The National Park Service

YELLOWSTONE NATIONAL PARK

MONITORING PLAN FOR YELLOWSTONE BISON: THE EFFECTS AND EFFECTIVENESS OF MANAGEMENT ACTIONS

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Approved August 2008
Updated September 2011

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PURPOSE AND RATIONALE FOR MONITORING

The successful conservation of plains bison (*Bison bison*) in Yellowstone National Park from about two dozen animals in 1901 to 5,000 animals in 2005 has led to an enduring series of disagreements among various publics and management entities regarding bison abundance and distribution, and the potential transmission of the *Brucella* pathogen to domestic cattle (Plumb et al. 2009). Also, since the State of Montana and the National Park Service (NPS) agreed to the court-mediated Interagency Bison Management Plan (IBMP; United States Department of the Interior [USDI] and United States Department of Agriculture [USDA] 2000a, b), progress has been slow at completing the plan's successive adaptive management steps. Thus, the Government Accountability Office (2008) recommended that the IBMP agencies develop specific management objectives, conduct monitoring to evaluate the effects and effectiveness of management actions, and develop methods for adjusting the IBMP based on these assessments.

These recommendations were implemented by the IBMP agencies through an adaptive management plan in 2008 (USDI et al. 2008). Also, under provisions of the IBMP, the NPS is considering implementation of a long-term, remote delivery, vaccination program for brucellosis in free-ranging bison inside Yellowstone (USDI 2010). Thus, there is a need to estimate key parameters of bison and brucellosis dynamics, and evaluate the likely effects and effectiveness of a variety of management activities. This monitoring plan identifies a suite of long-term monitoring and research activities for Yellowstone bison that meet the mission of the NPS and inform adaptive management.

MONITORING ACTIVITIES

The various types of actions in the IBMP to ensure the conservation of a viable, free-ranging bison population while safely and effectively reducing infection from, and transmission of, the non-native *Brucella* bacteria can be grouped into three general categories: 1) managing brucellosis transmission risk; 2) reducing the prevalence and transmission of brucellosis; and 3) conserving a viable population of wild bison and the ecological processes that sustain them (Figure 1). Thus, we developed management and research objectives for these desired conditions that are multidimensional and involve trade-offs, whereby improving an outcome associated with one objective affects outcomes associated with other objectives (Williams et al. 2007).

