Evaluation of the brucellosis and bovine tuberculosis status of bison and elk in Elk Island National Park, Canada

Veterinary Services

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Executive Summary

Elk Island National Park (EINP) in Alberta, Canada, maintains herds of bison and elk that are recognized by Canada as free of brucellosis and tuberculosis. Animals originating from EINP have been used as part of repopulation efforts throughout North America since at least the mid-1900s. The testing of over 5,000 bison and elk since the 1990s has not led to the detection of either disease. Because the animals at EINP are considered wild and are not part of a captive herd, certification of disease-free status is not possible under current regulations. To assess the brucellosis and tuberculosis status of the elk and bison populations located within EINP, the Animal and Plant Health Inspection Service determined that a risk assessment evaluating the level of testing for both diseases was necessary.

Because bison and elk have been known to become established reservoirs of infection for both brucellosis and tuberculosis, it is important to determine the status of these animals with respect to the diseases prior to allowing their importation into the United States. If one or both diseases were present in translocated animals, it could threaten the repopulation efforts for this endangered species as well as the disease status of surrounding livestock.

Tuberculosis has never been detected at EINP. Although brucellosis was detected in EINP bison in the plains bison herd in the 1940s and wood bison in the 1960s, the infection was cleaned up through a rigorous test and slaughter program. The entire population of EINP was subsequently recognized as free of brucellosis and tuberculosis by CFIA due to extensive testing for both diseases.

EINP contains two separate enclosures: the Main Park Area, to the north, which houses the plains bison herd, and the Wood Bison Area, to the south, which houses the wood bison herd. The enclosures are separated by wildlife fencing and a four-lane highway. Both park areas also include other ungulates, such as elk, moose, and deer that were enclosed or introduced at the time the enclosure was established.

Disease surveillance has been conducted on the ungulate population of the EINP, particularly bison and elk, since its inception. Surveillance measures have included testing of nearly all animals surplussed from the herd, visual evaluation of animals for signs of illness by park staff, aerial surveys to monitor for increased mortality, and testing at slaughter. To date, over 1,000 wood bison, 2,000 plains bison, and 2,500 elk have been removed from EINP and tested for brucellosis or tuberculosis with no infections detected in any of the animals.

This assessment primarily addresses the sensitivity of surveillance for brucellosis and tuberculosis in the bison and elk populations of EINP, although surveillance and detection among the other ungulates in the park are also discussed. Although the majority of surveillance data are available for the bison and elk populations, some disease evaluation, such as visual surveillance, aerial counts, and historical slaughter testing, occurs in the moose and deer populations. This information is considered in the

assessment; however, since specific test results are not available for these species, they are not included in the model calculations.

We evaluated EINP surveillance data from 1995 to 2008 to assess the likelihood that brucellosis and tuberculosis are present within the population of bison and elk. In addition, because adequate data was available on exit testing, this surveillance component was evaluated using a mathematical model. Only relatively recent test data were used from 1995-2008 due to their completeness and availability. The risk assessment also describes additional surveillance mechanisms and mitigations.

Based on analysis of surveillance data collected during exit testing, confidence levels that brucellosis will be detected if it exists in bison or elk in the Wood Bison Area population at a prevalence of 1 percent or greater is over 99.99 percent. For tuberculosis, analysis of exit data revealed that if the disease were present in the Wood Bison Area at a prevalence of 1 percent or greater, the likelihood that it would be detected by exit testing alone is 99.88 percent. This conclusion is based on the results of caudal fold tuberculin testing, without considering the fluorescence polarization assay (FPA)-tuberculosis testing that was conducted in January 2008 in the wood bison population. When the FPA-tuberculosis results in wood bison are included in the analysis, the confidence level increases to over 99.99 percent. Therefore, the results of this analysis revealed that the likelihood of the presence of either brucellosis or tuberculosis in 1 percent or more of the combined elk and wood bison population of the Wood Bison Area of EINP is extremely low (less than 0.01 percent) given the exit testing surveillance results to date.

For the Main Park Area, the likelihood that disease is present at a prevalence of 1 percent or greater given the negative results received from exit testing is not quite as low, but is still less than 1 percent for both tuberculosis and brucellosis. When tuberculosis or brucellosis is present in bison and elk herds under conditions similar to those at EINP, the prevalence of either disease generally averages over 30 percent. Therefore, the selected detection prevalence of 1 percent was intended as a conservative estimate and does not reflect the expected prevalence that would occur under normal conditions.

In addition to the exit testing, other forms of surveillance, such as visual surveillance, aerial surveys, slaughter testing for tuberculosis, and disease testing of animals post-relocation are used, increasing the overall sensitivity of disease detection at EINP. Further, the risk of either brucellosis or bovine tuberculosis entering EINP is extremely low given the closed nature of the park ungulate herds since their inception, the fencing that encloses the park, the biosecurity practices imposed by park officials, and the disease-free status of cattle herds in Alberta, Canada, including those surrounding the park. The low probability of introduction combined with a history of negative surveillance outcomes since 1995 increases our confidence that the likelihood of brucellosis or tuberculosis existing within the bison and elk populations of EINP is low.

Introduction

Elk Island National Park (EINP) was established in 1906 in an attempt to preserve native elk (*Cervus elaphus*) in Alberta. At that time, plains bison were introduced to share the area with the native elk. Introduction of wood bison occurred in 1965 as part of the wood

bison recovery effort. The park consists of 75 square miles of aspen thickets, rolling meadows, and boreal forests entirely surrounded by a 7-foot fence. It is located in central Alberta, approximately 30 miles east of Edmonton. The park is divided into two distinct areas by the Yellowhead Highway; the Main Park Area, to the north of the highway, contains the park's plains bison (*Bison bison bison*) population, while the Wood Bison Area, to the south, contains the wood bison (*Bison bison athabascae*) population. Each area also contains separate populations of elk, moose, and deer. Both areas are entirely fenced with no contact between their respective ungulate populations [2, 10].

Although the ungulates at EINP are managed as wild animals rather than as a domestic herd, the fencing around the park allows park staff to maintain control of these populations through periodic roundups for surplussing purposes (which includes disease testing), annual aerial herd counts, and regular visual surveillance of the animals. Because of these factors, along with the reputation of EINP as free of brucellosis and tuberculosis, the park has been used as a source for reestablishing bison and elk populations throughout North America.

This risk assessment is being conducted on the bison and elk populations within EINP to evaluate their status for brucellosis and bovine tuberculosis. Because the bison commingle with elk, their testing information is combined for the purposes of the quantitative analysis. Because the Main Park and Wood Bison Areas of EINP are completely separate from each other, these are considered separately.

Whole herd testing conducted in the wood bison herd during the 1970s and exit testing subsequently conducted on approximately 4,000 bison and 2,500 elk from EINP have failed to detect any evidence of brucellosis or tuberculosis. The elk and bison herds at EINP have been closed herds with no emigration of animals into these herds since the year of their inception, which was 1906 for elk, 1907 for plains bison, and 1965 for wood bison [1, 2].

This document assesses the brucellosis and bovine tuberculosis status of the ungulate population of EINP. Although the focus of the risk assessment is the status of the bison and elk populations, results for other ungulates residing within the same area are also provided. A description and history of brucellosis and bovine tuberculosis in EINP are provided, and a mathematical model using historical surveillance data acquired from bison and elk is used to assess the potential disease status of these animals. Information and test results for moose and deer residing in EINP are described, and this information is incorporated into the risk assessment. However, due to a lack of testing data in these species, they are not included in the model calculations.

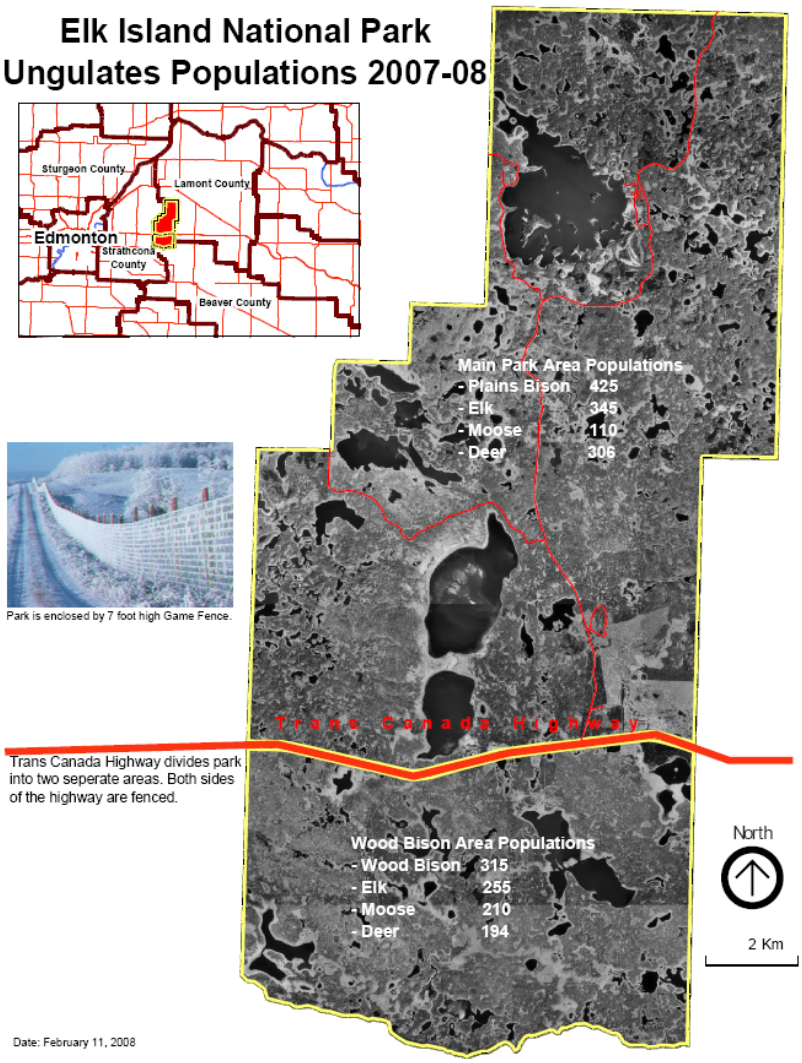
Background

Elk Island National Park

History

EINP was the first federally controlled area in Canada enclosed to protect a native mammal, the elk, and the first large mammal sanctuary established in Canada. The park is comprised of 75 square miles (48,000 acres) of mixed habitat (including aspen thickets, rolling meadows, and boreal mixed forest) entirely surrounded by a 7-foot high fence and is located in Alberta, approximately 30 miles east of Edmonton. The park is divided into two enclosures (the Wood Bison Area, which houses wood bison, and the Main Park Area, which houses the plains bison) that are separated by a four-lane highway (the Yellowhead Trans-Canada Highway) and another set of 7-foot fences on either side [10]. (See figure 1.)

Figure 1: Aerial view of EINP



EINP is home to over 40 species of mammals and 200 species of birds. The ungulate population currently includes approximately 315 wood bison, 255 elk, 194 deer, and 210 moose within the Wood Bison Area and 425 plains bison, 345 elk, 306 deer, and 110 moose entirely enclosed within the Main Park Area [10].

In the early 1900s, when the population of both the wood bison and the plains bison were at their nadir, the Canadian Government established a wildlife sanctuary that eventually became the EINP. Approximately 16 square miles (42 square kilometers) were cordoned off within a 2.2-meter fence. The enclosed area included 24 elk, 2-3 moose, and 35 mule deer within its boundaries at the time of enclosure. The elk have never been crossed with other elk and are considered today to be one of the few elk herds that has not been hybridized [10].

In 1907, the first bison were brought to EINP when 377 plains bison from a herd in Montana were transported to EINP to be held until fencing was completed around Buffalo National Park, their final destination. The Canadian Government established Buffalo National Park in eastern Alberta near Wainwright to regenerate the bison population. In 1909, when fencing was completed, the plains bison were moved there from EINP; however, a group of 45-50 bison that avoided capture remained and became the founding stock for the current herd of plains bison at EINP [2, 10, 11].

Around the same time, most of the few remaining wood bison were located in an area of northeastern Alberta and southern Northwest Territories that later became Wood Buffalo National Park (WBNP). As a result of legislation protecting the wood bison, the population in the area had increased to around 2,000 animals by the time the park was established in 1922. By this point, the herd of plains bison located at Buffalo National Park was also increasing rapidly, to the point that it had exceeded the park's carrying capacity [2, 10, 11].

To ease the crowding at Buffalo National Park, over 6,000 plains bison were transplanted to WBNP in the late 1920s. It was believed that the distance between the release site and the nearest wood bison herd would prevent interbreeding of the two subspecies. Unfortunately, this proved not to be the case, and cross-breeding with the resident wood bison herd occurred. In addition, although it was unknown at the time, the introduced plains bison brought bovine tuberculosis and brucellosis along with them [2, 11].

Meanwhile, EINP was designated a national park in 1930, and in 1947, the park expanded to include 60 square kilometers south of the Yellowhead Trans-Canadian Highway, which is today known as the Wood Bison Area. This enclosure is maintained separately from the original park, which is now referred to as the Main Park Area, and is the habitat for the plains bison herd. The 7-foot fence along both sides of the four-lane highway maintains these two subspecies as separate herds [10].

In the late 1950s, biologists located a small isolated herd of apparently pure wood bison in the northwestern corner of WBNP. These animals exhibited all of the physical traits of pure wood bison. In 1965, 23 of these wood bison were translocated to EINP. This was the last introduction of animals into EINP [2, 10, 11].

The elk herd at EINP consists of the descendants of the 24 elk that were enclosed when the park was first established as a fenced facility in 1909. In 1976, a portion of the elk herd located in the Main Park Area north of Yellowhead Trans-Canadian Highway was moved into the Wood Bison Area to establish an elk herd in this part of EINP. Other than this movement, the elk herd at EINP has remained behind the fence since the early 1900s and has never been crossed with other elk [10].

Buffalo National Park was eventually closed in 1940, and its remaining bison herd was sold off for slaughter. WBNP, however, continues to operate and maintains the largest bison population in Canada, although this is mostly a hybrid population of wood bison crossed with plains bison. Brucellosis and bovine tuberculosis remain endemic in the free-ranging herds in and around WBNP [3, 12].

The game fence that surrounds EINP is intended to restrict the movement of large herbivores (elk, bison, moose, and deer) into and out of the park. Because the park is surrounded by agricultural land, rural communities, and suburban developments, the fence is necessary to keep bison, elk, and other large herbivores from dispersing onto outlying lands where they are not welcome. It also keeps domestic animals (such as cattle) from entering the EINP. It is possible for deer to crawl under or jump the fence, while moose and elk are theoretically capable of straddling the fence; however, this type of movement is minimal according to park staff [10, 11].

Large herbivores graze together within each enclosure, separated by the two fences and the highway between the Wood Bison and Main Park Areas. Current herds consist entirely of descendents of the original park population, which was established in the early 1900s for plains bison and other ungulates and the 1960s for the wood bison. No emigrations into the park have occurred since those times [11].

Disease management at EINP

Although tuberculosis has never been detected at EINP, brucellosis was detected in the plains bison herd in the late 1940s. A test and slaughter program was in place throughout the 1950s and 1960s, as well as the use of Strain 19 vaccine. In 1972, the plains bison herd was determined to be free of brucellosis [10].

Shortly after the wood bison's arrival at EINP in 1965, brucellosis was detected in the wood bison herd. Over the next few years, an intensive modified test and slaughter program was implemented. As each cow produced a calf, the cow was destroyed and the calf was bottle raised. The result of the salvage plan was the removal of all founding herd members, with only their bottle-raised calves remaining [1, 10].

As a result of the eradication program, combined with a series of whole-herd tests for both brucellosis and bovine tuberculosis in the wood bison herd during the 1970s, and testing of nearly all bison and elk (as well as other large herbivores at the park including deer and moose) that die, are slaughtered, or are exported from the herd, the herd is believed to be free of both diseases by Canada. Since 1969, approximately 4,000 bison (both subspecies) and 2,500 elk from EINP have been tested for brucellosis and bovine tuberculosis prior to translocation, and all tests have been negative. Additionally, since

there have been no new additions to EINP since the early 1900s for plains bison and elk and 1965 for the wood bison herd, and limited contact with cattle and wild ungulates outside the park, there is a negligible chance of reentry of either of these diseases into the herd [2, 10, 11]. Based on this information, the Canadian Food Inspection Agency (CFIA) considers the EINP herds to have a bovine tuberculosis and brucellosis status that is equivalent to negative status under the Negative Status Program. This designation is the basis upon which the CFIA issues permits for movement of EINP elk within Canada for wildlife translocation projects [11].

The current elk, moose, and mule deer herds are the descendants of animals fenced in when the park was originally established. White-tailed deer subsequently found residence in the park and now far outnumber the mule deer [13].

Disease status of Canada

Brucellosis

Canada's national cattle herd (including captive bison) was declared brucellosis free by the CFIA in 1985 (subsequently recognized by the United States in 1997). However, small pockets of the disease in wildlife are known to persist; namely, among free-ranging bison located in and around WBNP [14, 15].

Bovine tuberculosis

In Canada, there are two regional foci where wildlife populations are considered to be disease reservoirs for tuberculosis: free-ranging populations of wood bison in and around WBNP and elk and deer in and around Riding Mountain National Park (RMNP) in Manitoba [16-18]. In the RMNP area, the disease was first found in a hunter-killed elk in 1992 in the vicinity of infected cattle herds. In 1997, another outbreak occurred in cattle, with subsequent surveys in elk finding a prevalence of less than 1 percent. From 1992 to 2001, 10 elk near RMNP were found to have bovine tuberculosis. Transmission of the disease from elk is believed to be the source of diagnosed cases in cattle in that area in 1997, 2001, and 2008. No cases of tuberculosis in cattle have been associated with the WBNP outbreak [19].

