis on PA due to its selection for use in previous studies. The author categorized results as "negative", "grey", "intermediate" or "high" based on values listed in Table 3-1. For the purposes of this study, "negative" and "grey" were considered negative titres, while "intermediate" and "high" were both considered positive. Both sets of serological data used slightly different, but comparable, methods of analysis. Values from each set were categorized as discussed, in order to be able to combine the data for analysis.

Table 3-1 Classification of anti-PA titre results.

Value	**Classification by Rijks**	**Classification in this Study**
0	Negative	Negative
>0 to <1:80	Grey	Negative
>=1:80 to <1:320	Intermediate	Positive
>=1/320 to <=1:2560	High	Positive

Historical herd population and composition data was provided for use by the Government of the Northwest Territories (2014) (GNWT, 2014) (GNWT, 2014) (GNWT, 2014) (GNWT, 2014). Field age in adult bulls is often categorized by the following guide (Table 3-2):

Table 3-2 Field age classification of adult bulls.

B1	Juvenile Bull
B2	Sub-adult Bull
B3	Adult
B4	Prime

Field ages in the dataset were categorized as per Table 3-3 below. B3 (Adult bulls) and B4 (Prime bulls) were combined, since no samples were classified with a written description as “Prime”, only “Adult”, suggesting that the distinction between the two age ranges may not have been made in some of the collected data. If a sample was conflictingly described as “B3 (sub-adult)”, it was classified as the age in parenthesis (“sub-adult” in this example). No male calf samples were in the database.

Table 3-3 Classification of field ages in dataset and for use in this study.

	Classification in the Dataset	**Assigned Classification in this Study**
Males	Yearling	Yearling
	B1	B1
	B2 or Sub-adult	B2
	B3 or Adult or B4	B3/B4
Females	Calf	Calf
	Yearling	Yearling
	Sub-adult or Young Adult	Sub-adult
	Adult	Adult

Statistical calculations were performed with Ausvet epitools (Sergeant 2013). Differences between proportions were calculated using a two-tailed z test, and 95% confidence intervals for proportions were calculated with the 'Wilson' Score interval as described by Brown et al (Brown, Cai et al., 2001). Statistical calculation of a trend over time of seropositive herd proportion was calculated using "ptrend" in STATA (StataCorp, 2013).

3.4 RESULTS

A) SEROLOGY

Serum samples from 278 wood bison in the Mackenzie bison population were tested for antibodies against PA from *B. anthracis*. Of the 278 samples, 196 were collected by hunters after they had shot a bison, one was from a bison killed by a motor vehicle on the highway, 39 were collected as part of other research studies, and 42 did not specify the sample source. Samples were collected in 19 different years (Table 3-4). Overall, 191 samples were positive for antibodies against *B. anthracis* and 87 were negative. The proportion of positive samples by year is described in Table 3-4, and shown in Figure 3-1.

In an effort to account for random variation, rolling averages of the proportion of positive submissions each year were calculated by averaging the values of three adjacent years. This method decreased the number of years in which values were obtained, since the first and last years could not have an average calculated, as well as any years in which an adjacent year had missing data (Table 3-4, Figure 3-2 and Figure 3-3). Averages were calculated for 19 different years, and the proportion of positive samples ranged from 0.05-0.90. The overall average proportion positive based on the rolling method was slightly lower than that of the annual proportion positives (0.28 versus 0.32).

A statistically significant change over time was observed with respect to the proportion positive, calculated using the "ptrend" function (StataCorp, 2013). For the contingency table (Table 3-4), the overall χ^2= 69.93 ($p<0.001$); for trend over time, χ^2= 35.65 ($p<0.001$); and for departure from linear, χ^2= 34.28 ($p=0.046$). There was a statistically significant trend in decreasing proportion positives in the herd over time (slope=-0.0298, SE=0.005, z=5.971).

Table 3-4 Overview of number of serum samples collected each year and proportion seropositive. Rolling average calculated by averaging values from 3 adjacent years.

Year	Number Tested	Proportion Positive	95% Confidence Interval	Rolling Average of Proportion Positive
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	-
1989	-	-	-	-
1990	3	0.67	0.21-0.94	-
1991	-	-	-	-
1992	-	-	-	-
1993	-	-	-	-
1994	20	0.9	0.7-0.97	-
1995	-	-	-	-
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	-
(Unknown Year)	7	0	0-0.35	-
Average (all years)	13.9	0.32	n/a	0.28