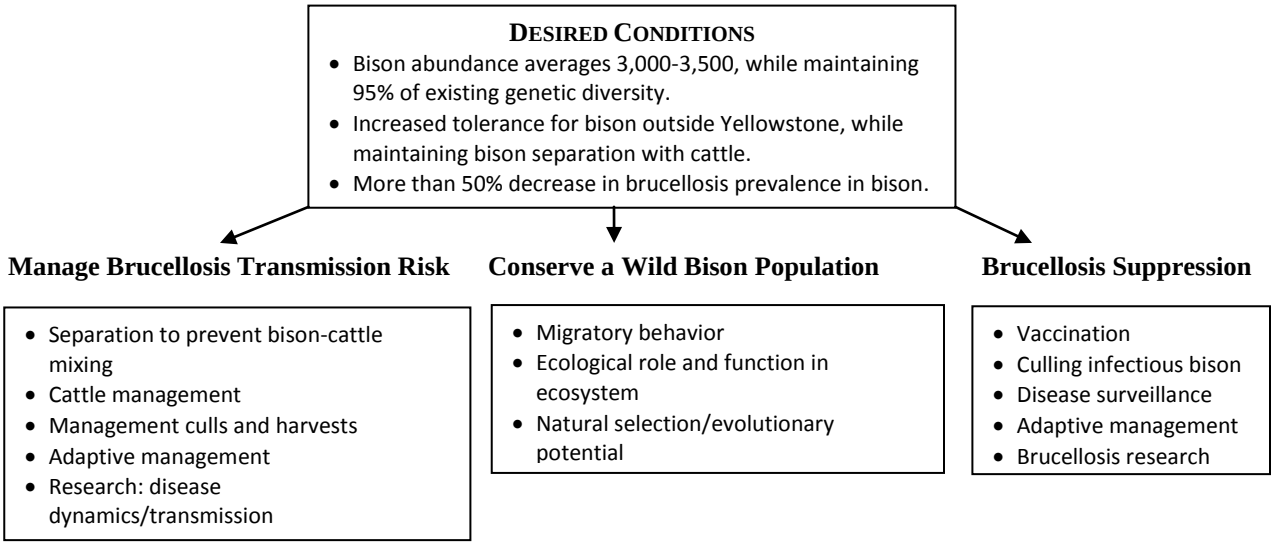


Figure 1. Conceptual model of conservation and disease management for Yellowstone bison.

We then developed the following monitoring activities to provide timely and useful information to help develop adaptive management adjustments.

Conservation (Preserve a Functional, Free-Ranging Bison Population)

1. Estimate the abundance, demography, and limiting factors for the overall bison population and two primary subpopulations (i.e., central and northern breeding herds).
2. Describe migratory and dispersal movements by bison at a variety of temporal and spatial scales in and outside the park.
3. Estimate the existing heterozygosity, allelic diversity, and long-term probabilities of genetic conservation for the overall bison population and identified subpopulations.

Risk Management (Prevent Brucellosis Transmission from Bison to Livestock)

4. Estimate the probabilities (i.e., risks) of brucellosis transmission among bison, cattle, and elk, and between the elk feed grounds in Wyoming and northern Yellowstone.
5. Estimate age-specific rates of bison testing seropositive and seronegative for brucellosis that are also culture positive, and the portion of seropositive bison that react positively on serologic tests due to exposure to cross-reactive agents other than *B. abortus* (e.g., *Yersinia*).
6. Estimate the timing and portion of removals from the central and northern herds each winter, including the portion of removals from each age and sex class and calf-cow pairs.
7. Document bison use of risk management zones outside the northern and western boundaries of Yellowstone and commingling with livestock during the likely brucellosis-induced abortion period for bison each spring.
8. Estimate the effects of hazing or temporarily holding bison in capture pens at the boundary of Yellowstone (for spring release back into the park) on subsequent bison movements or possible habituation to feeding.

Brucellosis Suppression (Reduce Disease Prevalence)

9. Determine the strength and duration of the immune response in bison following parenteral (e.g. syringe delivery) vaccination for brucellosis.
10. Determine the strength and duration of immune response in bison following remote delivery (e.g. bio-bullet) vaccination for brucellosis.
11. Document long-term trends in the prevalence of brucellosis in bison, and the underpinning effects of remote and/or parenteral vaccination, other risk management actions (e.g., harvest, culling), and prevailing ecological conditions (e.g. winter-kill, predation) on these trends.

SAMPLING OBJECTIVES AND METHODS

We developed one or more sampling objectives for each monitoring activity.

Sampling objective 1a: Estimate the number of Yellowstone bison with 95% confidence intervals no wider than + 10% of the estimated number of total bison.

Justification: Trustworthy population analyses depend on reliable estimates of population and subpopulation sizes that can be used to calculate rates of change in abundance and support independent measures of demographic rates (Eberhart 2002). A population can be defined as a

group of organisms of the same species that live together, reproduce, and share a common gene pool (Snyder et al. 1985, Keeton and Gould 1986). Evidence suggests there are 2-3 genetic subdivisions within the Yellowstone bison population (Halbert 2003, Gardipee 2007), but interchange rates between herds have increased significantly since the 1980s (Coughenour 2005, Fuller et al. 2007*a*, Taper et al. 2000; unpublished data). Thus, we characterize the bison inhabiting Yellowstone as a single population with significant substructure (Pérez-Figueroa et al. 2010).

Methods: We will estimate bison abundance annually using 2-3 replicate flights during June and July. These counts will coincide with the high visibility of bison during the breeding season and will be analyzed using a replicate count modeling approach (Hess 2002).

<u>Sampling objective 1b: Obtain estimates of age structure, pregnancy, recruitment, and survival with 95% confidence intervals no wider than + 5% of the estimated mean rates</u>.