As of 1997, the U.S. Department of Agriculture (USDA) recognized all Canadian cattle herds as being free of bovine tuberculosis. However, in response to the finding of bovine tuberculosis in cattle around RMNP, Canada eventually downgraded the area around RMNP, known as the Riding Mountain Eradication Area (RMEA), to tuberculosis accredited advanced status, leaving the rest of Manitoba as tuberculosis free. Movement and additional testing restrictions were placed only on cattle herds within the RMEA [18]. In 2006, after several years of enhanced surveillance, Canada reinstated tuberculosis-free status to cattle herds located in the RMEA [20]. Currently, however, the USDA requires testing for breeding cattle throughout Manitoba prior to importation into the United States.

Although tuberculosis was identified in a single animal in a cattle herd in Alberta in 2001 and more recently in 2007 in a bull in British Columbia that was born in Alberta, both investigations found no additional cases of disease beyond the index cases despite extensive testing. Except for a few cases within WBNP, bovine tuberculosis has never

been found in free-ranging elk, moose, or deer in Alberta, and Alberta's status remains tuberculosis free [15, 21].

Hazard Identification

Brucellosis

Etiology

Brucellosis can be caused by any one of several members of the *Brucella* genus. *Brucella* are intracellular Gram-negative coccobacilli that can infect both animals and humans. In cattle, bison, and cervids, brucellosis is most commonly caused by *Brucella abortus*. Other *Brucella* species affecting livestock include *B. melitensis* (sheep and goats) and *B. suis* (pigs) [22, 23].

Host range

Brucellosis has been detected in a wide range of species. Although most *Brucella* species have a primary host, infection can spill over into alternate hosts. For instance, *B. abortus* has also been reported in horses, sheep, goats, pigs, raccoons, opossums, dogs, coyotes, foxes, wolves, and moose [23, 24].

Prevalence in North America

A wide range of species can produce *Brucella* antibodies, and some can harbor the pathogen. Serological surveys provide most of the published information on brucellosis in wildlife, but these studies should be interpreted cautiously when assessing the significance of a species as a reservoir of bovine brucellosis. Often, sample sizes have been too small to permit generalization. Further, the sensitivity and specificity of serological tests are frequently not known when applied to wildlife species. Some titers may result from nonspecific agglutinins or from cross-reactions with antigens other than those of *Brucellae*. Serological reactions may indicate exposure, but not necessarily current or active infection and not the species of *Brucella* involved. Isolation of the pathogen in conjunction with serology provides better information, but few studies have done this [24].

Bison

Early studies of brucellosis in wild bison demonstrated that brucellosis does not appear to be randomly distributed in bison populations, but that it increases with age. In 1925, the rate of serological reactors observed at a study in WBNP increased from 24 percent in yearlings to 43 percent in 2-year-olds and 44-59 percent in older bison [25]. Later studies confirmed this observation and revealed that prevalence did not appear to be directly related to the density of bison although a minimum population of 200 was suggested as necessary to sustain brucellosis in a population. When brucellosis occurred in a bison herd, the prevalence was usually high, which was believed to be due in part to the gregarious nature of bison [12, 16, 25, 26].

Brucellosis is enzootic among free-ranging bison (*Bison bison*) herds in a few remaining areas in North America. These include Yellowstone National Park (YNP) and Grand Teton National Park in the United States and WBNP in Canada. Brucellosis was first isolated in bison in 1930 by Creech [27]. Most evaluations of brucellosis prevalence in bison are based on the use of seroagglutation titers.

In several studies, brucellosis has been found in over 50 percent of the bison in the Greater Yellowstone Ecosystem, which covers parts of Wyoming, Montana, and Idaho [28]. Surveys conducted throughout the 1930s, involving sera collected from nearly 500 surplus bison from YNP, identified the prevalence of brucellosis as ranging from 54 percent to over 75 percent in both positive and suspect categories according to seroagglutination testing [29, 30]. More recently, in the 1990s (using particle concentration fluorescent immunoassay and complement fixation), over 75 percent seroprevalence was detected in female bison, with 46 percent of those tested found to be culture positive, which is similar to levels seen in cattle [31].

Bison in the free-ranging Jackson herd in Teton County, Wyoming, evaluated from 1989 to 1990 were found to have a seroprevalence of 77 percent (including positives and suspects) [32].

Several studies have been conducted at WBNP over the years. In 1925, the rate of serological reactors ranged from 24 percent to 59 percent, depending on the age group sampled [25]. In a survey of slaughtered bison sampled by tube agglutination from 1959 to 1974, seroprevalence ranged from 6.1 percent to 62.0 percent in 2,365 bison [12]. Opportunistic sampling of complete or partial remains of 72 bison hunted or found dead in and around WBNP during the 1980s revealed evidence of brucellosis in 25 percent. This was believed to be a conservative estimate due to the young age of most animals sampled [33]. A study of live-captured bison from 1997-1999 found 30.9 percent of 346 bison serologically positive for brucellosis [26]. Adjoining WBNP are the Slave River lowlands in the Northwest Territories. Bison have been tested in this area as well, with serological tests revealing a brucellosis prevalence of 25.9 percent and 39.3 percent in 1970 and 1974, respectively [12].

Areas in Canada that previously harbored brucellosis in free-ranging bison include RMNP, Manitoba, and EINP, Alberta. Brucellosis was identified in bison in EINP beginning in the 1940s, with 15 percent of 20 bison in the 1950s found to be seroreactive. Through test and slaughter, combined with a vaccination program, the disease was eliminated from the EINP in the 1970s [34, 35]. In studies conducted at EINP from 1946-1947 and 1956, 42.4 percent of 380 total bison tested were either positive or suspect on tube and plate agglutination tests [35, 36].

Additional studies conducted outside these enzootic foci have not found evidence of disease, indicating that widespread infection is unlikely. Between 1986 and 1988, 51 wood bison from a herd located at the Mackenzie Bison Sanctuary, Northwest Territories, were euthanized and necropsied, with no pathologic, histological, or serological evidence of brucellosis found. No serological evidence of brucellosis was found in an additional 112 wood bison that were evaluated between 1986 and 1990. Unlike the Slave River lowlands, this area does not adjoin WBNP [25].

Brucellosis has been detected in captive bison herds as indicated by the finding of six infected farmed bison from North Dakota that tested positive on slaughter. As part of the traceback investigation, over 90 percent of the 21 bison in the herd of origin were also found to be serologically positive [37].

Elk

As with bison, the prevalence of seropositive elk (*Cervus elaphus*) was observed to increase with age [38]. Several studies have indicated that brucellosis transmission is enhanced by the practice of feeding animals in the winter, which encourages close contact [24, 38, 39].

Brucellosis testing in elk has been conducted at YNP over several years. In 1930, brucellosis testing of 67 elk from within the park identified 19.4 percent that were either positive or suspicious on serological evaluation [29]. Free-ranging, hunter-harvested elk from areas near YNP were sampled from 1991-1995, with antibodies detected on average in 1 percent of samples from areas near the park, and none in areas farther away. In total, 388 elk were sampled [40]. Brucellosis in elk in the GYA has generally been found at much lower levels than what is typically seen in bison. However, it can be found in much higher levels in elk in concentrated feeding grounds in Wyoming [28].

Brucellosis was first detected in elk in Idaho (around the GYA) in 1998. Hunter-harvested elk were surveyed for the presence of antibodies from 1998 until 2002, with an average prevalence of between 2-3 percent per year. Another group of elk from areas where artificial feeding is practiced were also trapped during the study and tested for brucellosis using serology and tissue culture. Prevalence ranged from 12-80 percent in these areas. From 1989-1997, 242 elk were trapped and tested from northern and central Idaho in areas that were not close to the GYA and where feeding did not occur No serological evidence of brucellosis was found in this population [39].

Similarly, samples from hunter-harvested deer and elk in Wyoming that were not exposed to concentrated feeding grounds were all negative for brucellosis [41], whereas the incidence of brucellosis in elk on two winter feed grounds in Wyoming was identified at 31 percent over a 5-year period based on serological testing [38]. In the National Elk Refuge and Grays River herds where feeding is practiced, approximately 50 percent of mature cow elk have been found to have serological evidence of infection [24].

In the Waterton Lakes National Park area in southwest Alberta, 17.6 percent of 17 elk collected in 1957 were serologically positive or suspicious for brucellosis [24]; while at EINP, 13.1 percent of 221 elk were serological reactors to brucellosis on tube and plate agglutination tests [35]. As described above, the disease has since been eradicated from the EINP herd.

Deer

White-tailed deer (*Odocoileus virginianus*) and, to a lesser extent, mule deer (*O. hemionus*) are some of the most extensively studied ruminant species. Numerous studies have been conducted in the United States and Canada with relatively low rates of brucellosis detected based on serological testing [24, 42].

A study of brucellosis in white-tailed deer in North Dakota was conducted during the 1947-1948 hunting season. Of the 436 deer sampled, only one positive reactor (0.22 percent) was detected [43]. A 1948-1949 survey of 58 deer from southeastern Saskatchewan did not find any reactors on tube agglutination tests despite a high prevalence of brucellosis in cattle in that area at the time [44]. Subsequent sampling from hunter-harvested deer in Wyoming all turned up negative for brucellosis [41]. In the southeastern United States, a serological survey was conducted among white-tailed deer using the plate-agglutination technique with a resulting prevalence of detection at 0.25 percent [45]. In Missouri, blood samples were collected from white-tailed deer harvested during the hunting seasons of 1950-1953. Of 996 deer sera tested, all but one tested negative using the standard plate agglutination test [46]. Among hunter-harvested deer sampled in Wisconsin, only one reactor out of 600 samples collected was found. From these results and the reports of other investigators, it appears that brucellosis is not the important disease in white-tailed deer that it is in cattle and has been reported to be in bison and elk [47].

Moose

Reports of brucellosis in moose are relatively rare, and it appears that brucellosis is a much more severe, often fatal, disease in moose than it is in bison, elk, and domestic animals. Its high mortality rate explains the absence of reactors among relatively healthy moose and the low prevalence in moose populations, likely resulting from the low level of transmission from these dead-end hosts [24, 35].

A study of 25 moose from northern Minnesota found negative serological and bacteriologic results, except for one bull moose with a unilateral testicular infection from which the organism was cultured [48]. Of 39 moose sampled in Alaska, one (3 percent) tested positive for antibodies to *Brucella* spp. [49].

Additional studies failed to identify brucellosis in sampled moose, including 124 moose from EINP, 146 from British Columbia (B.C.), and 208 from Quebec, Canada, despite the fact that many of the B.C. samples were collected from an area that had been heavily infected with brucellosis in domesticated cattle and which had experienced considerable mixing of moose and range cattle [24, 35, 50].

Transmission

B. abortus is most commonly transmitted by licking or ingesting placenta, aborted fetus, fetal fluids, and vaginal discharges from infected animals after abortion or giving birth. These tissues contain the highest concentration of bacteria and can contaminate the environment. Less commonly, *B. abortus* may be shed in milk, urine, semen, feces, and hygroma fluids of infected animals. In utero and venereal transmission may also occur, as well as transmission through fomites, such as feed and water; however, these are relatively uncommon. Survival of the organism in the environment increases with low temperatures, high humidity, and a lack of sunlight. The organism can remain viable for several months under the right conditions [14, 23, 51].

Transmission of brucellosis from bison and elk to cattle has been observed experimentally at rates equal to those seen in cattle-to-cattle transmission, suggesting that transmission from these species to cattle (as well as between bison and elk) may occur under natural conditions [24, 28, 51, 52].

In free-ranging bison in Wyoming, abortions due to brucellosis in elk suggests that transmission to cattle or other species may occur through contact with infective abortive tissues [32]. Other studies, however, have failed to detect transmission from bison to cattle under natural conditions in Montana during 1989 [53].

Incubation period

In cattle, abortions and stillbirths can occur between 2 weeks to 5 months after infection. Since reproductive losses typically occur during the second half of gestation, the incubation period is highly dependent on the stage of gestation at which infection occurs [23].

In a study evaluating both experimentally and naturally infected elk, the average incubation period was 68-125 days (average 89) for artificially exposed animals. In naturally exposed cows, the most likely incubation period was 44 days (in one animal) [51].

Clinical signs

In cattle, infection with *B. abortus* can lead to abortion (usually during the second half of gestation), retained placenta, metritis, orchitits, epididymitis, and impaired fertility. Some calves are born weak and may die soon after birth. After the first abortion, subsequent pregnancies are generally normal; however, cows may continue to shed the organism in milk and uterine discharges. Lameness, associated with hygromas of the leg joints, may also be seen, with arthritis as a potential sequelae. Deaths are rare except in the fetus or newborn. Infections in nonpregnant females are usually asymptomatic [14, 22, 23].

Bison and elk respond similarly to cattle in response to *B. abortus* infection, with abortion the most important sign, followed by reproductive sequelae in the female such as metritis, retained placenta, vaginal discharge, and fetal death [12, 24, 28, 32, 51-54].

In male animals, orchitis, epididiymitis, and seminal vesiculitis were seen frequently in bison while not as much in elk [30, 32, 53, 55].

Hygromas and arthritis have also been noted in bison and elk [33, 51].

As previously mentioned, brucellosis in moose tends to progress to fulminant infection and death [23, 24, 35].

Diagnosis

Serology is often employed in the presumptive diagnosis of brucellosis or in the screening of herds. Serological tests used include the buffered *Brucella* antigen tests (tube or plate agglutination), complement fixation, indirect or competitive enzyme-linked immunosorbent assays (ELISAs), or the fluorescence polarization assay (FPA). Other serological tests include Rivanol precipitation, acidified antigen procedures, and the serum agglutination test (tube or micro-titer test) [23].

Supplemental tests, such as complement fixation or Rivanol precipitation, are often used to clarify the results from plate or card agglutination tests. ELISAs or the *Brucella* milk ring test (BRT) can be used to screen dairy cattle herds by detecting antibodies in milk. Polymerase chain reaction (PCR) techniques and other genetic techniques are available in some laboratories [23].

Culture of *Brucella* organisms from lesions or tissue is confirmatory for infection. Similar tests are used to diagnose *B. abortus* infections in species other than cattle, but each test must be validated in that species [23].

The standard procedure for brucellosis testing in cattle is to use the buffered plate antigen test (BPAT) as a screening test and the complement-fixation test as the confirmatory test for sera that agglutinate in the BPAT[26]. However, since serological tests for *B. abortus* were developed for cattle, testing other ungulates can be problematic, and interpretation of test results difficult. Although many of the cattle tests have been used on bison in seroprevalence studies, their results should be evaluated cautiously as some tests have been shown to behave differently than in cattle, and heterologous test results in bison and elk have been observed [26].

Tests used for brucellosis detection at EINP in bison and elk include the BPAT and FPA for screening purposes and complement fixation and competitive ELISA for follow up in positives. The following is a description of the use of these tests in bison and elk as compared to cattle as the reference population.

Early studies of brucellosis serology in bison and elk used tube and plate agglutination tests, interpreted as for cattle, so that agglutination at a serum dilution of 1:25 or less was considered negative, 1:50 suspect, and 1:100 or greater a positive reactor [30, 35, 53].

Several different serological tests have been evaluated in bison as compared to cattle. The overall finding was that no single serological test was consistently reliable in diagnosing *B. abortus*-infected bison [52]. Many of the recent serological studies conducted have used an approach that combines multiple test methods [16, 32].

In bison from which *B. abortus* was cultured, serological titers tend to be higher than in those from which *B. abortus* was not cultured; however, a small percentage of bison with high titers remain culture negative, which is similar to results in chronically infected cattle herds [31].

Several studies have been conducted in elk evaluating the serological tests available for bovine brucellosis, including tube and plate agglutinating tests, complement fixation, and Rivanol. The overall recommendation was to use multiple test methods to diagnose brucellosis in elk. Generally, at least two tests were recommended with slight variations in which two tests, depending on which ones were studied; but in most cases, complement fixation was recommended [24, 38, 42, 56, 57].

Treatment

Eradication of *B. abortus* is generally accomplished in cattle by quarantine of infected herds, vaccination, and test-and-slaughter, combined with surveillance and traceback investigations. Options in wildlife generally consist of test-and-slaughter or depopulation with vaccination also used on occasion [23].

Two *B. abortus* vaccines, Strain 19 and RB51 (both live), are available and can be used to control the disease in endemic areas or as part of an eradication program; however, they are not routinely used in wildlife [23]. Strain 19 vaccination has been used to control bison outbreaks since the 1960s; however, documentation on this use is lacking, and the protective effect of the vaccine is not known. In addition, vaccination was always combined with test-and-slaughter activities [58]. Under experimental conditions, Strain 19 vaccination was observed to cause a high rate of abortion (higher than that of natural infection) in pregnant bison cows and failed to stimulate immunity in calves [59]. Strain RB51 vaccination has been used in private bison herds for several years, and its use in these herds, in addition to experimental studies, resulted in the appearance of safety, although it does not appear efficacious in preventing infection or abortions [58, 60, 61]. Strain 19 has been used on elk around the GYA since the 1980s, although its efficacy has yet to be determined [58]. Strain RB51 is also being investigated although based on results so far, its use cannot be recommended in elk [62, 63].

Public health issues

B. abortus is pathogenic for humans. Humans usually become infected by ingesting organisms (including contaminated, unpasteurized dairy products) or by the contamination of mucous membranes and abraded skin [23]. Ingestion of the organism through consumption of dairy products once accounted for the vast majority of human cases of brucellosis in North America; however, pasteurization of milk has all but eliminated the disease in humans [64]. Currently, occupational exposure is most likely to lead to human brucellosis. Rates are highest in laboratory workers, farmers, veterinarians, and others who contact infected animals or tissues [23].