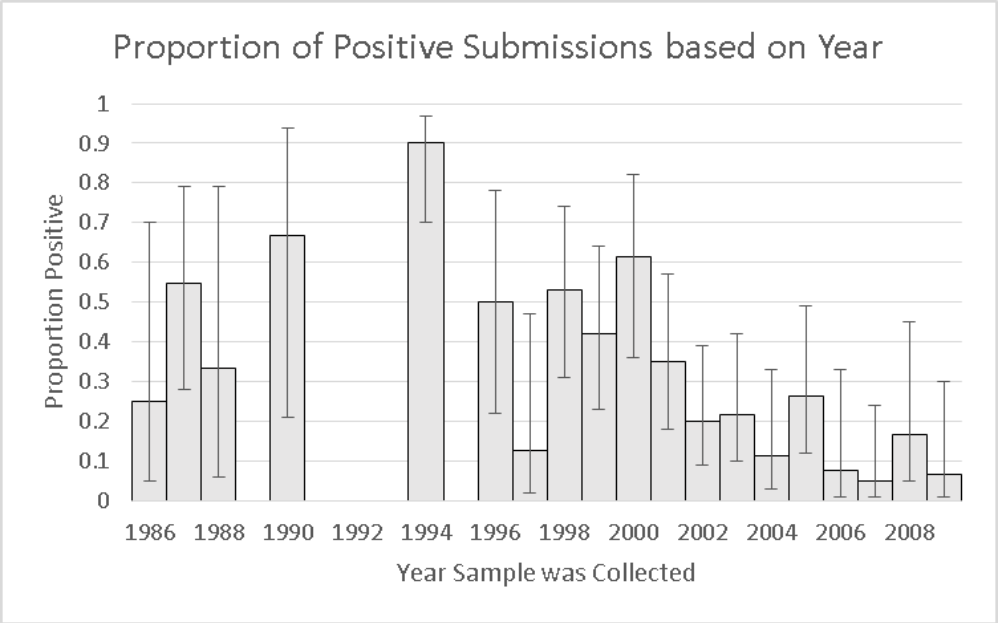


Figure 3-1 Proportion of positive submissions by year including both genders and all ages (n=271). No samples were tested from 1989, 1991-1993, and 1995. Error bars indicate 95% confidence intervals.

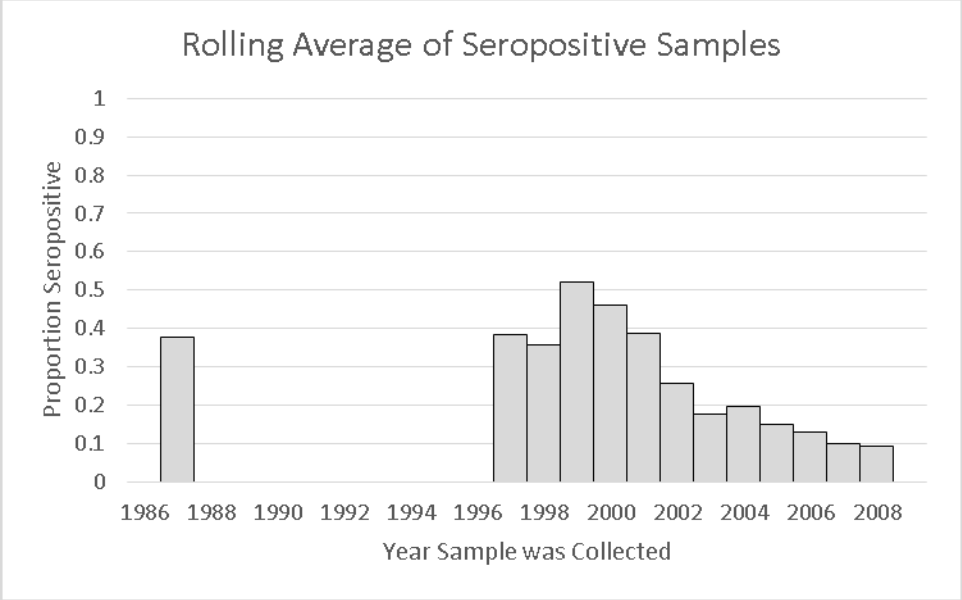


Figure 3-2 Three-year rolling average of proportion seropositive samples by year in the Mackenzie bison population.