Justification: It is essential to monitor key demographic rates for evaluating the relative effects of disease, harvests, management removals, predation, and environmental factors (e.g., drought, snow pack) on bison demography and population trends; thereby ensuring effects do not harm the integrity of this valuable resource. A key vital statistic for assessing population dynamics is adult female survival because survivorship of prime-aged animals has a major affect on population growth (Eberhardt 2002). Likewise, variability in recruitment plays a substantial role in population dynamics because high recruitment during good conditions for juveniles provides the resiliency for populations to grow at relatively rapid rates when abundance is relatively low (Saether 1997, Gaillard et al. 1998, 2000). Accurate information on the age of animals is essential because Yellowstone bison are relatively long-lived animals and the demographic parameters (e.g., survival, reproduction) that drive the dynamics of the population are age specific with juvenile and older age classes having lower birth and survival rates than the prime-age classes (Fuller et al. 2007*b*, Geremia et al. 2009).

Methods: We will conduct park-wide, ground-based classification surveys during each calving season (mid-April to mid-May) and estimate recruitment by comparing the ratio of the number of calves and yearlings per 100 adult female bison in subsequent years. We will also conduct post-calving (late-June to early-July) classification surveys to estimate the overall sex and age-class composition of the population. Adult female survival will be estimated from a sample of 45-60 radio-collared bison located bi-monthly through the year. Blood serum from captured bison will be used to estimate brucellosis exposure and pregnancy using the specific protein B (PSPB) assay.

<u>Sampling objective 1c: Monitor the composition and distribution of bison groups each year to document seasonal changes in group sizes and the maintenance of herds and matrilineal groups</u>.

Justification: Bison are inherently gregarious and population substructure occurs in Yellowstone at a landscape scale due to the traditional use of particular parts of a range by segments of the population during breeding and at a finer scale because bison form family groups (i.e., matrilineal groups) within herds that may include several generations of cows and calves. However, these forms of population structure increase the likelihood that non-random

removals from the population (e.g., culls, harvests) will differentially affect a particular segment of the population, either demographically or genetically. Thus, it is important to document spatial-temporal variations in this social structure.

Methods: We will monitor bison distribution and group sizes by conducting population-wide aerial surveys four times per year (early summer, mid-summer, early winter, and late winter). We will also conduct park-wide, ground-based classification surveys during the calving (mid-April to mid-May) and rutting (mid-July to mid-August) seasons.

Sampling objective 1d: Monitor the foraging and movements of bison each year to ensure they have access to a broad variety of habitats to exploit the heterogeneity of resources and respond to changes in the distribution, quality, and quantity of forages.

Justification: Bison traditionally exploited broad- and fine-scale variation in resources, sometimes moving long distances in response to environmental heterogeneities. Thus, the ability of Yellowstone bison to exploit environmental heterogeneity during foraging will be largely determined by population density, habitat characteristics, and environmental conditions in and outside the park. In turn, there is a need to ensure bison ranges include a broad variety of habitats so bison can exploit seasonal and multi-year heterogeneity in forages from the patches to landscape scale.

Methods: We will maintain a sample of 45-60 adult, female bison radio-collared with VHF or GPS collars that will collect spatial data for two or more years. We will locate radio-collared animals bi-monthly through the year. Also, we will monitor bison distribution and habitat use by conducting population-wide aerial surveys four times per year (early summer, mid-summer, early winter, and late winter). In addition, we will begin evaluating feasible techniques for assessing forage and range conditions across the landscape.

Sampling objective 1e: Monitor bison composition each year during the rut to assess if there is adequate competition (e.g., free movements and fighting) between adult bulls for mates and estimate the sex ratio of adult bison.

Justification: Differential reproduction resulting from mate competition may be the most important evolutionary process for bison and the intensity of competition for mates will be largely determined by population structure and density. Thus, there is a need to ensure that each subpopulation in the relatively large Yellowstone population includes >20 mature and reproductively active bulls (6 years and older) for every 100 adult females (Gates et al. 2005).