Typically, brucellosis in humans begins as an acute febrile illness with nonspecific flu-like signs. Drenching sweats can occur at night. Some patients recover spontaneously, while others may develop longstanding infection leading to chronic manifestations. The infection can be treated with antibiotics; however, relapses can be seen even in successfully treated cases. The mortality rate is low [23].

Tuberculosis

Etiology

Bovine tuberculosis results from infection with *Mycobacterium bovis*, a Gram positive, acid-fast bacterium [65].

Host range

M. bovis has an exceptionally wide host range. Although cattle are considered the maintenance host for *M. bovis*, many other species are susceptible, including other ruminants, deer, elk, bison, dogs, pigs, badgers, opossums, ground squirrels, rats, tigers, lynx, humans, and nonhuman primates. Most of these species are spillover hosts in which infection generally is not self-maintaining; however, some species, such as deer in the United States and bison in Canada, have become reservoirs for infection [65, 66].

Prevalence in North America

Bison

A large herd of plains bison (*Bison bison bison*) at the National Buffalo Park (NBP) at Wainwright, Alberta, was found to be affected with tuberculosis during the 1920s and 1930s. Tuberculous lesions were detected in 53.7 percent of over 12,000 bison killed. The lesions appeared similar to those seen in cattle [70]. In 1939, after shipping more than 6,000 young bison thought to be disease free to WBNP, the bison were depopulated due to the prevalence of tuberculosis in the park, and it was eventually closed [71].

Bovine tuberculosis was first identified in bison at WBNP in 1959 or 1960, most likely originating from the NBP herd additions. An infection rate of 51 percent was initially detected [21]. As with brucellosis, the prevalence of tuberculosis appeared to increase with age in the bison population of WBNP. Herd prevalence tends to be high in enzootic infection of bison populations due to the chronic nature of the disease and the relatively high rate of transmission in these gregarious animals [26, 72]. During a study conducted at WBNP from 1959-1974, tuberculosis reactor rates averaged 40 percent [25]. Later studies conducted on the bison population of WBNP identified tuberculosis in 49 percent and 37.8 percent based on tuberculin testing and 21 percent and 50.2 percent based on identification of tuberculous lesions on necropsy [26, 33, 55].

In 1959, 500 plains bison at EINP were slaughtered and evaluated for tuberculous lesions on post mortem exam, with no lesions found on any of the animals [21, 55, 73, 74].

Tuberculosis has also been found in captive bison in Central Alberta, South Dakota, and Oklahoma [21, 73, 74].

Elk

M. bovis lesions were identified in 5.5 percent of elk that ranged with the infected bison at the Buffalo Park near Wainwright in 1939 [70]. Although few positive elk have been found within WBNP, the tuberculosis prevalence was found to range from 25-40 percent in the Slave River lowlands, which adjoins WBNP [25, 75].

Infected elk were first found at RMNP in 1992 when a wild elk shot in the vicinity of an infected cattle farm was found to be infected with tuberculosis. Of 1,463 hunter-harvested elk sampled between 1992 and 2002, 0.7 percent were positive for *M. bovis* by culture. Results indicated that tuberculosis prevalence in elk (as well as other cervids) increased with age, especially in males, as was seen in both bison and elk for tuberculosis and brucellosis [17, 67, 68].

Bovine tuberculosis has rarely been diagnosed in free-ranging cervids in North America. In the instances described above, *M. bovis*-infected cattle, bison, or captive elk herds were located nearby, and wild elk were considered likely to be spillover cases [40]. Additional surveys conducted in areas around YNP and South Dakota of hunter-harvested elk revealed no positives among 789 animals evaluated histologically [40, 76].

In 1990, tuberculosis occurred in nine herds of farmed elk in Alberta and one herd in Saskatchewan. During the outbreak, one veterinarian contracted tuberculosis after clinically examining retropharyngeal lesions in an affected elk [67, 77].

Deer and Moose

Lesions due to *M. bovis* were found in 5.6 percent of 107 moose and 0.8 percent of 242 mule deer that ranged with the infected bison at NBP [70].

In 1934, two cases of tuberculosis in wild white-tailed deer were reported in New York State. They were shot in an area that was known to have a high prevalence of tuberculosis in cattle at the time. Two additional cases of *M. bovis* infection in wild white-tailed deer were confirmed in New York in 1961 [66]. Since then, additional infected free-ranging white-tailed deer and mule deer have been found throughout North America in small numbers, usually in the vicinity of infected cattle, bison, or captive cervid herds [17, 40, 78]. The exception is Michigan, where white-tailed deer are now recognized as the reservoir for bovine tuberculosis, likely due to enhanced transmission at feeding sites. This is the first self-sustaining outbreak of tuberculosis in free-ranging cervids in North America [68, 69].

Tuberculosis also occurs in captive herds of deer in several States and Provinces in North America [74, 79].

Transmission

Tuberculosis is usually transmitted by the respiratory route although infection may also occur through ingestion, cutaneous exposure, and congenital transmission. *M. bovis* has a complex epidemiological pattern that can include transmission of infection within and between farm animal and wildlife populations. Intensive, as opposed to extensive, livestock systems facilitate close contact between animals and promote the spread of the disease. Not all infected animals transmit the disease, and asymptomatic and anergic

carriers may occur. *M. bovis* can survive for several months in the environment, particularly in cold, dark, and moist conditions [65, 66].

Tuberculous lesions in both bison and elk are predominantly located in the lungs, suggesting that aerosol transmission is possible [33, 67]. Under experimental conditions, bison have been shown to be capable of transmitting tuberculosis to cattle [80]. Transmission is also likely to occur from both bison and cervids to cattle under natural conditions due to the coexistence of disease in multiple species (farmed and wild) on several occasions [17, 73].

Incubation period

The clinical signs of tuberculosis usually take months to develop. Infections can remain dormant for years, reactivating during periods of stress or in old age [65].

Clinical signs

Bovine tuberculosis is usually a chronic debilitating disease, but it can also be acute and fulminant on occasion. Early infections are often asymptomatic, later developing into emaciation, weakness, fever, and inappetence. A cough may be present with or without dyspnea, and diarrhea or constipation may be seen. If lymph nodes are greatly enlarged, they can obstruct blood vessels, airways, or the digestive tract, causing associated problems [65].

Bovine tuberculosis is characterized by the formation of tuberculous granulomas where the bacteria are localized. These granulomas are usually yellowish and caseous; they may be calcified and are often encapsulated. In deer, lesions appear more like abscesses. Granulomas are most often found in the mediastinal, retropharyngeal, and portal lymph nodes, as well as the lung, spleen, liver, and the surfaces of body cavities [65].

The severity of disease varies with the infectious dose and immunity of the individual. Infected animals may remain asymptomatic, become ill after stress or in old age, or develop a fatal, chronically debilitating disease [65].

The appearance and distribution of lesions in bison and elk were generally found in the thoracic cavity and are comparable to those seen in cattle [33, 67, 70, 72, 81].

Diagnosis

Tuberculosis is usually diagnosed in the field using the purified protein derivative or tuberculin skin test, which measures delayed hypersensitivity to *M. bovis* antigen. Estimates of the sensitivity of tuberculin testing in cattle range from 32-99 percent, while specificity is estimated to be 75.5-99.9 percent. The sensitivity of the test is influenced by the interpretation key used, the potency and dose of tuberculin administered, the time since infection, observer/operative variation, and other factors. In addition to the above factors, specificity is also affected by exposure to *M. avium*, *M. avium* subsp. *paratuberculosis*, or environmental mycobacteria, and comparative tests using different antigen can be used to distinguish between these and *M. bovis*. False negative reactions may occur in animals that have poor immunity or are anergic, old, or have recently calved. Each batch of tuberculin must be assayed in *M. bovis* infected animals against a

reference preparation or working standard since variability in potency may be considerable between batches [65, 66].

In some cases, blood samples may be taken for diagnostic blood tests, including ELISA and FPA. The FPA has been used to evaluate the prevalence of tuberculosis in bison in some studies [16, 26] . Under experimental conditions in cattle, it has been shown to perform as well as or better than tuberculin testing, with a sensitivity ranging from 61.5 to 92.9 percent and a specificity of 98.3 to 100.0 percent [82, 83]. In a study evaluating the use of this test in bison, sensitivity and specificity were both found to be 100 percent as compared to culture of *M. bovis*; however, the sample size used was very small (6 negative and 3 positive bison), which limits the ability to extrapolate these results to a larger population [84].

Occasionally, the sputum and other body fluids may be collected for microbiological examination. Bovine tuberculosis can be diagnosed in tissue samples by histopathology, microscopic demonstration of acid-fast bacilli, isolation of mycobacteria, and identification by biochemical tests. PCR methods have also been described [65].

Diagnosing tuberculosis in susceptible wildlife species can be problematic due to the lack of proven test methods in these species [24].

Screening tests used for bovine tuberculosis detection at EINP in bison and elk are the caudal fold tuberculin (CFT) test and mid-cervical test (MCT) or single cervical test (SCT), respectively, with comparative cervical testing (CCT) used as an ancillary test in both. Necropsy of positive reactors also occurs, including histopathologic examination and culture. The following is a description of the use of these tests in bison and elk as compared to the reference cattle population.

Most of the tests used to diagnose tuberculosis in bison surveys have been variations of tuberculin tests (CFT testing and CCT), sometimes with supplementation by other test methodologies (such as FPA) [26, 74].

Other studies used identification of tuberculous lesions in necropsied animals as their testing standard. When the necropsy results were compared with tuberculin testing, the conclusions were that the sensitivity and specificity of tuberculin (CFT) testing was 66.6 percent and 89.6 percent, respectively [55].

In a study evaluating CFT tests and CCT in a small number of bison samples, both methods accurately diagnosed infection in all experimentally inoculated bison and were negative for control bison. Gross tuberculous lesions were only detected visually in 66.7 percent of bison evaluated at 12 months post exposure although histopathologic lesions were detected in 83.3 percent and *M. bovis* was successfully isolated from all inoculated animals. These findings indicate that gross pathologic evaluation may be less sensitive than tuberculin testing initially (at least in an experimental setting) [80]. Additional studies have found the CCT test to be both sensitive and specific in bison [74].

In a naturally occurring outbreak of *M. bovis* in captive wild elk, both SCT and CCT were evaluated. Both performed well in detecting *M. bovis* sensitization in elk; however,

it was concluded that when elk have repeatedly received tuberculin skin tests, the CCT results may be varied [81].

Treatment

Although drug treatment has been used in cattle, it does not always eliminate the infection. No suitable or effective medications or vaccinations are available for wildlife [21, 65].

Public health issues

Although unusual, *M. bovis* can infect humans usually through ingestion of unpasteurized milk or dairy products and infrequently through aerosols and breaks in the skin. As with brucellosis, the introduction of pasteurization helped eliminate the majority of *M. bovis* infections in humans. Now, the highest risk for infection occurs in occupational groups who work with infected livestock either on the farm or in the slaughter house [66].

Infections in humans may result in asymptomatic infections, pulmonary tuberculosis, or disseminated infections. Untreated infections may be fatal [65].

Human infection with *M. bovis* resulting from contact with farmed elk were reported as part of investigation and response activities related to two separate outbreaks [73, 85].

Release Assessment

EINP is a unique facility. While it is entirely fenced, it contains wild animals that are infrequently handled, making it not quite a free-roaming population, yet not a farmed herd. Further, the population of ungulates within the park has been closed to new additions for over 40 years in the case of wood bison and almost 100 years in the case of the remaining populations. All animals in the park are descendants of the original translocated (or in the case of moose and deer, enclosed) animals.

Because no natural predators of bison and elk are within the park (wolves and bears, primarily), the populations of elk and bison have reached very high population densities compared to free-roaming animals (close to their maximum carrying capacity). To reduce the population to a more natural level, park officials conduct annual or biennial roundups of the bison herd (with concurrent trapping of the elk herd) to select animals to be removed from the park. Currently, bison and elk removed from the park are released into the wild through cooperative transplant projects with provincial, State, and foreign governments, and bison may also be sold at live-sale auctions. During the collection period, park officials also take a census of the population and evaluate the health of the animals.

The process of collecting, selecting, and testing the animals is stressful and dangerous (to both animals and staff) and results in a temporary, but unnatural, confinement of the animals. It is therefore intended to be discontinued once herd levels are reduced to a more natural state. The roundups represent the only handling animals in the park receive.

Although deer and moose are also part of the EINP population, they are not included in the surplussing process. Testing in these species is not included in the model that assesses disease surveillance due to the scarcity of test data for these animals at EINP; however, testing and surveillance within these species is discussed and included in the overall analysis.

Surveillance methods

Ongoing surveillance for both brucellosis and bovine tuberculosis has been conducted at EINP since the 1930s. A variety of surveillance tools have been used, including testing of all animals destined for translocation from EINP (brucellosis and tuberculosis), daily visual evaluation of park ungulates by staff members for signs of illness, annual aerial counts of ungulate populations with sensitivity sufficient to detect excess herd mortality, and slaughter testing for tuberculosis and brucellosis (although this was discontinued in the 1970s).

Shortly after brucellosis was eradicated from the nascent wood bison population, three whole herd (or near-whole herd) evaluations were conducted on the wood bison herd. These evaluations were conducted in 1970, 1972, and 1975. The herd size at that time was 33, 41, and 72, respectively, with all animals sampled in the first two studies and 71 of 72 sampled in the third. (Since then, the herd has grown too large to continue regular whole herd evaluations.) No positive results were identified. It was concluded that brucellosis (as well as bovine tuberculosis, if present) had been eradicated from the herd.

Although no longer conducted, surplus cervids (including elk, moose, and both mule and white-tailed deer) were once slaughtered at an onsite abattoir under CFIA supervision, with tests conducted for brucellosis and tuberculosis and evaluations performed. Between the 1950s and the 1970s over 3,000 cervids were evaluated, with none found positive for brucellosis or tuberculosis.

Additional surveillance mechanisms currently in place include the testing of bison and elk selected for surplus from the park; visual surveillance of both the wood bison and plains bison; and to a lesser extent, the elk, deer, and moose populations within the EINP.

Brucellosis testing is done at CFIA Lethbridge Laboratory and the CFIA Ottawa Laboratory. The CFIA Ottawa Laboratory houses the Brucellosis Centre of Expertise, which is a World Organization for Animal Health (OIE) Reference Laboratory for brucellosis. The Lethbridge Laboratory is a subsidiary of the Ottawa Centre of Expertise.

All tuberculosis diagnostic laboratory work (histopathology, isolation and identification, and FPA analysis) is done at the CFIA Mycobacteria Centre of Expertise at Ottawa.

Exit testing

In natural ecosystems, ungulate populations are limited to a sustainable carrying capacity mainly through dispersal and predation. Herbivores in a natural ecosystem will expand their range (or seasonally migrate) and disperse if there are too many in one area. In addition, large predators also reduce the number of herbivores in the population. Both of these factors are absent in EINP due to the fence, which restricts movement and dispersal of the animals within the park and excludes large predators from the park. Therefore, both bison and elk must be regularly surplussed from EINP in order to maintain a natural/sustainable carrying capacity [34].

The bison and elk populations of EINP have been rounded up for surplussing purposes approximately every other year or every year since at least 1994. The two bison herds are rounded up separately, with the plains bison round up occurring first, followed by the wood bison round up. The bison are lured into a holding pen in their respective areas of the park using hay in late fall. Generally, a number of mature bulls are not able to be captured. Once the bison have been confined, a separate operation targets the elk population through trapping of individual or small groups of animals. In addition to selecting animals for surplussing, other activities conducted during the round up include assessing the health status and demographics of captured animals and disease testing [11, 34].

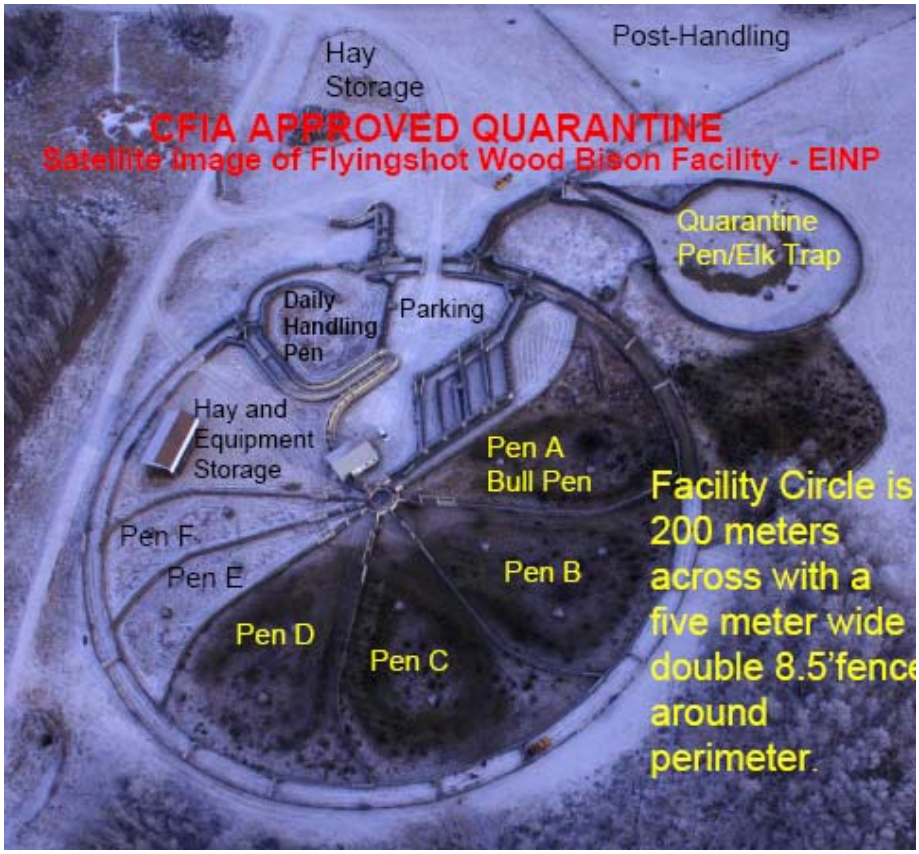
Testing of bison and elk located in EINP since 1994 has resulted in no positive animal detected, despite testing in over 1,000 wood bison, 2,000 plains bison, and 2,500 elk. On average, between 10-30 percent of each bison herd and 10-40 percent of the elk are tested and removed from EINP each year (this does not include the whole herd test in the wood bison herd in 2008).