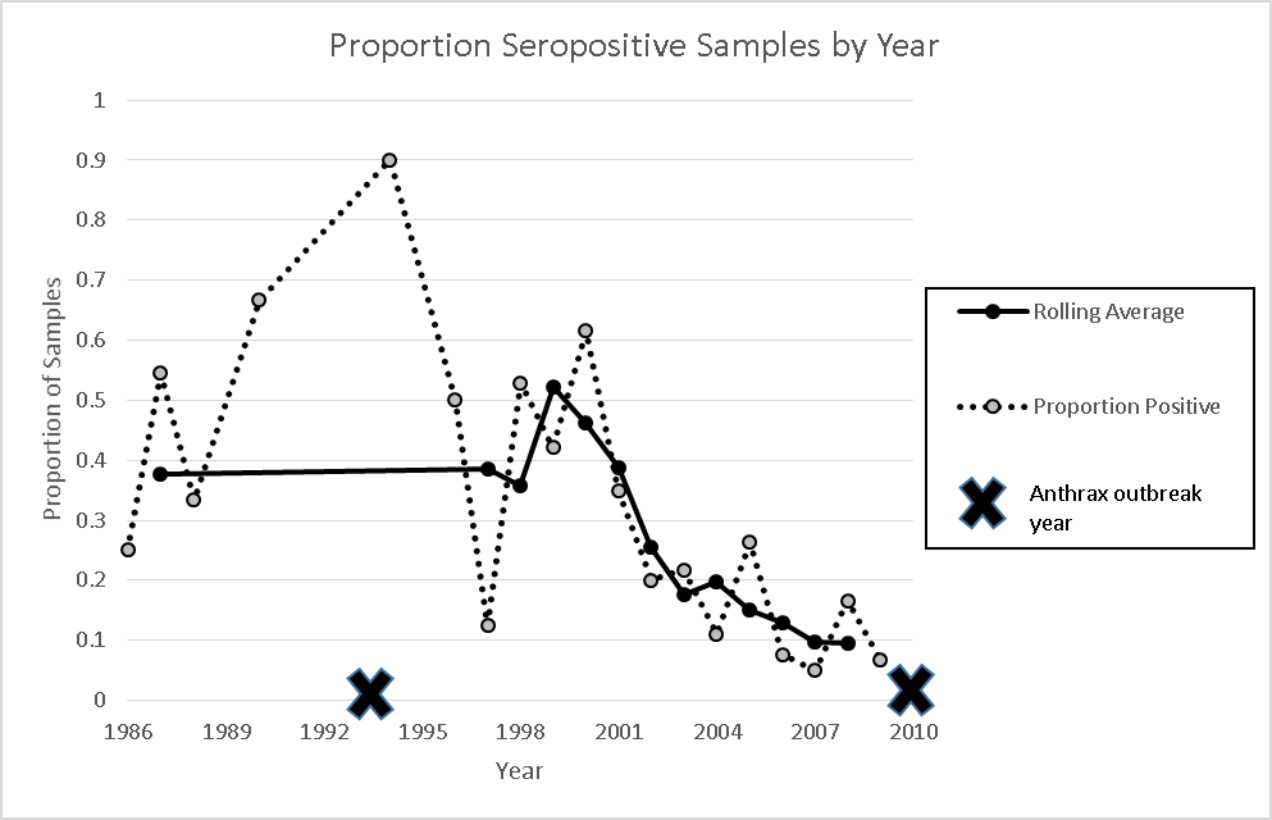


Figure 3-3 Proportion of seropositive samples each year. Rolling average calculated by averaging three adjacent years. "X" on horizontal axis indicates year when mortalities due to anthrax occurred in the Mackenzie bison population.

In all years except 1987, 1988, and 1996, there were more samples from male bison than from females. This trend can be visualized in Figure 3-4.

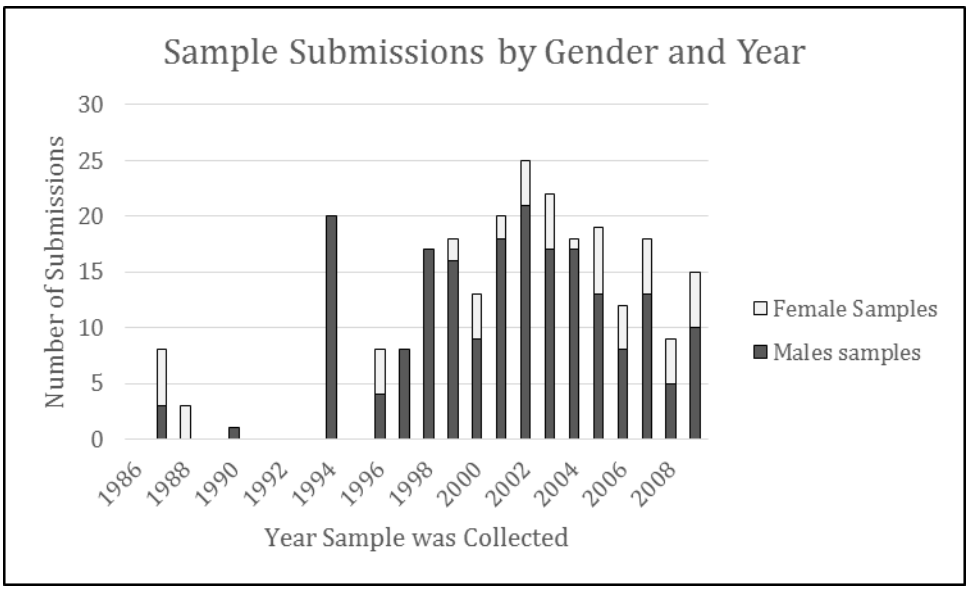


Figure 3-4 Number of samples submitted each year by gender (n=254). No samples were available from 1989, 1991-1993 or 1995. All samples submitted in 1986 were missing a recorded gender.

With respect to gender, 55 samples were from females, 203 were from males, and 20 did not specify. Of the samples from females, 18.2% were positive, while 35.5% of the samples from males were seropositive. Proportion of seropositive females each year ranged from 0 to 50%; the same proportion for males was 0 to 100%. Samples missing a gender variable were positive 25% of the time.

An estimated animal age in years was recorded for 128 samples, which excludes records with age ranges or the value “0”. The proportion of positive animals by age is shown in Figure 3-5.