Methods: We will monitor the composition of bison each year during the rutting (mid-July to mid-August) season using park-wide, ground-based classification surveys.

Sampling objective 1f: Monitor the function and role of bison in changing habitat and biological diversity.

Justification: Bison are likely a keystone species in Yellowstone that could have a disproportionately large effect on other species and, as a result, largely determine the structure

and function of this ecosystem over time. However, there is a need to investigate how bison affect biogeochemical cycling, ecosystem trophic structures, patterns of species diversity, and species composition in Yellowstone. Some of the major types of ecological processes that bison could influence are the creation of landscape heterozygosity (e.g., patches, disturbances), nutrient redistribution and turnover, competition with other ungulates, prey for carnivores, habitat enhancement for grassland birds and other commensals, provision of carcasses for scavengers, stimulation of primary production, dispersal of plant seed, modification of grassland fire regimes, maintain floral diversity, and opened access to vegetation through snow cover (Sanderson et al. 2008).

Methods: We are collaborating with Dr. Doug Frank at Syracuse University to evaluate the role bison play in shaping plant communities within Yellowstone. The research will begin in summer 2011 and is planned to elucidate the influences of recent decreases in elk abundance and distribution, and increases in bison abundance and distribution, on ecosystem processes such as the spatial pattern and intensity of ungulate grazing and grassland energy and nutrient dynamics. The project will replicate previous work describing plant production on grazed and ungrazed sites sampled 10 and 20 years ago (Frank and McNaughton 1993, Frank 2008) to compare the effects of grazing across multiple decades and evaluate the effects of changes in grazer densities (e.g., bison, elk) on nutrient cycling and plant productivity.

<u>Sampling objective 2: Monitor bison movements each year to identify changes in population distribution, dispersal and migratory patterns, and the effects of biotic (e.g., density), abiotic (e.g., environmental conditions), and management (e.g., snow packing on roads) factors</u>.

Justification: Yellowstone bison are migratory and their successful long-term conservation depends on bison occupying lower-elevation, public and private lands outside the park, particularly during severe winters or when bison numbers are at the lower end of the conservation spectrum (Gates et al. 2005). However, these migratory movements may create a "sink" for bison if they move to the park boundary, resist brucellosis risk management actions, and are culled from the population. Thus, there is a need to monitor bison movements and the factors influencing them to determine the (1) spatial-temporal patterns of bison movements and use of areas outside the park, (2) differential effects of boundary management removals on each herd given their respective movement dynamics, and (3) fidelity of individual bison to particular seasonal movement strategies and destinations. There is also a need to evaluate if management actions are facilitating the movements of bison to the boundary or resulting in the integration of the central and northern herds over time.

Methods: We will maintain a sample of 45-60 adult, female bison radio-collared with VHF or GPS collars that will collect spatial data for two or more years. We will locate radio-collared animals bi-monthly through the year. Also, we will monitor bison distribution by conducting population-wide aerial surveys four times per year (early summer, mid-summer, early winter, and late winter). Further, we will conduct additional aerial and ground surveys during November through May to periodically monitor bison abundance, distribution, and movements north onto private and Gallatin National Forest lands in the Reese Creek area, and west onto private and Gallatin National Forest lands in the Horse Butte area.

Sampling objective 3a: Obtain estimates of population heterozygosity, allele frequency, and genetic subdivision within the Yellowstone bison population, with 95% confidence intervals no wider than + 5% of the estimated mean rates. Conduct periodic sampling for cross-species hybridization to identify any evidence of breeding with cattle.