The entire process can take several weeks to complete and involves rounding up the entire bison herd (minus a few animals that are not collected due to handling difficulties

(large bulls) or danger to the animal (cows with very young calves)), selection of animals for surplus, and disease testing. Animals collected in the roundup that are not selected for surplus are temporarily confined in a fenced enclosure until test results have been completed. The bison are held there until test results have been received for animals undergoing testing. The handling facilities for the Wood Bison Area are shown below in figure 2.

Unlike bison, elk are not rounded up, but are trapped in small groups from the general park area once the bison have been confined (otherwise the bison tend to consume the bait left in the elk traps). Captured elk are then transported to their own holding pen and handling area in goose-neck trailers.

Figure 2: Aerial view of wood bison handling facility



Younger animals tend to be overrepresented among the surplussed (and therefore tested) bison, likely due to their desirability in conservation efforts. However, the age breakdown of surplussed elk was similar to that in the overall EINP population. Because of this, park staff generally include 10-15 percent aged animals in the surplussed bison groups to enhance disease detection among those tested (since disease is more likely to be present in older animals), as well as to decrease the age of the resident herd. The difference in disease risk according to age is accounted for in the model that assesses disease surveillance.

Brucellosis testing

Sensitivity and specificity data were provided by CFIA, based on test estimates established by CFIA experts at the Brucellosis Centre of Expertise.

For most of the period under discussion (1995-present, when most complete and recent exit data were made available), the brucellosis screening test used in both bison and elk at EINP was the BPAT. In 2007, the FPA-brucellosis test replaced BPAT as a screening test for all situations except where the importing country requires the use of BPAT testing. Samples from EINP have been tested using both BPAT and FPA-brucellosis for screening purposes since 2007, as the laboratory that tests the samples works to incorporate automated equipment before it can acquire large volume FPA-testing capacity. The BPAT has a sensitivity of 92.1 percent and a specificity of 91.7 percent in bison and 99.0 percent and 99.9 percent in elk. The FPA-brucellosis has sensitivity/specificity of 94.5 percent/99.5 percent in bison and 99.0 percent/99.3 percent in elk.

Ancillary tests for brucellosis used at EINP have historically been the complement-fixation test until 2000. Beginning in 2001, competitive enzyme-linked immunosorbent assay (c-ELISA) was evaluated side by side with the complement-fixation test, and in 2007, c-ELISA was adopted as the sole ancillary test for brucellosis. The sensitivity/specificity of the complement-fixation test is reported as 92.3 percent and 99.4 percent in bison and 99.0 percent and 99.3 percent in elk, while the c-ELISA is reported as 94.5 percent/97.4 percent in bison and 99.0 percent/99.4 percent in elk. Any positives on ancillary testing would subsequently be necropsied, which would include culture and histopathology, although this has not yet occurred.

In the case of the 62 wood bison held for export to Alaska, the brucellosis screening test used was BPAT combined with FPA-brucellosis, and c-ELISA for ancillary testing.

From 1995 through 2008 brucellosis testing was conducted in over 2,000 elk and wood bison in the Wood Bison Area and over 3,000 elk and plains bison in the Main Park Area. See Tables 1 and 2 for annual test data and population estimates according to species and park area.

Table 1: Brucellosis testing in Wood Bison Area of EINP

Year	Wood Bison		Elk	
	number tested	population	number tested	population
1995	73	441	120	580
1996	34	367	107	532
1997	79	421	88	568
1998	74	428	55	502
1999	85	354	101	439
2000	112	350	81	449
2001	67	335	117	396
2002	70	337	58	311
2003	0	345	0	289
2004	113	429	120	355
2005	0	303	0	209
2006	30	307	0	384
2007	0	311	161	415
2008	265	315	81	255

Table 2: Brucellosis testing in Main Park Area of EINP

Year	Plains Bison		Elk	
	number tested	population	number tested	population
1995	222	722	176	1263
1996	188	728	103	1355
1997	396	666	144	1290
1998	137	634	82	1169
1999	214	577	249	1470
2000	109	477	469	1146
2001	97	471	149	707
2002	0	419	86	607
2003	126	430	0	631
2004	0	527	88	550
2005	244	503	0	338
2006	39	548	0	322
2007	0	476	0	333
2008	56	425	0	345

Tuberculosis testing

Sensitivity and specificity data for tuberculosis tests were also provided by CFIA. Intradermal tuberculin tests are estimates that can vary greatly due to the many factors that can influence test performance. All data are derived from generally accepted estimates, except for MCT and CCT in cervids, which were derived from an investigation of over 500 naturally infected farmed elk associated with a tuberculosis outbreak in western Canada. For the FPA-tuberculosis test, estimates generated as part of the validation work for this test are provided; however, the data were obtained using cattle.

Tuberculosis screening tests conducted in bison (except for the wood bison herd test conducted in January 2008, which used the FPA-tuberculosis test) have been the caudal fold tuberculin (CFT) test. In elk, the screening test used has been MCT. The CFT has a reported sensitivity of 70 percent and specificity of 95 percent in bison, and the MCT estimates are 81 percent and 95 percent, respectively, in elk. The FPA-tuberculosis has a reported sensitivity of 70 percent and specificity of 99.8 percent in cattle.

The ancillary test used in bison has been the comparative cervical tuberculin test (CCT) performed according to the same standards and interpretation as used in cattle, as well as the same provision for conducting the CCT either within 10 days of, or at least 60 days following, the CFT. The sensitivity of the CCT in bison is reported at 70 percent, with specificity at 98 percent. The official protocol is for any CCT reactors to be necropsied; however, historically, bison found positive on the CFT, even if negative according to CCT, have been necropsied, including histopathologic examination and culture, to provide additional assurance that the animals were negative. Since 1994 there have been 5 CFT responders identified in wood bison, with none positive according to CCT (and all negative on necropsy).

The ancillary test used in elk has been the CCT, performed according to standards and interpretation specific for cervids (a scattergram adapted for cervids similar to USDA's scattergram interpretation). Prior to 1995, the CCT was not conducted until at least 60 days following the MCT. Since that time, the MCT and CCT have been conducted simultaneously in elk to minimize handling. The reported sensitivity of the CCT in elk is 89 percent (recording suspicious reactors as positives) and specificity is 95 percent. Animals testing positive to both tests are necropsied, including histopathology and culture of lymph nodes. Since 1995, six elk were classified as tuberculosis suspects based on reaction to simultaneous MCT/CCT testing; no positives were identified based on detailed post mortem evaluations involving histopathologic examination and culture of lymph nodes.

In 2007, the procedures for tuberculosis testing of plains bison scheduled for surplussing was changed to decrease the amount of handling and time required to complete tuberculosis testing (a minimum of two handling events are required to administer and evaluate the CFT test). Instead of performing the CFT on all animals selected for surplus, EINP began selecting 20 percent of the oldest animals from among those collected and submitting them for slaughter. If tuberculosis were present in the herd, these would be the animals most likely to exhibit lesions consistent with the disease. Ante-mortem and post-mortem examinations are conducted on these animals at slaughter; and if no tuberculous lesions are found, the herd is released. This testing is not included in the model; however, it is included in the discussion of surveillance system sensitivity.

All tuberculin tests at EINP are conducted by CFIA staff or a CFIA accredited veterinarian.

As with brucellosis, over 5,000 tests for tuberculosis have been conducted in elk and bison located in both the Wood Bison Area and Main Park Area of EINP from 1995

through 2008. The annual test data and population estimates are provided below in Tables 3 and 4, according to park area.

Table 3: Tuberculosis testing in Wood Bison Area of EINP

Year	Wood Bison		Elk	
	number tested	population	number tested	population
1995	73	441	120	580
1996	34	367	107	532
1997	79	421	88	568
1998	74	428	55	502
1999	85	354	101	439
2000	112	350	81	449
2001	67	335	117	396
2002	70	337	58	311
2003	0	345	0	289
2004	113	429	120	355
2005	0	303	0	209
2006	30	307	0	384
2007	0	311	161	415
2008	265[1]	315	81	255

[1] TB testing conducted in wood bison using FPA-TB in 2008

Table 4: Tuberculosis testing in Main Park Area of EINP

Year	Plains Bison		Elk	
	number tested	population	number tested	population
1995	222	722	176	1263
1996	188	728	103	1355
1997	406	666	144	1290
1998	137	634	82	1169
1999	214	577	249	1470
2000	109	477	469	1146
2001	97	471	149	707
2002	46	419	86	607
2003	126	430	0	631
2004	0	527	88	550
2005	244	503	0	338
2006	39	548	0	322
2007	0[1]	476	0	333
2008	0[2]	425	0	345

[1] data do not include 13 plains bison evaluated at slaughter in 2007
[2] data do not include 20 plains bison evaluated at slaughter in 2007

Visual evaluation

In addition to disease testing as part of the surplussing process, park staff visually evaluate bison in the park regularly to assess their condition and identify potentially ill animals. Elk tend to be more reclusive and are not consistently evaluated in this way; however, they are included among necropsy results for other reasons (injury during capture and transport, etc.).

The park staff, including rangers, trail crews, fence crews, visitor services staff, and technical services staff, have been trained to look for "poor-doers" (that is, animals in poor condition or acting abnormally), as part of their job. Approximately 65 park staff are present from May to September, and 25-30 staff are on the grounds of the park from October through April.

If staff spot a suspicious animal, the park biologists or the park veterinarian will evaluate the animal. If the animal is euthanized (or if a dead animal is found prior to scavenging), it will be delivered to the Alberta Agriculture Laboratory in Edmonton for necropsy. If any lesions suggestive of bovine tuberculosis (such as granulomas or lymphadenopathies with suppurative lesions in cervids) are observed, tissues are collected and submitted to the CFIA Laboratory in Ottawa for histopathology and culture. If lesions or other evidence suggestive of brucellosis (such as endocarditis, arthritis, or orchitis) are observed, blood samples and tissues are collected for brucellosis testing and submitted to the CFIA Laboratory at Lethbridge.

The following animals have been necropsied under this protocol with no positives identified for either brucellosis or tuberculosis: 29 wood bison since 1987, 33 plains bison since 1994, 1 moose since 2004, and 52 elk since 1989 (with additional negative results from 185 elk reported from recipient jurisdictions). Results for elk included animals that were euthanized subsequent to injury during capture or transport, or for other reasons [10].

Design prevalence of disease for detection purposes

Both brucellosis and bovine tuberculosis have been documented in wild and farmed herds of bison and elk in North America. Because the animals in EINP more closely approximate wild herds due to the overall lack of handling despite the fact that they reside within a fenced area, disease occurrence in wild herds will be discussed in the following sections.

In free-ranging or wild herds, the observed prevalence can vary greatly when one of these diseases is present within a herd depending on a number of factors. In general, the prevalence of both brucellosis and bovine tuberculosis tends to be greater in free-ranging bison herds compared to elk herds. This is likely due to the gregarious nature of bison, compared to elk, which enhances disease transmission [12, 25, 26, 72].

However, this trend can be affected by management factors. Feeding wild animals, especially deer and elk, artificially congregates animals and can increase transmission. The effect can be dramatic as has been seen in free-ranging elk that are infected with either brucellosis or bovine tuberculosis and have been exposed to artificial feeding practices [24, 28, 38, 39].

During the collection, sorting, and testing period for bison and elk in EINP, management is much more intensive than the rest of the year. Animals are confined in relatively small pens, and concentrated feeding around bales of hay replicates a concentrated feeding environment. The process can take up to 2 months to complete and can be expected to increase the prevalence of both brucellosis and tuberculosis in the bison and elk herds if those diseases are present in the population.

Also, as both of these diseases are chronic in nature, their prevalence is increased in older animals [17, 25, 26, 38, 67, 68, 72]. Herd density may have an effect on the prevalence of these diseases as well; however, the relationship is not straightforward. For brucellosis, prevalence is more likely affected by the density during the calving season, and for tuberculosis, a minimum herd population threshold appears important [26]. Other factors that could potentially increase the prevalence of disease in these herds are the heavy carrying capacity for each species and the relatively aged population of the animals.

Bison and elk located in each of the park areas share the same range land and are known to commingle. Therefore, surveillance data for the two species are combined. However, the two areas, Main Park Area and Wood Bison Area, are considered independently due to their physical separation. Differences in observed prevalence for the two species are accounted for in the model, as are differences in the age of the park population as compared to the age of animals tested.

Brucellosis prevalence in free-ranging bison and elk

The prevalence rates observed in outbreaks of brucellosis in free-ranging bison and elk in the United States and Canada have varied widely according to the factors discussed above. In bison, prevalence rates from 15 to 77 percent have been observed with an overall weighted prevalence of 35 percent. Among adult bison, the average brucellosis prevalence is greater than that seen in juveniles (2 years old and younger). The relative risk of infection in adults compared to juveniles was estimated in the model at 1.5:1 to account for this difference, based on differential rates provided in the literature. The estimated prevalence rates used in the model are based on serological diagnosis in all cases since serological screening is practiced in the EINP bison herd [12, 25, 26, 29-33, 35, 36].

In elk, the overall prevalence rate of brucellosis in free-ranging herds was found to be about 17 percent, with rates varying from 1 to 80 percent. When prevalence was divided into populations that were known to have been exposed to feeding practices and those without exposure to feeding, the prevalence rates averaged 47 percent and 2 percent, respectively. The EINP herd more closely resembles wild herds that are exposed to feeding than those that are not, although the true value may lie somewhere in between. Although no differentiation was made according to age in the available studies, it is assumed for the purposes of the model that the relative risk of adults compared to juveniles is similar for elk as for bison; therefore, it is set at 1.5:1. As with bison, prevalence rates were derived from studies using serological evaluation [24, 29, 35, 38-40].

Based on the prevalence information, the prevalence used in the model as a threshold for detection of brucellosis in both the bison and elk herds is set at 1 percent, which is a conservative estimate given that the prevalence of brucellosis when it is present in bison and elk herds tends to occur at significantly higher rates.

Tuberculosis prevalence in free-ranging bison and elk

As with brucellosis, tuberculosis rates seen in free-ranging bison and elk in the United States and Canada have varied greatly, depending on the distance of the herd from a reservoir population, feeding practices, age of the herd, etc. In bison, the average prevalence observed in the various surveys was approximately 43 percent, with an increasing prevalence observed in adults compared to juveniles similar to that seen with brucellosis. Therefore, as with brucellosis, the relative risk between adults and juveniles is assumed to be 1.5:1. Studies evaluating prevalence using tuberculin testing were used to assign these estimates since this is the screening test used for bison in EINP; however, studies that used the identification of tuberculous lesions at necropsy had very similar results in terms of detected prevalence in infected herds at 42 percent [25, 26, 33, 55, 70].

In elk, the overall prevalence of tuberculosis was approximately 18 percent. Data were not available for either age or feeding history of the herds; so assumptions were made that the relative risks of exposure to feeding and in adult animals were similar to that seen for brucellosis in elk and tuberculosis in bison. In addition, studies available for the determination of prevalence primarily used identification of tuberculous lesions at necropsy. Since prevalence rates of tuberculosis in bison were almost the same, whether tuberculin testing or lesion identification at necropsy was used for diagnosis, tuberculous lesions are used as a proxy for prevalence in elk based on tuberculin testing [17, 25, 70].

For tuberculosis, the prevalence used in the model as a threshold for detection in both the bison and elk herds was established at 1 percent. Reasoning for this is the same as for brucellosis.

Risk of introduction of disease into EINP

The risk of either brucellosis or bovine tuberculosis entering EINP is extremely low given the closed nature of the park ungulate herds since their inception, the fencing that encloses the park, the biosecurity practices imposed by park officials, and the disease-free status of cattle herds in Alberta (including those surrounding the park).

Fencing

The animals in EINP are enclosed by a 7-foot high livestock fence that is maintained by a dedicated fence crew throughout the year. Although the fence cannot entirely prevent movement of ungulates (particularly deer) into and out of the surrounding nonpark area, it does prevent bison and elk from moving between the two park areas due to the four-lane divided highway between the Main Park Area and Wood Bison Area. Additionally, although EINP staff reported that movement of nonbison species into the park could not be prevented entirely, it is believed to occur at a very low level, contributing an insignificant risk to introduction of disease into the park given the disease status of cattle and farmed bison and cervids surrounding EINP.

Closed herd status

The population of ungulates at EINP has been closed to the introduction of animals since 1965 in the case of wood bison and 1909 in the case of plains bison. The elk, moose, and mule deer that were present in the enclosure when the plains bison herd was fenced in 1909 are the progenitors of those animals present in the park today. The large herbivores in the park today are descendants of those original inhabitants, with the exception of white-tailed deer, which subsequently found residence in the park from the surrounding area and now outnumber the mule deer.

Biosecurity measures

Several measures have been officially employed to reduce the possibility of introduction of disease into EINP through human activities via a management directive issued by park officials. These measures include a prohibition on livestock trailers entering the park or EINP vehicles being used outside the park in association with livestock or farming, and a requirement to steam clean transport vehicles used to move surplus ungulates out of the park. In addition, clothing and footwear associated with livestock may not be worn into the park unless thoroughly cleaned, and park issue clothing and footwear may not be worn outside the park around livestock.

Alberta cattle herd status

Canada declared freedom from brucellosis in 1985 and from bovine tuberculosis in 1997. In 1997, USDA removed testing requirements for both brucellosis and tuberculosis in animals originating from Canada, effectively recognizing their disease-free status.