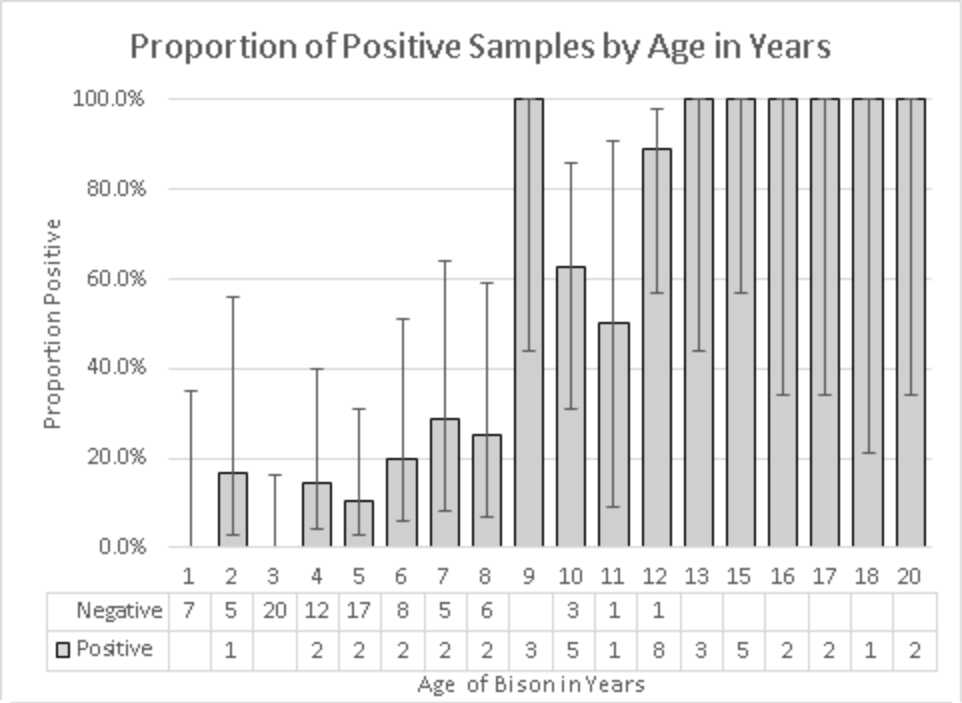


Figure 3-5 Proportion of seropositive samples based on age of bison in years (n=128). Table beneath graph indicates the number of submitted samples which were negative or positive. No bison were characterized as 14 or 19 years old. Error bars indicate 95% confidence intervals.

Field age was provided for 191 samples from males. Included in the dataset were 8 yearlings, 4 juvenile bulls (B1), 56 sub-adult bulls (B2), and 123 adult or prime bulls (B3/B4). The proportion of positive males by age is shown in Figure 3-6. There were proportionally more seropositive B3/B4 males than B2 ($p=0.0003$, $z=3.6$, two tailed-test). There were not enough samples available to compute statistical significance of the difference between B3/B4 proportions and B1 or yearlings ($z*p<=5$).

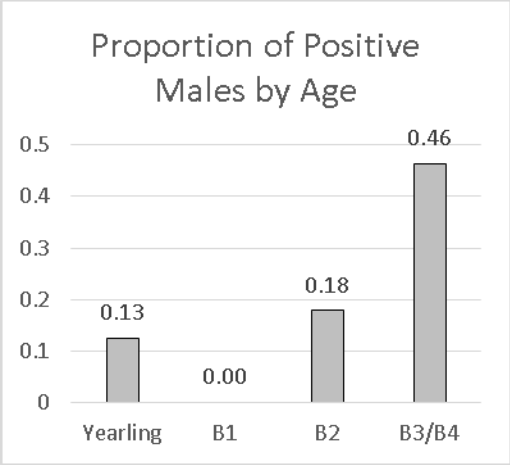


Figure 3-6 Proportion of positive samples submitted from male bison classified by field age (n=191). No male calves were included in the database.

Field age was recorded for 43 of the samples from females. One calf, 2 yearlings, 12 sub-adults and 28 adults were in the database. Only samples from adult females tested positive; 28.6% of these had a positive titre.

B) MORTALITY

There have been 3 documented outbreaks of anthrax in the Mackenzie bison population, based on mortalities (Gates, Elkin et al., 1995; GNWT, 2014). The first outbreak occurred in 1993, which affected 172 wood bison. The disease killed 150 males, 20 females, and 2 which did not have a specified gender. Only 2 calves were discovered, and no yearlings were found. All of the bison with a recorded gender as female were adult. Of the 150 males, 15 were juvenile or sub-adult, while 135 were adult or mature. In 2010 a second outbreak in the herd was discovered. Only 9 bison were found dead, all of which were adult males. Finally, the herd experienced its largest anthrax outbreak in 2012 which killed 451 bison. Of 336 records with a listed gender, 158 were female, and 178 were male. The outbreak killed 41 calves and 27 yearlings, as well as 133 cows. Of the males, 13 were juvenile or sub-adult, while 150 were adult or mature.

C) MACKENZIE BISON POPULATION HERD DATA

Herd population estimates were available for the Mackenzie bison population from 10 different years spanning 1983-2013. The population ranged from 714-2431 animals (mean 1672; SD 509). Herd composition estimates were available for 13 different years between 1989 and 2013 (Table 3-5).

Table 3-5 Herd composition of the Mackenzie bison population per 100 cows, 1989-2013.

	Annual Composition		
Year	**Calves**	**Yearlings**	**Bulls**
1989	44	22	83
1999	42.9	30.7	94.3
2000	28.9	18.3	75.1
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
2009	42.1	24.3	88.0
2010	-	-	-
2011	37.6	27.5	88.1
2012	-	-	-
2013	11	8.5	86.8

We averaged the bull composition numbers since 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 (Terry Armstrong, July 23 2014, personal communication). As such, the cow:bull composition ratio for the population from 1989-2013 was 1.2:1.