Justification: The loss of genetic variation can negatively affect the ability of wildlife populations to adapt to changes in their environment (DeYoung and Honeycutt 2005, Allendorf and Luikart 2006). Small herd size, artificial selection, and cattle-gene introgression are some of the factors threatening the long-term genetic integrity of North American bison (Freese et al. 2007). Yellowstone bison are one of only two populations managed as public trust resources without evidence of hybridization with cattle and have been identified as having a high level of genetic variation (Halbert 2003, Halbert et al. 2005). Thus, Yellowstone bison are an invaluable resource for the long-term conservation of the species. However, conserving the genetic diversity of Yellowstone bison may be complicated by management practices that deliberately reduce abundance and inevitably result in genetic changes to the population (Harris et al. 2002, Allendorf et al. 2008). Exploitative selection due to human harvest or culling could change the genetic diversity of the central and northern subpopulations by affecting productivity and migratory patterns that, in turn, influence gene flow between breeding groups (Schwartz et al. 2006, Allendorf et al. 2008). Also, population substructure (e.g., distinct breeding herds) could result in a disproportionate harvest of animals from the subpopulations and increase the loss of genetic diversity (Gross et al. 2006).

Methods: We will randomly sample bison from primary breeding locations during July and August to identify genetic subdivisions within the Yellowstone population (DeYoung and Honeycut 2005). It will be necessary to sample bison across decades to determine if existing subpopulations are converging or becoming more divergent (Halbert 2003, Schwartz et al. 2006, Allendorf et al. 2008). We will collect blood from all radio-collared bison and store it on FTA (i.e., DNA-stabilizing) cards. We will also collect blood from a sample of bison captured during boundary management operations. In addition, we will collect fecal samples from bison during the breeding season to identify population subdivision and rates of gene flow among subpopulations. We will use blood samples collected from known individuals to monitor genotyping error rates from fecal samples. Microsatellite data will be used to identify population heterozygosity, allele diversity, and population subdivision with estimated F_{ST} values (DeYoung and Honeycut 2005). Rates of gene flow will be quantified using subpopulation assignment and maximum likelihood tests (Luikart and England 1999).

Sampling objective 3b: Obtain estimates of interchange rates between herds with 95% confidence intervals no wider than + 5% of the estimated mean rates.

Justification: Evidence suggests there are 2-3 genetic subdivisions within the Yellowstone bison population (Halbert 2003, Gardipee 2007). This structure likely reflects the severe population bottleneck and subsequent isolation of endemic and reintroduced bison herds in Yellowstone from the late 1880s through the 1970s (Meagher 1973), and strong female philopatry (i.e., fidelity) to breeding areas (Gardipee 2007). However, there is some indication that interchange rates between herds began increasing in the 1980s (Coughenour 2005, Fuller et al. 2007*a*) and have continued to increase with bison abundance (Taper et al. 2000; unpublished

data). Subpopulation structure reduces effective genetic population size from that estimated by the overall population size and, in turn, could contribute to loss of genetic variation and inbreeding depression unless considered during management actions (Allendorf and Luikart 2006, Gross et al. 2006). Thus, there is a need to consider the rate of genetic interchange during the long-term management of Yellowstone bison. We will use microsatellite data to obtain a more-precise measure of genetic differences between the two breeding groups of Yellowstone bison, and as an indicator for assessing migration rates (i.e., gene flow).

Methods: We will estimate gene flow by measuring the difference in allele frequency between groups (F_{ST}). We will also use additional measures to increase confidence in inferences derived from F_{ST} estimates. For example, we may monitor private (or rare) alleles associated with specific genetic groups of animals. Rare alleles typically arise through mutations and, if gene flow is high, then private alleles should be rare. Thus, supplementing genetic analyses with direct observation of migrants provides the greatest level of confidence in rates of gene flow. However, there are limitations to direct observations especially in species where immigration is pulsed or infrequent.

<u>Sampling objective 3c: Document and track the number and location of bison populations established or augmented outside Yellowstone using *Brucella*-seronegative Yellowstone bison to ensure the long-term conservation of the bison genome</u>.

Justification: Less than 2% of the 500,000 plains bison in existence today can be classified as likely free of domestic cattle gene introgression (Halbert and Derr 2007). Also, the retention of allelic diversity may be important for bison to adapt to future alterations in environmental conditions. Thus, a precautionary approach dictates that viable satellite herds should be created from each of the existing genetically important bison herds in North America, with the highest priority given to populations such as Yellowstone bison that are genetically diverse and have no evidence of introgression (Allendorf et al. 2001, Freese et al. 2007).