Brucellosis

Surveillance for brucellosis is achieved through periodic national serum surveys. The last survey was conducted from 2002-2003. Of 15,105 cattle sera screened using FPA-brucellosis, 24 positive reactors were identified; none of which were positive on confirmatory testing (c-ELISA). Based on the sample size and results, Canada was able to determine with 95 percent confidence that its cattle population was free of brucellosis if the disease existed in the national herd at or above a prevalence of 0.02 percent. A 2007-2008 survey is currently underway (sampling of over 15,000 serum specimens has been completed, which meets the collection goal, and laboratory analysis is in progress) with no suspect brucellosis reactions found thus far out of over 1,000 samples analyzed to date [86, 87].

In addition, cattle herds in the area around EINP are also subject to brucellosis surveillance through an ongoing auction market testing program.

Tuberculosis

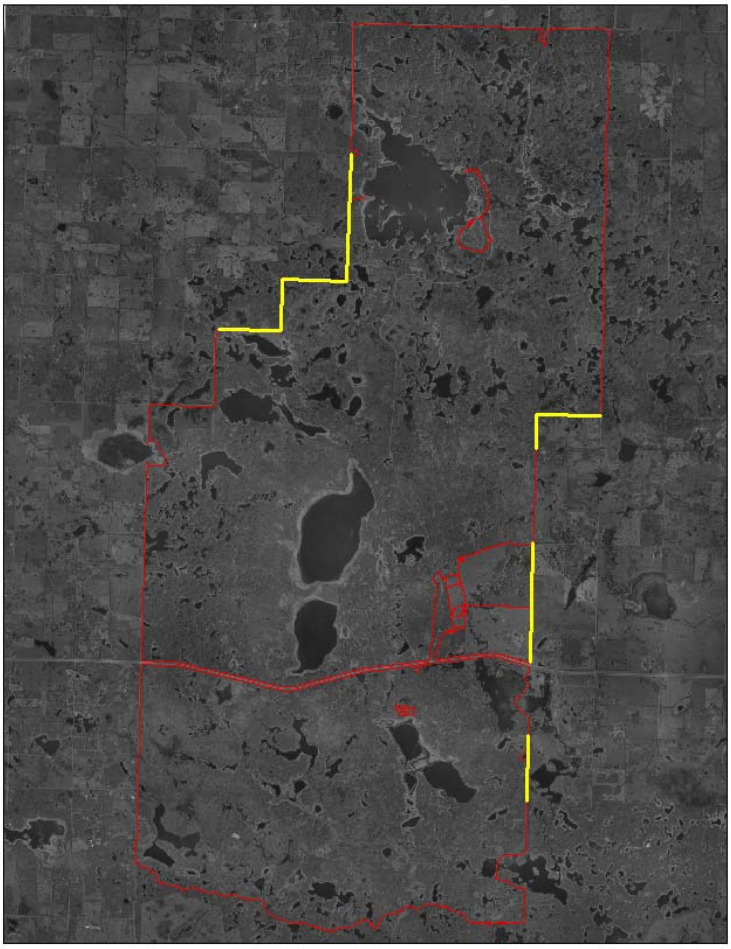
Canada has been considered tuberculosis free by USDA since 1997 with the exception of Manitoba. Cattle herds in the area surrounding EINP are subject to surveillance for tuberculosis under a granuloma submission program at slaughter. Additionally, farmed cervids are subject to brucellosis and tuberculosis surveillance through the granuloma submission program (tuberculosis) and abattoir sampling program (brucellosis). The sampling plan on which Canada's granuloma submission program was established includes cattle and cervids, and is designed to detect disease prevalence of 0.01 percent or higher with 95 percent confidence.

EINP exposure to Alberta cattle

Alberta has been considered free of brucellosis and tuberculosis in its cattle herd since 1997 by USDA. It is highly unlikely that diseases exist outside of the park and could enter EINP from surrounding cattle farms, free-ranging cervids, or EINP ungulates that could potentially leave and reenter the park.

The fence around EINP is shared by a limited number of cattle herds allowing for the potential of nose-to-nose contact between park animals and cattle in adjacent farms. However, this only occurs for a small proportion of the fence line (see figure 3). This factor, combined with the level of testing in the cattle and bison herds (as well as cervids) in Alberta to maintain tuberculosis- and brucellosis-free status, plus the additional mitigation of negative test results in cattle on the adjoining premises that are tested at market, results in a negligible risk of spread to EINP inhabitants.

Figure 3: Areas of EINP fence line in contact with cattle herds (in yellow)



Deer and other cervids are unlikely reservoirs for both brucellosis and tuberculosis; most commonly, they are infected as spillover or incidental hosts, with cattle as the reservoir species [40, 47, 88]. If this is the case, disease testing in cattle could be expected to uncover the presence of disease in the area. It is extremely unlikely that a brucellosis or

tuberculosis reservoir could be present in the Alberta free-ranging cervid population without also being present in cattle. Transmission to nearby cattle would be expected to occur and would likely be detected through routine testing (as has occurred in the RMEA) or via the dramatic increase in abortions that would likely be seen if brucellosis infects a naïve herd. Therefore, the likelihood that brucellosis or tuberculosis would enter EINP as a result of contact with infected but undetected wild deer or other cervids surrounding the park is extremely low.

The probability of introduction of either brucellosis or tuberculosis into EINP, based on the factors outlined above, is negligible at this time and is estimated at 0.1 percent for the purposes of the model.

Quantitative analysis of the exit testing surveillance component

A quantitative approach [89] was applied to the exit testing component of EINP surveillance activities in the Wood Bison Area and the Main Park Area. All animals that leave the EINP population are tested for brucellosis and tuberculosis (except for plains bison since 2007, when CFT testing was replaced with slaughter surveillance). Because animals are surplussed regularly, we received sufficient data from CFIA and EINP staff to conduct a quantitative evaluation of this surveillance component for the years 1995-2008.

The detection sensitivity provided in this analysis only applies to the years for which testing data are available from CFIA and may not apply to subsequent years if either the testing protocol changes or the number of tests conducted declines.

Description of methodological approach

This methodology was developed as an alternative approach to analyzing complex, nonsurvey based surveillance data and quantitatively estimating the level of confidence in a surveillance process that has not demonstrated any evidence of a disease (that is, the absence of detection of that disease through the surveillance system). It allows estimation of the probability of obtaining the observed surveillance results (no positive outcomes) from a population if it were infected at a specified level. Alternatively, the approach provides an estimate of the probability of detecting disease (observing one or more positives) in a population if it were infected at a specified level.

The proposed approach uses scenario tree analysis and stochastic simulation as the basis for modeling the surveillance system (or component) under evaluation. The scenario tree is developed with the appropriate infection, category (risk or otherwise), and detection nodes that represent the important factors that would result in a positive outcome or test result. Using the associated tree probabilities and groupings according to risk factors and categories, a level of confidence that the surveillance efforts could reasonably be expected to detect disease if it was present in the population can then be estimated.

The underlying assumption of this approach is that disease is present in the population at a certain (low) level or design prevalence p*. The estimated sensitivity of the surveillance system or component, or the probability of detecting one or more positive animals given the disease is present, represents the confidence level for the statistical test of the null hypothesis (H_0: disease is present in the population at a level of p* or higher; H_A: disease is not present or infection levels are below levels of p*). The surveillance sensitivity can also be viewed as the probability that the surveillance system or component will identify a diseased animal given that the disease is present in the population at the specified prevalence p*. If so desired, one can further assume a prior estimate of the probability that the disease is not present and apply Bayesian inference to calculate the posterior probability that disease is not present given the negative results obtained from the surveillance system.

A second underlying assumption of this approach is that a surveillance system whose goal is to ensure that disease is not present in the population will conduct all necessary followup investigations and testing on all suspect positive results. Thus, the surveillance

system has, by definition, perfect specificity. The assumption of perfect specificity is predicated on the full and complete investigation of any positive surveillance result until it can be determined whether it is a true or false positive result. A false positive result (that is, one that is eventually confirmed as a negative result through further testing) subsequently becomes another negative outcome of the surveillance system that can be used with the rest of the negative outcomes to provide a level of confidence in that surveillance process [90].

With these assumptions in mind, the analytic method has two outputs. The first is a level of confidence in the ability of the surveillance system under evaluation to detect disease when it is present at p*, also called the sensitivity of the surveillance system. The second output is the probability that disease is absent given the negative surveillance results and a prior probability of the disease presence.

Description of the model and assumptions

Separate scenario trees were developed to model the process of detection of brucellosis and tuberculosis by the surveillance system component exit testing. Bison and elk residing together within each enclosure were considered within the same model due to their sharing of the same range land and intermingling, according to park staff.

The scenario tree is structured to include the factors affecting the probability that a surveillance unit (the individual animal) will be infected, as well as the factors affecting the probability that the surveillance unit will be detected. It describes the animals included in the surveillance system (those tested) and the population from which they were sampled. The scenario trees for both diseases consist of two risk category nodes (species and age) to account for the differential risk of disease between bison and elk and between adults and juveniles, an infection node that incorporates the assumed design prevalence p* for this analysis, and detection nodes that represent the sequential testing that is undertaken to investigate any suspect positive cases. Thus, the scenario tree represents the probabilities that an animal from a given species-age grouping will test either positive or negative (defined as either negative test result or animal not tested). Scenario trees and associated probabilities utilized in this analysis are presented in Appendix 1.

Selection of the design prevalence in this approach has the same influence on sample size as it does when conducting a survey for disease detection: a small p* means that a larger sample size will be required to detect the disease for a given confidence level. A design prevalence of 0.01 (1 percent) was used for both disease models. The design prevalence was selected based on a likely minimum prevalence for brucellosis or tuberculosis if they were to become established in the population.

Based on a review of the literature, different relative risks were assigned to each species (bison and elk). Relative risks were also applied according to the age of the animal since older animals are more likely to be infected with these chronic diseases than younger animals. The following relative risks were used as model inputs to represent the risk differential among species and age groups: a 1.5:1 ratio was assumed between bison and elk, and a 1.5:1 ratio was assumed between adults and juveniles. Test sensitivity data provided by CFIA were incorporated into the probability of detection. The effective

probability of an animal being infected was based on the adjusted relative risk and the design prevalence. Annual surveillance sensitivities that factor in the effective probability of infection by species and age were estimated using a hypergeometric approximation that adjusts for sampling in small herds.

The primary model outputs are twofold. The first is the sensitivity of the exit testing surveillance component (CSe), which is an aggregate measure of the group-level surveillance sensitivities (by species and age). The CSe is interpreted as the confidence (the probability) that one or more positive animals will be detected, given that the population is truly infected at a prevalence greater than or equal to the design prevalence p*. The second model output uses a Bayesian revision to estimate the probability that the disease is not present in the population, given the surveillance did not detect the disease.

Model outputs were calculated beginning with surveillance data from 1995 when accurate and stratified data on exit testing of bison and elk were made available. The value of historical information was also considered, and time discounting of the CSe's was incorporated into the model output applying the Bayesian revision with a one-year time lag. The starting (1995) prior probability that the disease was absent from the animal population in the Wood Bison Area was also assumed to be 0.5 (50 percent).

Finally, a sensitivity analysis was conducted using values of 0.005, 0.01, and 0.05 (0.5, 1, and 5 percent respectively) for the design prevalence and values of 0.0001, 0.001, and 0.01 (0.01, 0.1, and 1 percent respectively)

Model results

In general, when evaluating the detection sensitivity of a surveillance system in a population of animals, the proportion of animals tested depends partially on the size of the reference population. In a small population of animals (fewer than 1,000), a relatively large proportion is required to be tested to determine the absence of disease at a predesignated design prevalence as compared to a larger population to achieve the same confidence in the results. The confidence level is equivalent to the sensitivity of the surveillance system in detecting disease if present in the population at the established prevalence.

For example, tables provided by Cannon and Roe [91], based on a hypergeometric distribution, with assumptions of 100 percent test sensitivity and random selection, indicate how many animals to test in a population based on a predetermined disease prevalence (that is, established as a threshold for detection). To detect at least 1 case of disease in a population of 100,000 animals with 99 percent confidence, assuming the disease is present in at least 1 percent of the population, the number of animals required to be tested is 458 (less than 5 percent of the overall population). Therefore, if 458 animals from this population are tested and no positives are detected, then we can be 99 percent confident that the disease is not present in this population at a level of 1 percent or greater.

Conversely, to determine that disease is not present in a population of 100 animals at a prevalence of 1 percent or higher, all 100 animals must be tested to make this determination with 99 percent confidence.

In the first example with a larger population of 100,000 animals, a 1 percent prevalence is equivalent to 1,000 infected animals. Testing 458 animals would be expected to detect at least one of the positive animals 99 percent of the time the survey was conducted. In the population of 100 animals, however, a disease prevalence of 1 percent would represent a single animal. To achieve 99 percent confidence that the single infected animal was detected, all 100 animals would need to be tested.

The design prevalence for detection of disease within each area of EINP was set at 1 percent for evaluation purposes. It is likely that if either brucellosis or tuberculosis were present in the park, it would exceed 30 percent as discussed above. Therefore, surveillance to detect disease at 1 percent would be highly likely to identify either disease in this herd.

The population of elk and bison in each of the EINP enclosures has ranged from approximately 500 to 1,000 animals during recent years. In the Cannon and Roe tables, a population of 500 animals would require testing of 300 (60 percent) to determine with 99 percent confidence that disease is not present in 1 percent or more of the herd. A herd of 1,000 animals would require that 368 (37 percent) be tested.

To determine if disease (either tuberculosis or brucellosis) is present within either of the EINP populations at a prevalence of 1 percent or greater (5-10 animals infected total), the number of animals required to be tested to determine the absence of disease in at least 99 percent of animals with 99 percent confidence would range from approximately 300-350 animals. However, the number of animals (both bison and elk) that have been tested during recent years ranges from 150-200 on average (although during some years, no surplussing or testing has been conducted in one or both species). Testing this population more often is not feasible due to the animals' wild status and the difficulty (and danger) in handling them. Therefore, routine testing of this population only occurs in animals being handled for surplussing purposes at the time of the roundup. Although this level of testing would result in confidence levels ranging from 60-85 percent for each year if considered individually, the model used in this assessment uses confidence levels from multiple years' worth of surveillance data, resulting in a significantly higher confidence level (over 99 percent) at the end of the surveillance period (1995-2008).

In many cases, when the sensitivity of a disease survey is estimated, the results are considered on a stand-alone basis. In cases where the herd is open to new introductions on a regular basis, which can exist in both wild and farmed herds, this may be the best way to evaluate data due to a relatively high probability of disease introduction during the time between surveys. For example, if it has been determined that disease is not present in a herd at 1 percent or above with 99 percent confidence, but the likelihood of disease entering the population is high, then the probability that the disease is not present at levels below 1 percent would subsequently decline after the conclusion of the survey.

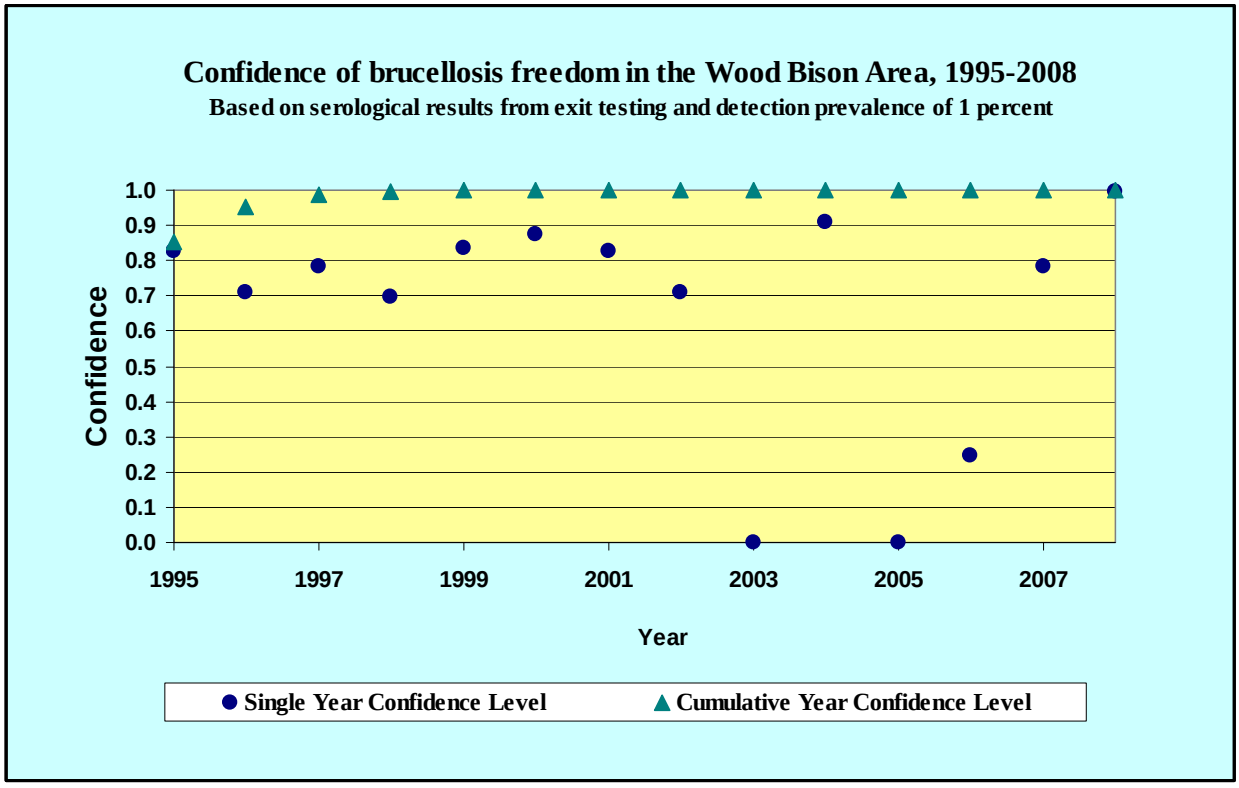
Unlike the situation above, the probability of disease introduction (either brucellosis or tuberculosis) into EINP is extremely low due to a number of factors. First, the portion of the park that houses the wood bison has been closed to new entrants since the 1960s (and the remainder of the park since the early 1900s), and all current residents are descendents of the original population. Second, 7-foot high wildlife fencing has been in place around the park (separate fencing for the Wood Bison Area and Main Park Area) since its inception. Although this fence is not sufficient to prevent 100 percent of wild animal movement or nose-to-nose contact with farmed cattle, the lack of tuberculosis or brucellosis within Alberta, including the farms surrounding EINP, makes the risk of introduction of disease through contact with surrounding wild or domestic animals exceedingly unlikely. The probability of introduction of both tuberculosis and brucellosis into the EINP herd was assumed for modeling purposes to be 0.1 percent on an annual basis.