D) COMPARISON

Female bison submission were 18.2% seropositive, which could estimate an overall population seroprevalence of 18.2% in all females in the herd. 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.

By comparison, 35.5% of the samples from males were seropositive. The 1993 outbreak killed 23% of the adult males, and 35% died from anthrax in 2012. No herd population estimate was available in 2010 to calculate what proportion of the adult males in the herd died, but the number would be small since only 9 bison were found dead.

Combining the proportion of seropositive animals with herd composition estimates allows us to further compare mortality and exposure status based on gender (Table 3-6). While there tend to be more cows in the herd than bulls, more bulls are seropositive for anti-PA than cows. More bulls have died than cows in all outbreaks, but the ratio between bull and cow deaths has varied significantly (1.1 to 6.8 bulls per cow).

Table 3-6 Ratio of cows versus bulls in relation to herd composition, prevalence of seropositive serum averaged in dataset, and mortality in outbreak years

Ratio		**Cows**	**Bulls**
Herd		1	0.8
Seropositivity		1	1.9
Mortality	1993	1	6.8
	2010	n/a	1
	2012	1	1.1

Only one calf was included in the serological dataset, which was seronegative. None of the female yearlings were seropositive, while 12.5% of the male yearlings had titres against PA. In the 1993 and 2010 outbreaks, essentially none of the calves or yearlings in the herd died from anthrax. However, the 2012 outbreak had an estimated 18% loss of calves and 16% loss of yearlings.

The Mackenzie bison population has fluctuated in herd size between approximately 700 to 2500 animals from 1983 to 2013. Figure 3-7 shows the herd size by year with respect to the proportion of seropositive animals in the herd as well as outbreak years.

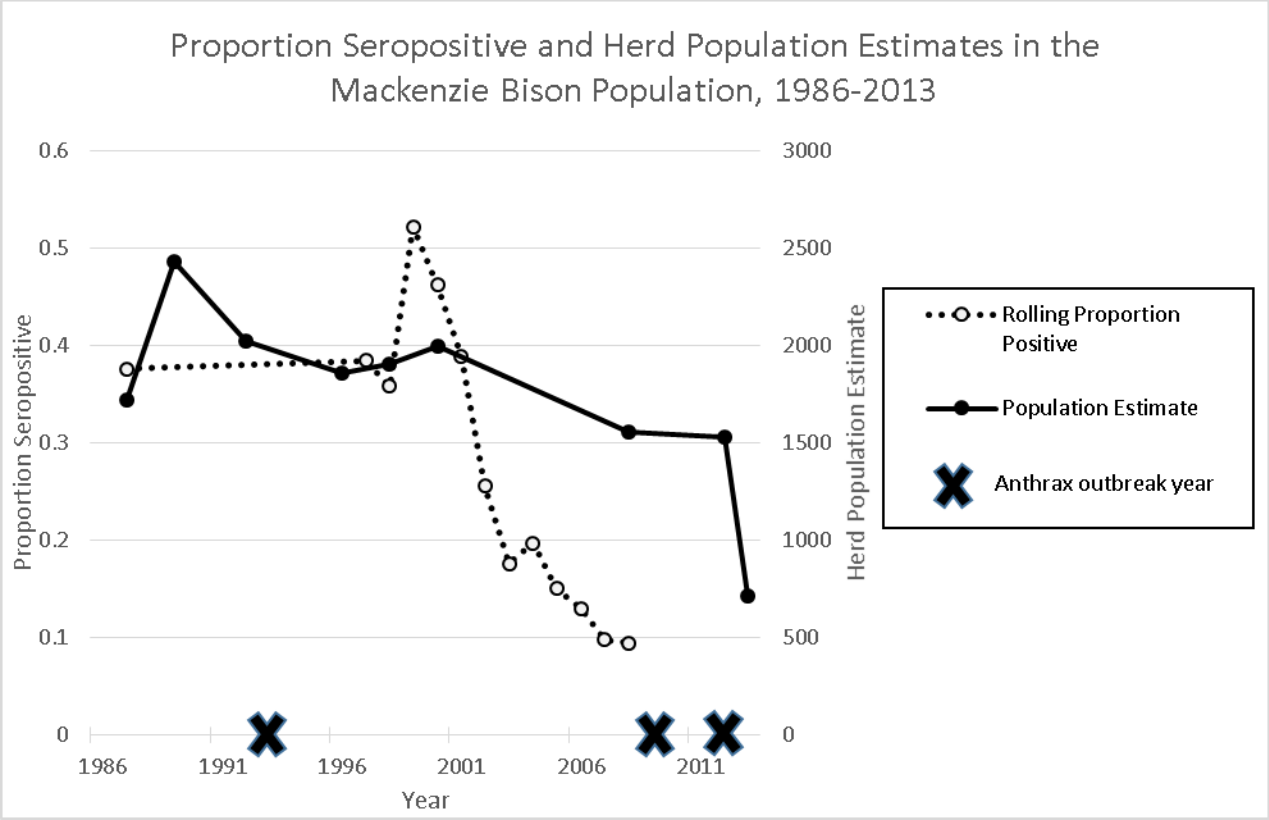


Figure 3-7 Proportion of herd seropositive for anti-PA, and herd population estimates for the Mackenzie bison population, plotted by year 1986-2013. Proportion positive calculated as average of 3 adjacent years. “X” on horizontal axis denotes year when mortalities due to anthrax were discovered.