Methods: In 2005 and 2006, bison from Yellowstone were transferred to the Montana Department of Fish, Wildlife, and Parks and Animal and Plant Health Inspection Service (2006) to test the feasibility of a bison quarantine protocol outlined in the IBMP. This quarantine program determined that bison could be reliably diagnosed as negative for brucellosis. We will work with the IMBP agencies and American Indian tribes to facilitate the transfer of "surplus" Yellowstone bison to quarantine facilities near the park and/or on tribal lands for monitoring and eventual release onto suitable habitat, or to terminal destinations on tribal lands for periodic harvest for food or ceremonial purposes.

<u>Sampling objective 4a: Develop a risk assessment model incorporating threat structure (i.e., levels of risk) to quantify interspecies transmission within wildlife and the associated hazard to domestic livestock outside the park</u>.

Justification: There is much debate about "who infects who" with brucellosis. This question is vital to the long-term conservation of Yellowstone bison and elk while management actions are taken to reduce brucellosis prevalence. The ability of *B. abortus* to infect multiple hosts increases the risk of transmission to cattle and complicates disease management. Despite

consensus among resource managers that the risk of brucellosis transmission from bison to domestic cattle is low, the U.S. Government Accountability Office (2008) indicated the IBMP agencies should conduct a risk assessment to better quantify the transmission risk. A quantitative assessment of the risk bison and elk pose to cattle will require estimating the frequency, duration, and routes of exposure from both wildlife species. The risk of brucellosis infection to cattle is directly related to the level of infection in the wildlife host populations. Understanding the interspecies transmission dynamics that maintain infection in bison and elk is necessary to evaluate the risk each wildlife species presents to cattle. Not all animals are equally likely to be infectious because the threat of brucellosis transmission is restricted to a subset of each wildlife host population (i.e., pregnant infected females). Thus, wildlife populations can be divided into high and low risk groups to create models with more realistic risk structure.

Methods: We will quantitatively assess the risk that bison and elk pose to cattle outside the park using a modeling approach that incorporates risk structure. This will allow us to identify high risk groups and target high risk animals for vaccination. We will use the following parameters to model brucellosis transmission with multiple host species: 1) the probability of a susceptible host contacting infectious material; and 2) the probability of infection given contact. Quantifying how bison-bison, bison-elk, and elk-elk transmission rates vary over time in the multiple-host system of the greater Yellowstone area will be difficult. These rates are influenced by several ecological parameters of the host populations (e.g., density, disease prevalence) and environmental factors (e.g., winter severity, food availability). Thus, empirical data collected from field studies will be valuable for parameterizing risk transmission models. These models will serve as valuable tools for assessing how landscape-scale properties and processes (e.g., topography, snow, foraging patches, predation risk) influence bison, cattle, and elk spatial overlap during the time interval of *Brucella* shedding. We will also use these models to evaluate how the widely disparate levels of brucellosis seroprevalence in elk (1-3%) and bison (40-60%) persist in the northern portion of the greater Yellowstone area. Whether long-term seroprevalence rates in bison and elk are independent of each alternate host is uncertain. The low seroprevalence in elk in the northern portion of the greater Yellowstone area may be explained by a proportion of infected elk dispersing from the Wyoming feed grounds (seroprevalence = 8-30%; Cross et al. 2007) into northern Yellowstone or by elk-elk or bison-elk transmission in northern Yellowstone independent of feed ground elk. Models assessing transmission dynamics will aid in identifying support for each hypothesis.