Surveillance system analysis results for the Wood Bison Area

Brucellosis

Given the exit testing data from 1995-2008, the sensitivity of detection of brucellosis in the Wood Bison Area of EINP if present in 1 percent or more of the bison and elk, is estimated at greater than 99.99 percent given a probability of introduction of 0.1 percent. What this means is that if brucellosis were present in either the wood bison or the elk residing within the Wood Bison Area of EINP at a prevalence of 1 percent or greater, the surveillance system used for animals exiting this portion of EINP since 1995 is likely to have detected at least one case of brucellosis with a sensitivity, or confidence level, of 99.99 percent. (See figure 4.)

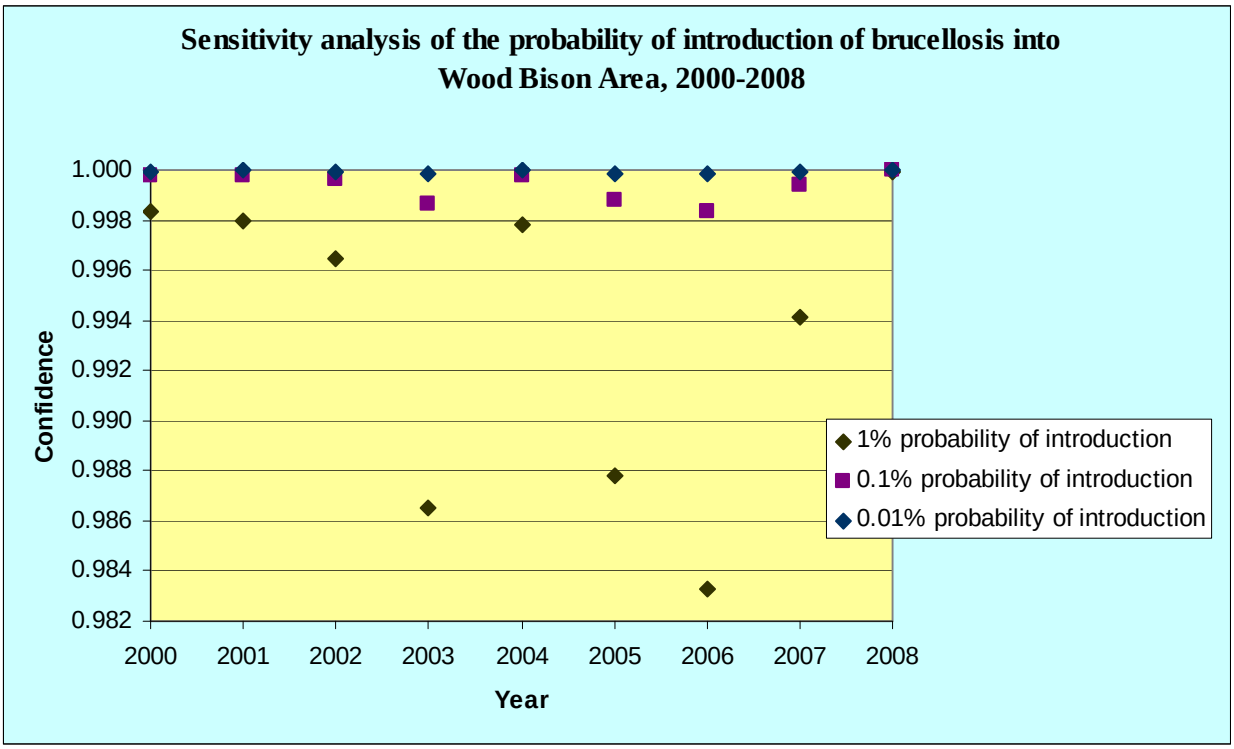
Figure 4: Confidence of brucellosis freedom in the Wood Bison Area, 1995-2008



If each year of exit testing data is evaluated independently, the confidence levels range from 0 to 99.74 percent, depending on the number of samples tested each year. However, one of the strengths of this population is its closed nature, which results in a very small probability of disease introduction into the population, thus allowing confidence levels for disease detection to be aggregated from one year to the next, resulting in a confidence level of 99.99 percent by the end of 2008.

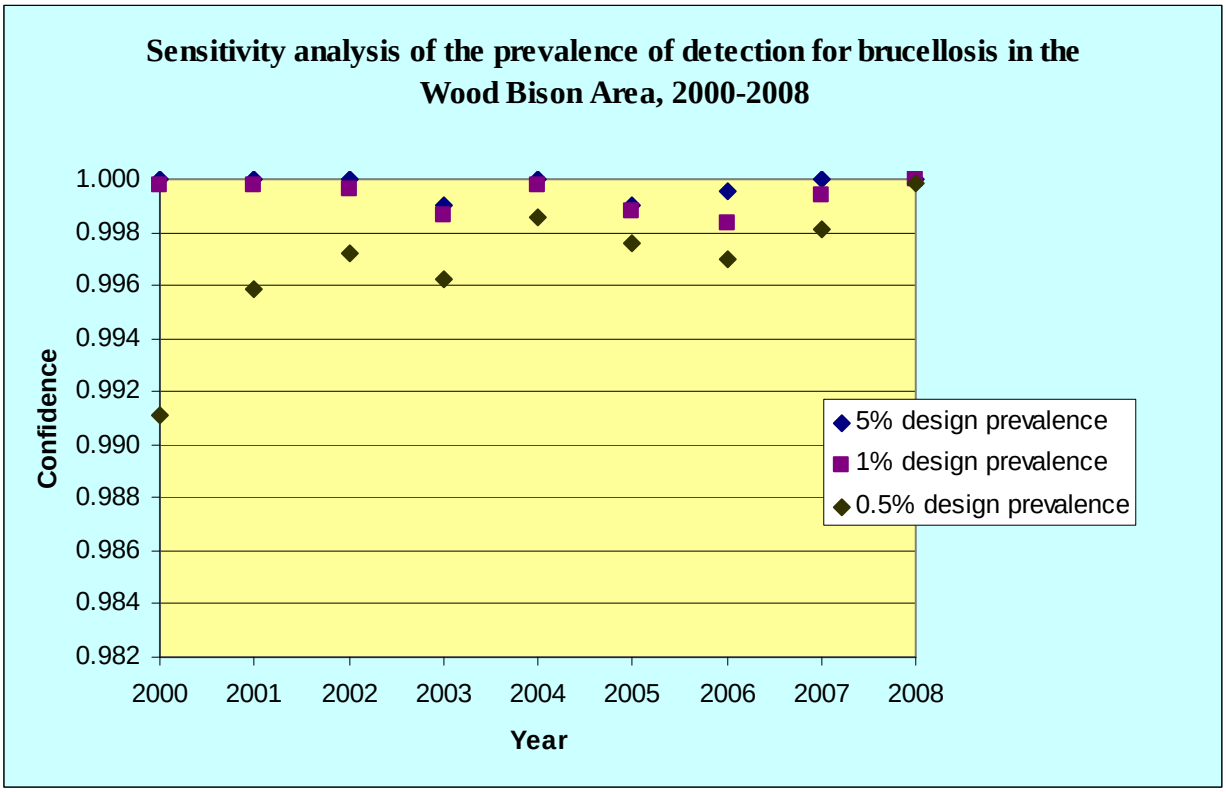
Because certain assumptions for design prevalence and probability of disease introduction were made in the model used for the surveillance system analysis, we conducted a sensitivity analysis of those assumptions to determine their influence on the model results. The two charts below represent the results of the sensitivity analysis. The first, figure 5, addresses the probability of introduction of disease, comparing our assumption of 0.1 percent chance of introduction per year with two alternate assumptions, 0.01 percent and 1 percent. The results show that even if our assumption of 0.1 percent probability of introduction of disease were off by a factor of 10 and the true probability were 1 percent, the probability that disease is not present in the Wood Bison Area population would not fall below 98 percent from 2000 on and would end up above 99.99 percent by 2008.

Figure 5: Sensitivity analysis of the probability of introduction of brucellosis into Wood Bison Area, 2000-2008



The second chart, figure 6, addresses the design prevalence assumption. The model evaluates the confidence of disease detection based on a hypothetical baseline prevalence of 1 percent. We provided alternate design prevalence levels for discussion purposes, including 0.5 percent and 5 percent.

Figure 6: Sensitivity analysis of the design prevalence for detection for brucellosis in the Wood Bison Area, 2000-2008



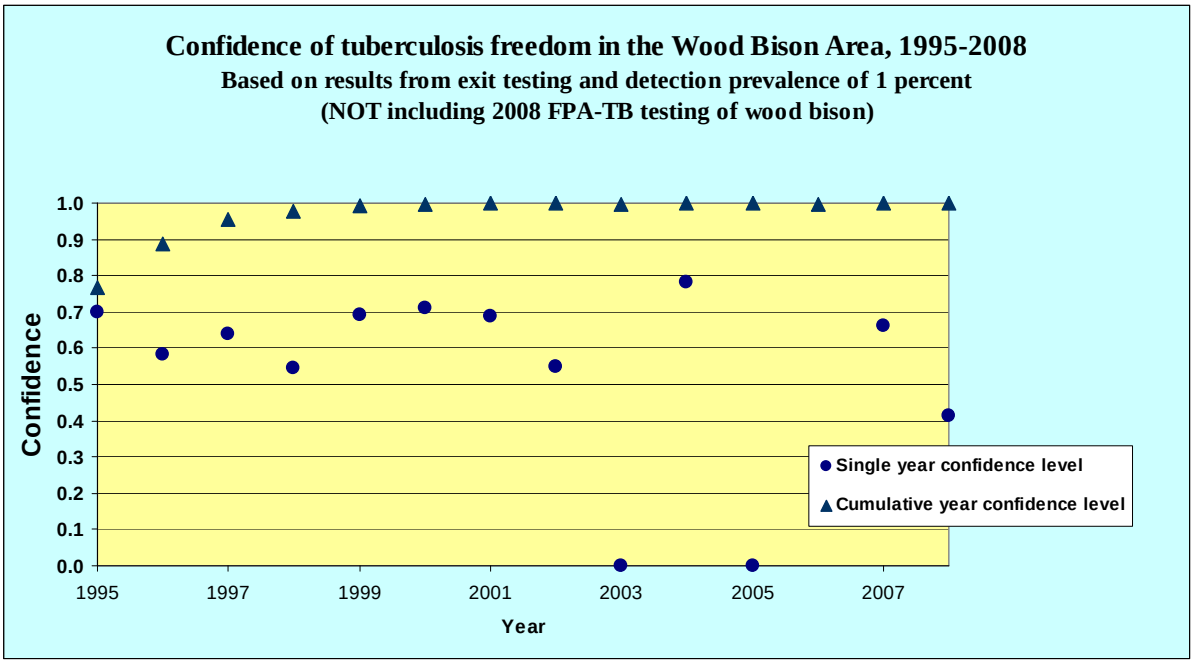
The results show that even if the assumed prevalence of disease were decreased from 1 percent to 0.5 percent, the probability that brucellosis is not present in the population of wood bison and elk by 2008 would remain at 99.99 percent (with no probabilities below 99 percent since 2000).

Tuberculosis

Estimates of the sensitivity of the surveillance system for detection of tuberculosis do not include the whole herd FPA testing for tuberculosis conducted in 2008 since this is not a USDA-recognized test for tuberculosis. Instead, data were initially analyzed through 2007 for bison and elk, with 2008 including only elk testing data.

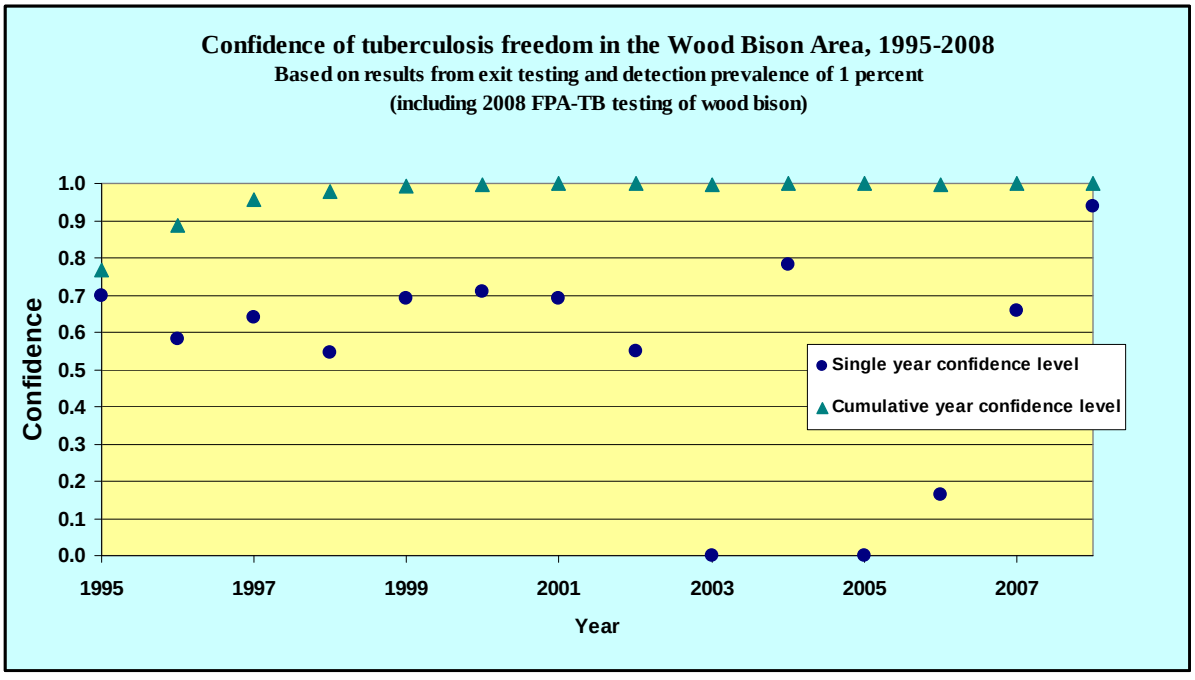
If the disease were present in at least 1 percent of the bison and elk in the Wood Bison Area of EINP, the cumulative sensitivity of detection of tuberculosis, given the test data from 1995-2008 (excluding bison test data from 2008) and assuming a probability of introduction of tuberculosis into EINP of 0.1 percent, is estimated at 99.88 percent (see Figure 7). As seen with the brucellosis data, the sensitivity, or confidence, of disease freedom for individual years ranges from 0-78 percent depending on the number of samples collected each year, which is lower than the corresponding analysis for brucellosis mainly due to lower test sensitivities of tuberculosis screening and confirmatory tests in elk and bison.

Figure 7: Confidence of tuberculosis freedom in Wood Bison Area not including 2008 FPA-TB testing of wood bison, 1995-2008



If the 2008 tuberculosis test results in bison are added, with the assumption that the FPA-tuberculosis test has a test sensitivity of 70 percent, the confidence that the exit testing surveillance would detect tuberculosis in this population if it were present at a prevalence of at least 1 percent would be increased to 99.99 percent (see figure 8).

Figure 8: Confidence of tuberculosis freedom in Wood Bison Area including 2008 FPA-TB testing of wood bison, 1995-2008



As with the brucellosis data, a sensitivity analysis was also conducted for the tuberculosis exit testing surveillance data to assess the effect of our assumptions of probability of introduction of disease and design prevalence on the model results. Both sensitivity analyses were run on data that did not include wood bison FPA-tuberculosis testing in 2008. Figure 9 displays the results of the analysis of the probability of introduction using 0.01 percent and 1 percent, and Figure 10 displays the results of the analysis of the design prevalence with 0.5 percent and 5 percent, as compared with the baseline input assumptions of 0.001 and 1 percent respectively.