3.5 DISCUSSION

Based on mortality data alone, the Mackenzie bison population has only experienced 3 anthrax outbreaks. However, serological data suggests that these bison have been exposed to *B. anthracis* much more frequently than previously understood. Positive titres against PA can indicate several different situations. The bison may have had clinical disease (Dragon, Elkin et al., 1999) caused by *B. anthracis*, and recovered. They may have experienced subclinical (i.e. inapparent) disease after exposure and then developed immunity against the pathogen, or they may have been actively infected at the time of sampling. There is no way to differentiate these scenarios based on the available data.

In every year from which samples were submitted, there was always at least one seropositive sample. The year with the highest proportion of positive titres was in 1994, the year following the only anthrax outbreak within the timeframe of our dataset. This suggests that bison that did not die from the 1993 anthrax outbreak were exposed to the bacteria and either recovered from clinical disease or experienced subclinical infection. No serological data was available after the 2010 or 2012 outbreaks. It should be noted that some positive results may have been false positives, occurring due to factors such as group cross reactions or non-specific inhibitors (Thrusfield, 2007).

For both males and females, the overall proportion of seropositive samples within the herd closely resembles the proportion of fatalities of each gender in 2012. Approximately 19% of the cows and 35% of the bulls died that year, in comparison with a seroprevalence of 18.2% and 35.5%, respectively. However, this trend is not exhibited in the other two outbreak years. In 1993, only 2% of the cows and 23% of the bulls died; in 2010 only 9 bulls were found dead. Since there was no serosurveillance conducted immediately prior to the 2012 outbreak, it is unknown whether there was an increased exposure to *B. anthracis* that year (expect a higher proportion seropositive), or a larger number of susceptible animals (lower proportion). One explanation may be that immunological resistance was decreased in most of the herd in 2012 due to some form of stress, which led to the mortality of most of the exposed animals. Perhaps in other years, only immunologically stressed or weakened animals succumbed to disease.

There were more seropositive adults than juveniles in both genders with respect to field age. This is similar to mortalities in all three of the outbreaks. In 1993, only 1.1% of the animals found dead were juvenile, and only adults were found in 2010. In 2012, 15.1% of the dead bison were calves or yearlings, while the majority was adults. It also appears that proportionally more adult bison were seropositive than juveniles when considering age in years (Figure 3-5), but lack of similar data in some outbreak years makes it difficult to compare these values with known mortalities. Low seropositivity in juveniles suggests that the relatively small numbers of calf and yearling deaths in outbreaks may not be because of resistance afforded from maternal antibodies against PA, but rather that fewer young animals may be exposed to *B. anthracis* than older animals in the herd.

One of the limitations in this field of study is 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 (Thrusfield, 2007). While some work has been done to test the duration of vaccine-induced titres against PA in bison, no evidence exists of the half-life of antibodies created by natural exposure. In one vaccine study in bison using different dosing protocols, all titres peaked 1-2 months after vaccination and were again seronegative after 9 months (Keith Haffer, July 21 2014, personal communication). It is possible that positive titres in our dataset reflect exposure to the pathogen within the previous year, but it is also possible that some bison retain immunity for several years. Vaccination results would suggest that seropositive bison in our dataset had been exposed to *B. anthracis* in the past 12 months, but there is no way to predict how immunological response differs after natural exposure to this pathogen in wood bison compared to vaccination. This creates uncertainty in knowing if the proportion of seropositive animals each year is a reflection of true incidence, rather than prevalence, of infection.

Furthermore, it is also unknown what minimum level of anti-PA antibodies would provide protection against *B. anthracis* in wood bison. Susceptibility to infection by *B. anthracis* is very species-dependent (Bagamian, Alexander et al., 2013), hence extrapolating protective values from another species such as cattle is likely not a valid estimate. It is possible that bison above a certain titre are immune to the disease, and hence would no longer be considered part of the

susceptible population. Knowing true protective antibody levels in combination with conducting comprehensive serosurveillance immediately before outbreak years would allow researchers to calculate tests such as incidence and case fatality rates. Unfortunately, it is currently not feasible to calculate protective immunity after natural exposure in bison since it is still unknown by which route of exposure they are infected, the dose they receive, nor differences between immunity conferred by vaccines versus natural exposure to *B. anthracis* in these animals. Furthermore, a clinical trial involving large animals with specific handling needs, in combination with a pathogen requiring a Biosafety Level 3 laboratory (Health Canada, 2001), would likely be cost prohibitive.

It is unknown why some bison die from anthrax in outbreaks while others are spared, nor how many infected bison actually die from disease. To calculate the case fatality rate in bison, both the number of mortalities as well as the number of diseased or exposed animals must be known. If a case of anthrax in wood bison is defined as an animal having a positive titre against PA, the case fatality rate could be calculated if serosurveillance was conducted in outbreak years before deaths occurred. Using serological data from the prior year may be an ineffective estimator if the cause of outbreaks is due to increased exposure to the pathogen in one particular year, rather than a modification of host resistance. As such, the true case fatality rate of anthrax in wood bison in the Mackenzie bison population remains unknown since there is a lack of serological data in outbreak years.

Based on the available evidence, it is likely that anthrax outbreaks in this bison herd are multifactorial in nature, influenced both by the dose of spores bison are exposed to and the status of host resistance in the animals.

3.6 CONCLUSIONS

It is likely that at least some of the Mackenzie bison population is exposed to *B. anthracis* in most years even when no mortalities are detected. It is still unknown why deaths seem to only occur in some years while not in others. Further research investigating indicators of stress in the wood bison may help to uncover if a modification of host resistance is the cause of mortalities due to anthrax.

CHAPTER 4 GENERAL DISCUSSION AND FUTURE RESEARCH

4.1 CONCLUSION

In addition to reviewing relevant literature about anthrax (*Bacillus anthracis*) and wood bison (*Bison bison athabascae*), the overall objectives of this thesis were: 1) to describe the epidemiology of the 2012 anthrax outbreak in the Mackenzie bison population herd and compare it to other known outbreaks, and 2) to describe the serological epidemiology of anthrax in the same group of animals. These objectives contributed to providing evidence needed to understand anthrax outbreaks in wild wood bison in Northern Canada.

The first chapter explored published literature about anthrax and wood bison in order to describe possible methods of transmission in these animals, and to explain the relevance of epidemiology in order to address anthrax outbreaks in these herds.

The second chapter described the 2012 anthrax outbreak in the Mackenzie bison population, and compared the results with other known outbreaks. This was the largest outbreak ever recorded in this herd of bison. Like previous outbreaks, proportionally more male bison died than females. However, more juvenile bison died in this epidemic than in other years, where predominantly adults were affected. This large outbreak killed a substantial number of animals, and may affect future conservation success in this population. As this herd began with fewer than 20 bison more than 50 years ago, maintaining genetic diversity should be a priority with these animals. If the 2012 anthrax outbreak killed certain animals rather than others because of unknown genetic differences among the bison, it is possible that some genetic diversity in the herd may have been lost.

When many adult males die from anthrax, it may result in a situation where otherwise potentially less fit males breed with cows due to decreased competition. This could negatively impact the long-term success of the herd. In 2012, both adult males and adult females died from anthrax. The number of cows killed in this outbreak will affect calving rates in future years, more so than

in other outbreaks where predominantly adult males are affected. Each adult female lost potentially decreases the number of calves born the following years.

Anthrax does not affect all wood bison conservation herds, so it is not a ubiquitous problem in this subspecies conservation effort. However, the bacterium can kill a substantial number of animals in the herds which it does affect, like the Mackenzie bison population. This group of animals was previously the largest conservation herd of wood bison in the world. Since the 2012 outbreak, it has been surpassed in population size by the Aishihik herd. An increased understanding of transmission and precipitating factors for outbreaks is needed to support management efforts to decrease mortalities from anthrax, in order to improve wood bison conservation.

The data from the 2012 outbreak demonstrated that anthrax affected bison in a large geographic range within the Mackenzie bison population, rather than being limited to one small defined area. In terms of the different hypotheses for outbreaks, this could suggest that there was extensive growth of anthrax in the soil throughout the entire region, or concentration of spores from dispersed flooding followed by drought. Conversely, if outbreaks happen due to a modification in host resistance, it may suggest that a significant number of animals in the herd were stressed from factors such as malnutrition or heat stress.

The third chapter described serological epidemiology of anthrax in the Mackenzie bison population. Approximately 18% of the samples from females and 35% from males tested positive for anthrax titres, indicating previous exposure to *B. anthracis*. The highest percentage of positive titres was in 1994, the year following the only known outbreak within the dataset.

Serology is a key piece of evidence needed to understand anthrax outbreaks in these animals. Without knowledge of previous exposure to *B. anthracis*, one may incorrectly presume that all exposed animals die from the disease. This research has demonstrated that each year data is collected, at least part of the herd tests seropositive for anthrax. This strongly suggests that some animals are exposed to the bacterium and are sub-clinically affected, or recover from disease. The fact that the proportion of positive serology titres vary from year to year, and that dead bison

have only been found in this herd three times, suggests that there may be a difference in exposure levels to the bacterium over time. For example, in outbreak years, there may be increased amounts of *B. anthracis* in the geographic vicinity of the bison. If deaths due to anthrax were caused only by changes in the pathogenicity of the bacterium rather than increased exposure, or solely due to a modification of host resistance in the bison, one would expect to see fairly constant seropositive proportions in the herd in all years, including immediately following the 1994 outbreak.

If bison do in fact recover from exposure to *B. anthracis* in most years, it warrants consideration about management for this disease. Presumably animals that do not die from the disease during outbreak years are exposed to moderate or high levels of the bacterium. For example, in 2012 there were bison seen grazing directly next to many of the dead bison laying at Mills Lake. As such, it is possible that these bison will have gained immunity to the disease and may be protected into the following year's outbreak season. Cows would offer maternal antibodies to newborn calves the following season, so they may be spared as well. Because of this, it is possible that incinerating carcasses in order to destroy as many spores as possible may not be required. The exception to this would be if *B. anthracis* does in fact grow successfully in the soil, wherein leaving spores from dead carcasses may cause an exponential increase in bacterial load in following years. Until the consequence of leaving spores from carcasses in the soil is fully understood, incinerating the carcasses could still be considered the best plan of action.