Figure 9: Sensitivity analysis of the probability of introduction of tuberculosis into Wood Bison Area, 2000-2008

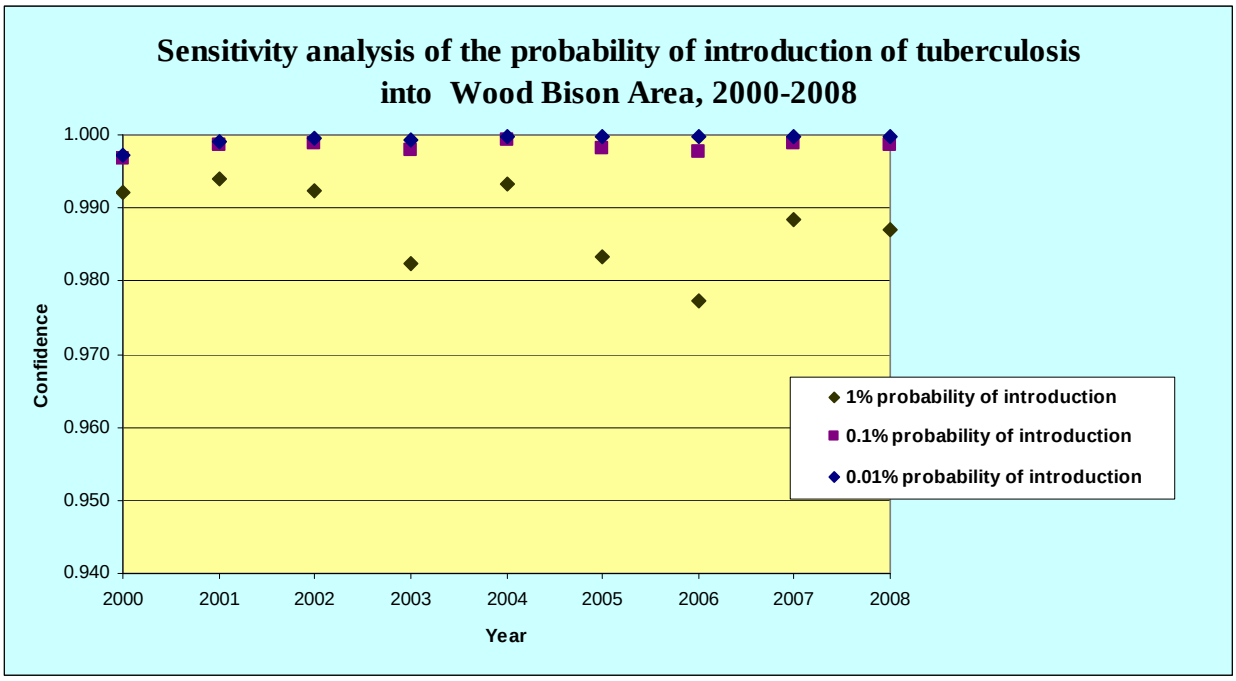
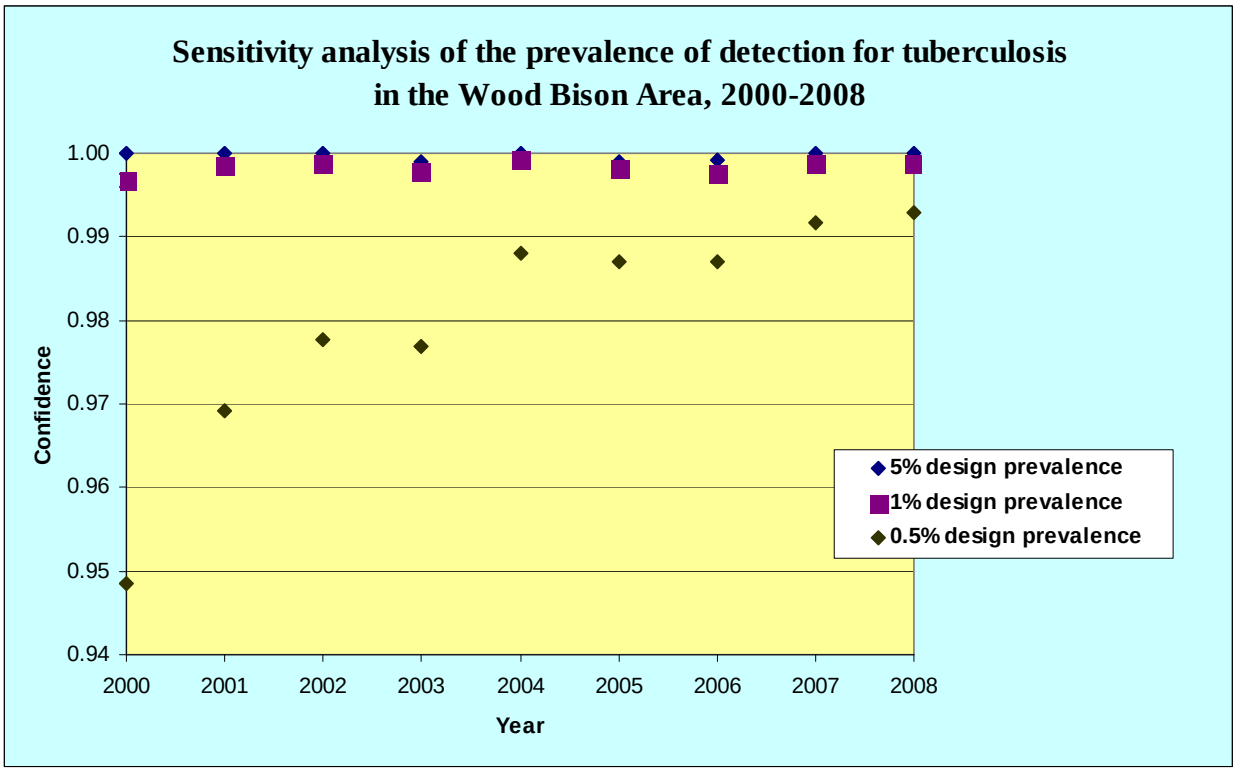


Figure 10: Sensitivity analysis of the design prevalence for detection for tuberculosis in the Wood Bison Area, 2000-2008



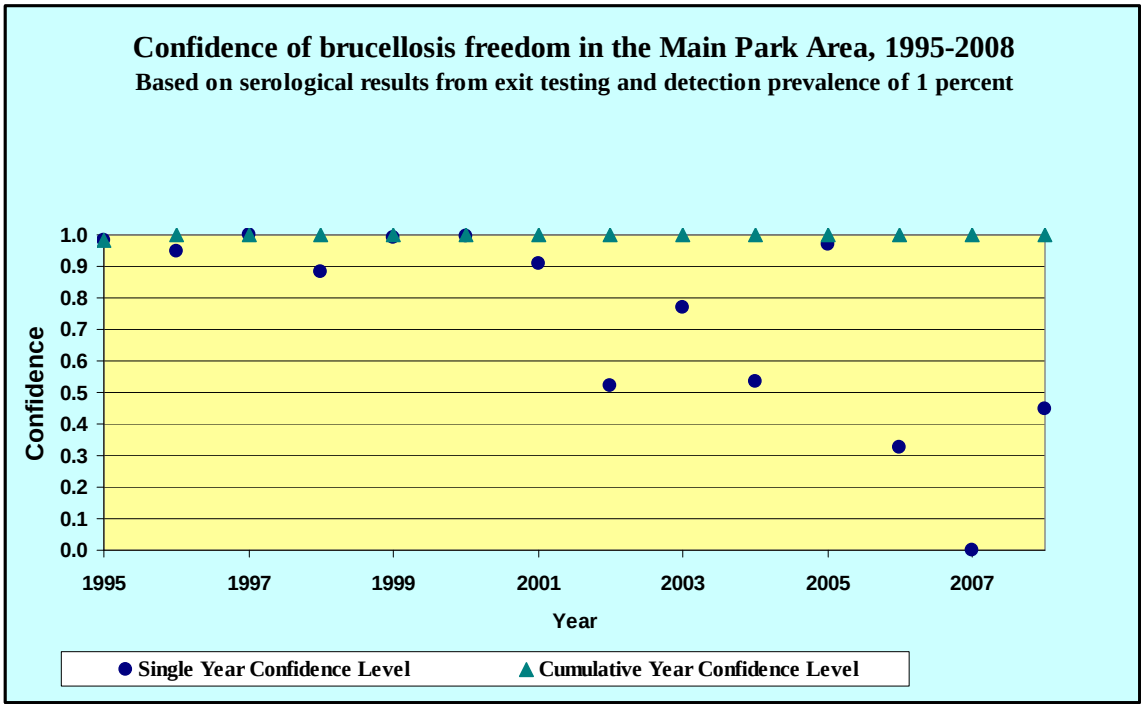
Even if the likelihood of disease entry were increased by increasing the probability of introduction to 1 percent, or the number of potentially infected animals available for detection were decreased, by lowering the prevalence threshold for detection to 0.5 percent, the probability that tuberculosis is not present in the Wood Bison Area population remains above 98 percent and 99 percent, given the respective changes.

Surveillance system analysis results for the Main Park Area

Brucellosis

As seen in the surveillance system analysis of brucellosis testing in the Wood Bison Area of EINP from 1995-2008, the sensitivity of detection of brucellosis in the plains bison and elk herds located in the Main Park Area if present in 1 percent or more of the bison and elk is very high, with an estimate of 99.85 percent given a probability of disease introduction of 0.1 percent. As with the Wood Bison Area, these results indicate that the surveillance of animals exiting this portion of EINP since 1995 is likely to have detected at least one case of brucellosis with a sensitivity or confidence level of 99.85 percent if brucellosis were present in either the plains bison or elk residing in the Main Park Area of EINP at a prevalence of 1 percent or greater (See figure 11.)

Figure 11: Confidence of brucellosis freedom in Main Park Bison Area, 1995-2008



The confidence levels for each year of surveillance, evaluated separately, range from 0 to over 99 percent, depending on the testing protocol and the number of samples tested each year. As described in the Wood Bison Area, the Main Park Area is also closed to new introductions of animals. Thus, assuming a probability of disease introduction into the population of 0.1 percent, confidence levels for disease detection during 1995 through 2008 result in a cumulative confidence level of 99.85 percent.

As above, a sensitivity analysis was conducted for the Main Park Area to determine the influence of assumptions regarding design prevalence and probability of disease introduction on the model results. Figure 12, below, addresses the probability of introduction of disease, comparing our assumption of 0.1 percent chance of introduction per year with two alternate assumptions, 0.01 percent and 1 percent. The results show the probability that disease is not present in the Main Park Area population would not fall below 98 percent from 2000-2008 if the true probability of introduction were 1 percent.

Figure 12: Sensitivity analysis of the probability of introduction of brucellosis into the Main park Area, 2000-2008

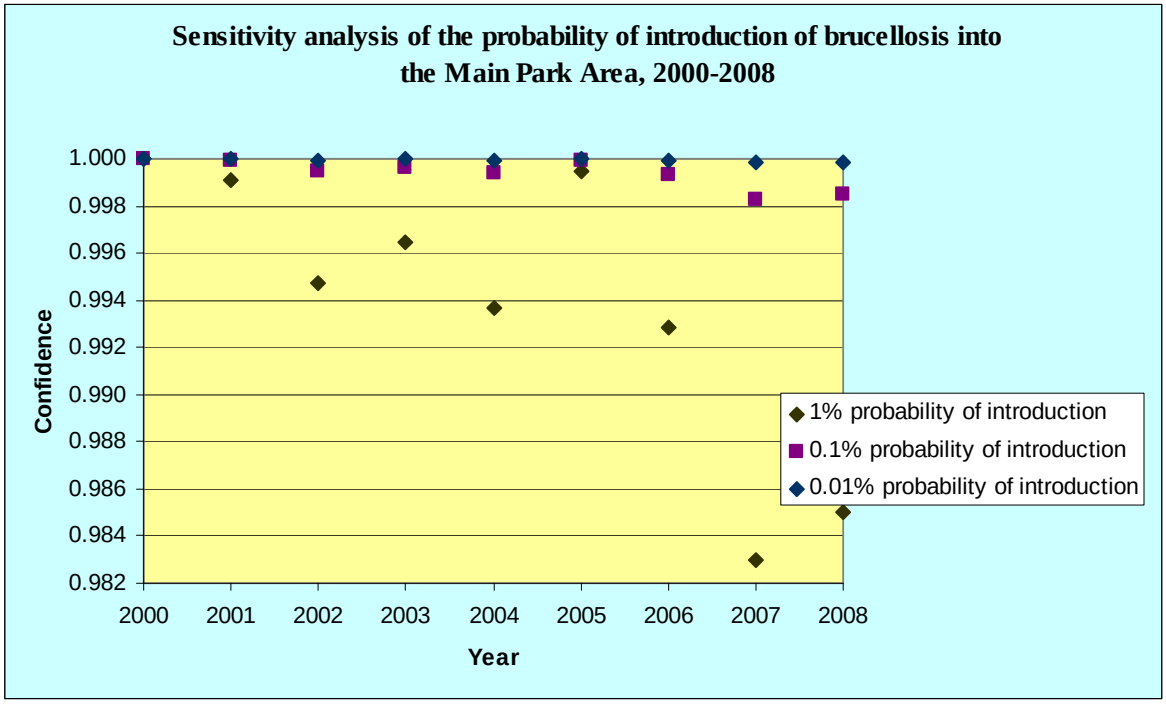
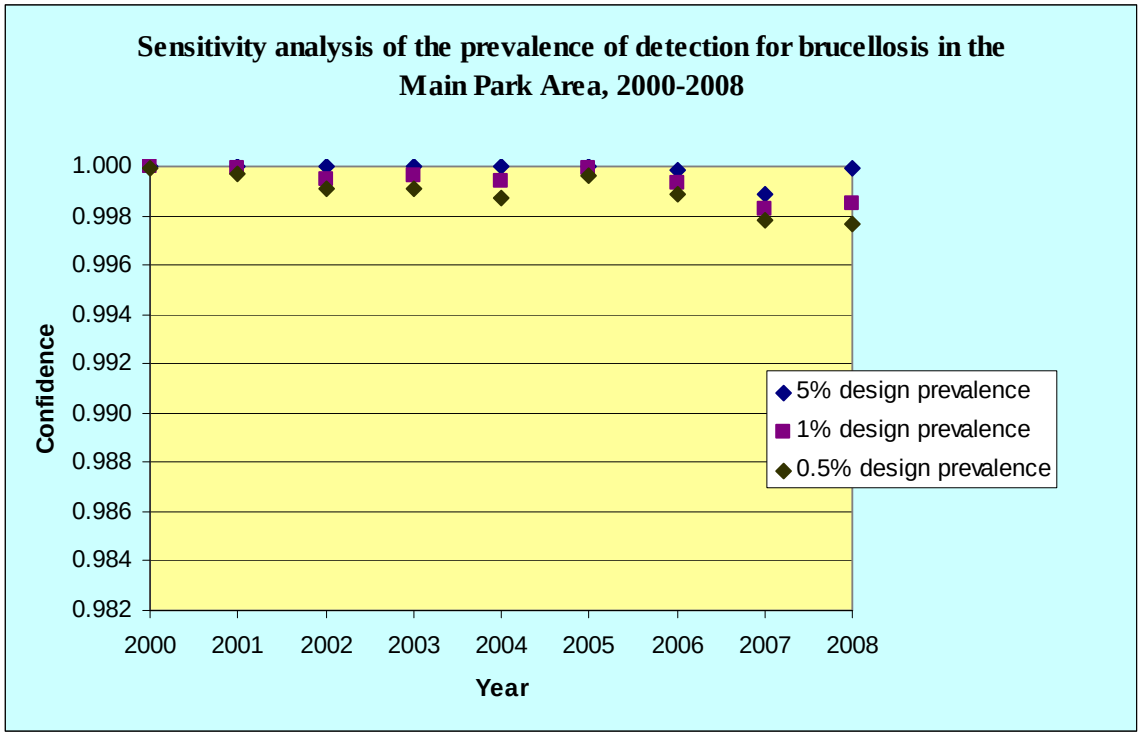


Figure 13 addresses the design prevalence assumption. The model evaluates the confidence of disease detection based on a hypothetical baseline prevalence of 1 percent. The results of the analysis show that even if the assumed prevalence of disease were decreased from 1 percent to 0.5 percent, the probability that brucellosis is not present in the population of plains bison and elk in the Main Park Area by 2008 would remain at over 99.7 percent since 2000.

Figure 13: Sensitivity analysis of the design prevalence of detection for brucellosis in the Main Park Area, 2000-2008

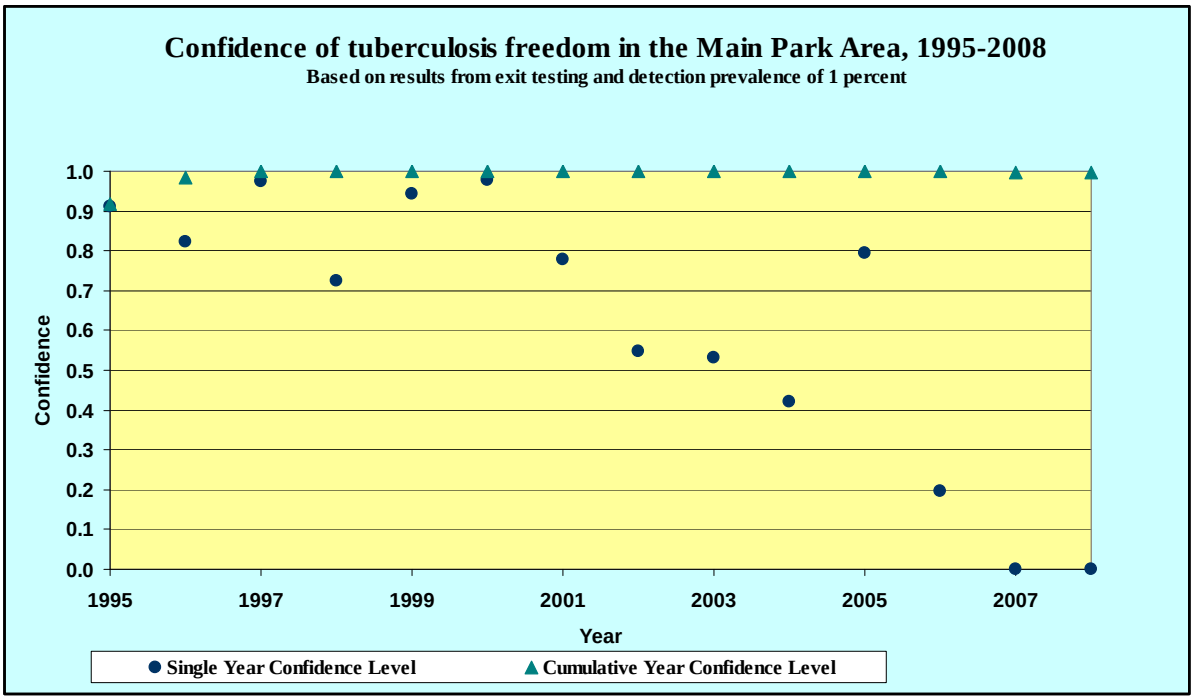


Tuberculosis

If tuberculosis were present in at least 1 percent of the bison and elk in the Main Park Area of EINP, the cumulative sensitivity of detection of tuberculosis, given the test data from 1995-2008 (not including evaluation of 13 and 20 plains bison slaughtered in 2007 and 2008, respectively), and assuming that the probability of introduction of the disease into EINP is 0.1 percent, is estimated at 99.69 percent (see Figure 14). As with the previous analyses, the sensitivity or confidence of disease freedom for individual years ranges from 0-98 percent depending on the number of samples collected each year, but the cumulative confidence is even higher than the greatest confidence for any individual year given the historical surveillance and the low probability of disease introduction.