Study limitations for the descriptive epidemiology included a lack of infrared imaging to assess if any carcasses were missed in densely wooded areas, and difficulty with record-keeping due to the nature of recording data in the field during an outbreak.

Limitations for the serological epidemiology included lack of serological data both immediately before and after outbreaks to calculate case fatality rates, lack of knowledge of the half-life of anti-PA antibodies in order to understand how recently bison with positive titres have been exposed to the bacterium, and no knowledge about protective serological levels in order to interpret the significance of a positive result. Furthermore, bison were included in the study as

part of a convenience sample, which may inherently introduce some bias (for example, more males hunted rather than females).

4.2 FUTURE RESEARCH

Further studies collecting serological samples from the Mackenzie bison population, as well as other wild wood bison herds, would help to provide a clearer picture about exposure to *B. anthracis* in these animals. Specifically, blood samples should be collected in the spring of each year, before the anthrax season. If any deaths are detected, serological samples should once again be collected in the fall in order to compare pre- and post-epidemic titres.

4.3 IMPLICATIONS

This research has demonstrated that anthrax does not always affect wood bison populations in the same way. In 2012, both genders and all age classes were affected by the disease, unlike in previous years. As well, investigating serological samples within the herd dispelled the myth that anthrax is a generally fatal disease in wood bison since in all years there were bison with positive titres. It is still not understood if the reason bison die in some years rather than others is due to a dose-response relationship, modification of host resistance, or alteration of pathogenicity in the bacterium.

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APPENDIX A

CARCASS DISPOSAL RECORDS

APPENDIX C. Anthrax Carcass Disposal Report SR #: _____

SEQUENTIAL CARCASS NUMBER (Cross reference to surveillance report if possible): DR #: _____

SAMPLING/TREATMENT CREW PERSONNEL: _____

Date Found: _____ Date/Time of Sampling/Treatment: _____

Latitude: _____ Longitude: _____ Location: _____

Weather Conditions: _____

Other Bison Present? Y / N Herd Size: _____

Age Class of Carcass: Calf / Yearling / Sub-adult / Adult / Unknown Est. Age of Animal (years): _____

Sex of Carcass: Male / Female / Unknown Est. Length of Time Dead: _____

State of Carcass:

Position: left side down / right side down / on belly / on back / unknown

Carcass Condition: (Circle choice)

Good (Whole carcass intact, slight bloating)

Fair (Decomposing but organs intact, moderate bloating)

Poor (Advanced decomposition, saw-horse bloating, hair sloughing from hide, fluid soaked soil)

Mummified (Dry skin holding bones)

Disarticulated (No soft tissue remaining, carcass spread out over kill site)

Evidence of Scavengers Y / N (note species):

Visible Signs of Disease:

Estimate the percentage of carcass mass remaining upon discovery (Circle portion of carcass left):

0 – 20% **60 – 80%**

20 – 40% **80 – 90%**

40 – 60% **90 – 100%**

Comments:

Site Description:

1. Clearing 2. Wooded Area 3. Burned Area 4. Muskeg

5. Hillside 6. In or Near Water: stream / pond / lake / other 7. Other:

Carcass Treatment

Carcass Treated with: Fuel Oil / Formaldehyde Time Spent Treating Carcass (hours):

Carcass Treated: Before / After Cremation

Samples Taken:

Mouth Swab: Y / N Nasal Swab: Y / N Anal Swab: Y / N

Blood Collected: Y / N Soil Collected: Y / N Other:

Fire Hazard/Potential: _____

Figure 5-1 Front of the carcass disposal report

DISPOSAL CREW PERSONNEL:_____

Date/Time of Disposal:_____

Weather Conditions:_____

Other Bison Present? Y / N Herd Size:_____

Carcass Condition: (Circle choice)

Good (Whole carcass intact, slight bloating)

Fair (Decomposing but organs intact, moderate bloating)

Poor (Advanced decomposition, saw-horse bloating, hair sloughing from hide, fluid soaked soil)

Mummified (Dry skin holding bones)

Disarticulated (No soft tissue remaining, carcass spread out over kill site)

Evidence of Scavengers Y / N (note species):

Visible Signs of Disease:

Estimate the percentage of carcass mass remaining prior to incineration (Circle portion of carcass left):

0 – 20% **60 – 80%**

20 – 40% **80 – 90%**

40 – 60% **90 – 100%**

Comments:

Carcass Incineration

Date/Time Burning Started: Time Spent Incinerating Carcass:

Fuels Used:

Wood (# of cords): Placement of Wood:

Coal (# of bags): Placement of Coal:

Diesel (# of litres):

Comments:

Carcass Re-incineration

Date/Time of Follow-up: Time Spent Re-incinerating Carcass:

Fuels Used:

Wood (# of cords):

Coal (# of bags):

Diesel (# of litres):

Comments:

Modified: 2010 August

Figure 5-2 Back of carcass disposal report