One reason for the lower detection confidence for tuberculosis in the Main Park Area compared to the Wood Bison Area is a recent change in protocol to stop CFT testing of all surplussed plains bison for tuberculosis (as is practiced in the wood bison and elk populations and was practiced in the plains bison population prior to 2007). The CFT testing was replaced by the selection of 20 percent of the surplussed plains bison for slaughter, with applicable ante-mortem and post-mortem testing. Generally, the oldest animals are selected for slaughter testing since these animals are most likely to exhibit lesions consistent with tuberculosis if it were present in the herd. Although this testing was not included in the model calculations, it is expected to increase the overall sensitivity of detection of disease within the Main Park Area population above the level determined by the model.

Figure 14: Confidence of tuberculosis freedom in the Main Park Area, 1995-2008



As above, a sensitivity analysis was conducted for the tuberculosis exit testing surveillance data to assess the effect of our assumptions of probability of introduction of disease and design prevalence on the model results. Figure 15 displays the results of the analysis of the probability of introduction using 0.01 percent and 1 percent, and Figure 16 displays the results of the analysis of the design prevalence with 0.5 percent and 5 percent, as compared with the baseline input assumptions of 0.001 and 1 percent, respectively.

Figure 15: Sensitivity analysis of the probability of introduction of tuberculosis into the Main Park Area, 2000-2008

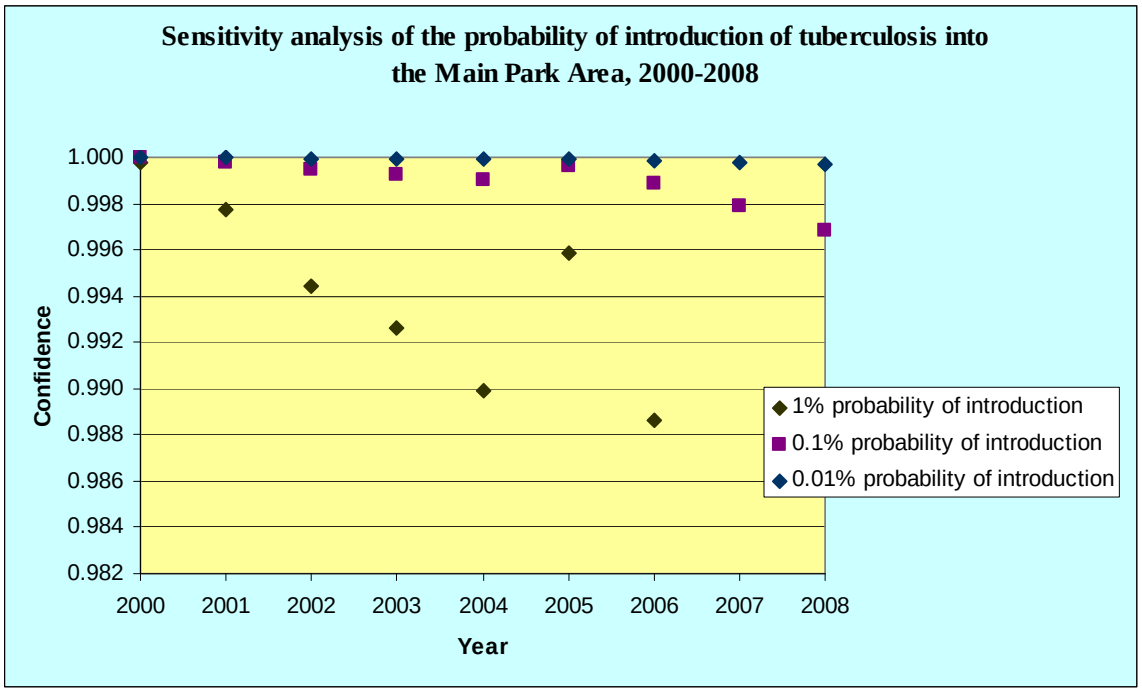
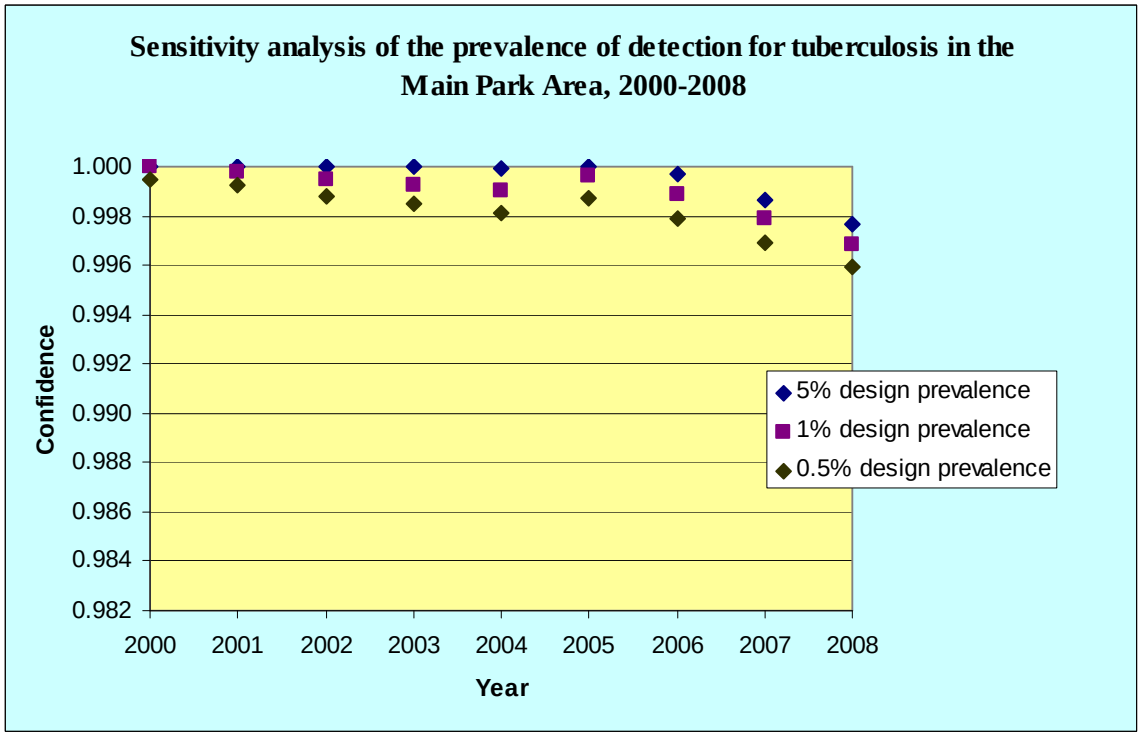


Figure 16: Sensitivity analysis of the design prevalence for detection for tuberculosis in the Main Park Area, 2000-2008



If the likelihood of disease entry were increased by increasing the probability of introduction to 1 percent, as displayed in figure 15, or the number of potentially infected animals available for detection were decreased, by lowering the prevalence threshold for detection to 0.5 percent as shown in figure 16, the probability that tuberculosis is not present in the Main Park Area population remains above 98.8 percent and 99.5 percent, given the respective changes.

Discussion of additional surveillance not included in the analysis

In addition to the exit testing analyzed in the "surveillance model," a number of different activities conducted at EINP would also be expected to detect disease in the park populations. These include the visual surveillance that park staff conduct on a year-round basis, particularly on the bison populations in the park, which identifies animals that are unthrifty or otherwise display signs of illness. They also include the annual aerial sampling of park ungulate populations. EINP staff report that this testing is sensitive enough to detect increased mortality in any of the herds (above the 10-15 percent annual mortality that is expected), including that which would likely result from an abortion storm if brucellosis were to be introduced into the park. In addition, animals that have been relocated from EINP are often tested once they reach their destination, and when animals die, their necropsy results are often forwarded back to EINP. So far, no positive animals have been detected at any of their destinations. These surveillance mechanisms, in addition to the exit testing surveillance, increase the confidence that disease would be detected in the park population.

Conclusions

Based on the availability of exit testing data for brucellosis and tuberculosis for bison and elk surplussed from EINP, we were able to assess the sensitivity of this method of surveillance for detection of either disease if present within either species within either the Wood Bison Area or the Main Park Area of EINP at a prevalence of 1 percent or greater.

To estimate the probability of absence of disease and overall confidence level for multiple years with temporal discounting of historical data, we evaluated several years of surveillance data from elk and bison exiting the park between 1995 and 2008, assuming a given detection prevalence and a low risk of introduction of disease into the park due to the disease-free status of surrounding cattle, bison, and cervid herds and the closed nature of the park.

The results of the analysis demonstrated that the likelihood of the presence of either brucellosis or tuberculosis within the combined elk and wood bison population of the Wood Bison Area of EINP is less than 0.01 percent if either disease were present in 1 percent or more of the animals. For the Main Park Area, the likelihood that disease is present at a prevalence of 1 percent or greater given the negative results obtained from exit testing is not quite as low, but is still less than 1 percent for both tuberculosis and brucellosis. The likelihood of disease in this population is further reduced by additional tuberculosis testing in the form of post-mortem inspections, conducted on approximately 20 percent of the plains bison selected for surplussing since 2007 that is not included in the model calculations.

When tuberculosis or brucellosis is present in bison and elk herds under conditions similar to those at EINP, the prevalence of either disease generally averages over 30 percent. Therefore, the selected detection prevalence of 1 percent was intended as a conservative estimate and does not reflect the expected prevalence that would occur under normal conditions.

Additional surveillance measures in place, including visual surveillance of park ungulates, aerial surveys to detect excess moralities, and testing of animals after relocation from EINP all serve to decrease the likelihood that disease is undetected in this herd.

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Appendix 1. Scenario Trees and Associated Probabilities for Brucellosis and Tuberculosis Exit Testing Model

A. Complete Scenario Trees

Park Section	Species		Age		Animal Status		Screening		Confirmatory		Limb	
	Branch	Prop	Branch	Prop	Branch	Prob	Branch	Prob	Branch	Prob	Outcome	Prob
Main Park Area (North)	Plains Bison	0.534	Adult	0.203	Infected	0.015	Positive	0.921	Positive	0.923	Positive	0.00139
									Negative	0.077	Negative	0.00012
							Negative	0.079			Negative	0.00013
					Uninfected	0.985					Negative	0.10656
			Juvenile	0.797	Infected	0.010	Positive	0.921	Positive	0.923	Positive	0.00365
									Negative	0.077	Negative	0.00030
							Negative	0.079			Negative	0.00034
					Uninfected	0.990					Negative	0.42156
	Elk	0.466	Adult	0.570	Infected	0.009	Positive	0.990	Positive	0.990	Positive	0.00246
									Negative	0.010	Negative	0.00002
							Negative	0.010			Negative	0.00003
					Uninfected	0.991					Negative	0.26301
			Juvenile	0.430	Infected	0.006	Positive	0.990	Positive	0.990	Positive	0.00124
									Negative	0.010	Negative	0.00001
							Negative	0.010			Negative	0.00001
					Uninfected	0.994					Negative	0.19916
												1.00000

Park Section	Species		Age		Animal Status		Screening		Confirmatory		Limb	
	Branch	Prop	Branch	Prop	Branch	Prob	Branch	Prob	Branch	Prob	Outcome	Prob
Wood Bison Area (South)	Wood Bison	0.447	Adult	0.317	Infected	0.014	Positive	0.945	Positive	0.945	Positive	0.00176
									Negative	0.055	Negative	0.00010
							Negative	0.055			Negative	0.00011
					Uninfected	0.986					Negative	0.14000
			Juvenile	0.683	Infected	0.009	Positive	0.945	Positive	0.945	Positive	0.00252
									Negative	0.055	Negative	0.00015
							Negative	0.055			Negative	0.00016
					Uninfected	0.991					Negative	0.30254
	Elk	0.553	Adult	0.574	Infected	0.009	Positive	0.990	Positive	0.990	Positive	0.00290
									Negative	0.010	Negative	0.00003
							Negative	0.010			Negative	0.00003
					Uninfected	0.991					Negative	0.31400
			Juvenile	0.426	Infected	0.006	Positive	0.990	Positive	0.990	Positive	0.00144
									Negative	0.010	Negative	0.00001
							Negative	0.010			Negative	0.00001
					Uninfected	0.994					Negative	0.23425
												1.00000

Legend:

Category Node
Infection Node
Detection Node

B-1. Unit sensitivity of detection - Brucellosis

Section	Year	Species	Age	BPA/FPA-Br +		CF/ELISA +		Limb		
		Branch	Branch	Branch	Prob	Branch	Prob	Outcome	Prob	
Main Park (North) and Wood Bison (South) Areas	1995 to 2000	Bison	Adult	Positive	0.921	Positive	0.923	Positive	0.86008	
						Negative	0.077	Negative	0.07092	
				Negative	0.079			Negative	0.07900	
										0.86008 Adult Bison
			Juvenile	Positive	0.921	Positive	0.923	Positive	0.86008	
						Negative	0.077	Negative	0.07092	
				Negative	0.079			Negative	0.07900	
										0.86008 Juvenile Bison
	2001 to 2006		Adult	Positive	0.921	Positive	0.946	Positive	0.87036	
						Negative	0.066	Negative	0.05088	
				Negative	0.079			Negative	0.07900	
										0.87035 Adult Bison
			Juvenile	Positive	0.921	Positive	0.946	Positive	0.87036	
						Negative	0.066	Negative	0.05088	
				Negative	0.079			Negative	0.07900	
										0.87035 Juvenile Bison
	2007 to 2008		Adult	Positive	0.946	Positive	0.946	Positive	0.89303	
						Negative	0.066	Negative	0.05188	
				Negative	0.066			Negative	0.05500	
										0.89303 Adult Bison
			Juvenile	Positive	0.946	Positive	0.946	Positive	0.89303	
						Negative	0.066	Negative	0.05188	
				Negative	0.066			Negative	0.05500	
										0.89303 Juvenile Bison
		Elk	Adult	Positive	0.990	Positive	0.990	Positive	0.98010	
						Negative	0.010	Negative	0.00990	
				Negative	0.010			Negative	0.01000	
										0.98010 Adult Elk
			Juvenile	Positive	0.990	Positive	0.990	Positive	0.98010	
						Negative	0.010	Negative	0.00990	
				Negative	0.010			Negative	0.01000	
										0.98010 Juvenile Elk

B-2. Unit sensitivity of detection - Tuberculosis

Section	Year	Species	Age	CFT/MCT +		CCT +		Limb		
		Branch	Branch	Branch	Prob	Branch	Prob	Outcome	Prob	
Main Park Area (North)	1995 to 2006	Plains Bison	Adult	Positive	0.700	Positive	0.700	Positive	0.49000	
						Negative	0.300	Negative	0.21000	
				Negative	0.300			Negative	0.30000	
										0.49000 Adult Bison
			Juvenile	Positive	0.700	Positive	0.700	Positive	0.49000	
						Negative	0.300	Negative	0.21000	
				Negative	0.300			Negative	0.30000	
										0.49000 Juvenile Bison
		Elk	Adult	Positive	0.810	Positive	0.880	Positive	0.72080	
						Negative	0.110	Negative	0.08810	
				Negative	0.190			Negative	0.19000	
										0.72090 Adult Elk
			Juvenile	Positive	0.810	Positive	0.880	Positive	0.72080	
						Negative	0.110	Negative	0.08810	
				Negative	0.190			Negative	0.19000	
										0.72090 Juvenile Elk

Section	Year	Species	Age	CFT/FPA/MCT +		CCT +		Limb		
		Branch	Branch	Branch	Prob	Branch	Prob	Outcome	Prob	
Wood Bison Area (South)	1995 to 2007	Wood Bison	Adult	Positive	0.700	Positive	0.700	Positive	0.49000	
						Negative	0.300	Negative	0.21000	
				Negative	0.300			Negative	0.30000	
										0.49000 Adult Bison
			Juvenile	Positive	0.700	Positive	0.700	Positive	0.49000	
						Negative	0.300	Negative	0.21000	
				Negative	0.300			Negative	0.30000	
										0.49000 Juvenile Bison
	2008		Adult	Positive	0.700	Positive	0.700	Positive	0.49000	
						Negative	0.300	Negative	0.21000	
				Negative	0.300			Negative	0.30000	
										0.49000 Adult Bison
			Juvenile	Positive	0.700	Positive	0.700	Positive	0.49000	
						Negative	0.300	Negative	0.21000	
				Negative	0.300			Negative	0.30000	
										0.49000 Juvenile Bison
		Elk	Adult	Positive	0.810	Positive	0.880	Positive	0.72080	
						Negative	0.110	Negative	0.08810	
				Negative	0.190			Negative	0.19000	
										0.72090 Adult Elk
			Juvenile	Positive	0.810	Positive	0.880	Positive	0.72080	
						Negative	0.110	Negative	0.08810	
				Negative	0.190			Negative	0.19000	
										0.72090 Juvenile Elk