The potential for infective contact between bison and elk in Yellowstone is relatively high because the distributions and habitat use of the two species overlap broadly during the likely period of abortion (Meagher 1973, Singer and Norland 1994, Cheville et al. 1998). However, Ferrari and Garrott (2002) demonstrated that seroprevalence of elk in the west-central portion of Yellowstone was low, despite bison and elk frequently commingling at high densities during the abortion period. The potential for elk in the northern portion of the greater Yellowstone area acquiring infection from infected elk in the southern portion of the greater Yellowstone area is unlikely because the calving areas of elk near the Wyoming feed grounds are well south of northern Yellowstone (Boyce 1989). Also, elk typically isolate themselves during calving and thoroughly clean birth sites (Johnson 1951, Clutton-Brock et al. 1982). Bison are nearly as meticulous at cleaning birth sites as elk, but often calve in close proximity to other group members making bison-bison contact likely, but bison-elk contact unlikely. To date, there have

been no quantitative assessments of these transmission hypotheses to determine whether the mechanisms appear reasonable. We will analyze potential explanations for the observed seroprevalence in bison and elk using simulation models. Also, we will explore similar models to assess the risk of brucellosis infection to domestic livestock from bison and elk. Simulation models parameterized with elk population and movement data will be used to assess the plausibility of elk dispersal as a mechanism maintaining seroprevalence in northern Yellowstone elk.

Sampling objective 4b: Determine the genetic relationships and likely transmission pathways among *Brucella* isolates from bison, elk, and cattle in the greater Yellowstone area.

Justification: Recent outbreaks in cattle from the greater Yellowstone area are hypothesized to have originated from wild elk, but this hypothesis has not been tested due to limited sensitivity of molecular techniques and difficulties in sampling wildlife. Molecular genetic markers may be useful for assessing the origin of transmission pathways of infectious disease outbreaks, even for pathogens like *Brucella* that are difficult to isolate and that have genomes with little variation. The recent discovery of variable 8-base pair repeats in *Brucella* genomes has created opportunities to use these markers for genotyping (DNA "fingerprinting") and transmission studies of brucellosis (Pappas et al. 2006). Genotyping *Brucella* isolates from bison, elk, and cattle in the greater Yellowstone area could have important management applications. For example, verifying that transmission from bison to elk is rare or nonexistent would provide justification for independently reducing brucellosis infection prevalence in Yellowstone bison and feed ground elk because re-infection from elk to bison would be unlikely.

Methods: We will collaborate with geneticists from the University of Montana to collect blood and fecal samples from various breeding groups of elk and bison in the greater Yellowstone area for the culture of *B. abortus* isolates. These geneticists have proposed to use the DNA marker system called "HOOF-Prints" (i.e., hypervariable octameric oligonucleotide fingerprints) to genotype 10 variable number of tandem repeat loci (Thorne et al. 1979). Genetic relationships among allelic combinations (i.e., haplotypes) will then be analysed using the software NETWORK V4.5 (Bricker and Ewalt 2005). The network analysis permits reconstruction of all possible genetic relationships among haplotypes and the visual representation of the frequencies of each haplotype. This will allow us to identify infection sources (i.e., host species) and brucellosis transmission dynamics over the greater Yellowstone area.

Sampling objective 5a: Obtain estimates of the proportion of seropositive and seronegative bison that are culture positive within 95% credible intervals using informative priors in a Bayesian analysis.

Justification: Seroprevalence is an indirect measure of brucellosis infection that provides minimal information on the state of infection in Yellowstone bison. Positive serologic responses indicate the presence of antibodies to *Brucella* exposure rather than active infection. Antibodies can persist for a long time after the host's immune system has cleared a disease agent. This allows for a more rapid immune response if the pathogen is re-encountered. The intracellular strategy of *B. abortus* allows it to hide from the host's immune system, preventing antibody

production, and persist for long periods undetected. This complicates diagnoses using serologic tests because positive results do not indicate the host is harboring the pathogen and negative tests are not completely reliable to determine an animal is pathogen free. Consequently, serologic tests overestimate the level of infection (i.e., false positives) and may not be sensitive enough to detect low numbers of *Brucella* in actively infected bison (i.e., false negatives). The proportion of culture positive bison in the population is expected to be a more sensitive indicator of active infection, but requires tissues collected from dead animals. We will combine culture assays with serology data to obtain a more accurate estimate of the state of brucellosis infection and better inform management of the risk bison pose outside the park. Also, we will use this information to monitor changes in population infection in response to brucellosis suppression management and identify age classes with the greatest probability of being actively infected. This information will help us develop the transmission risk structure for the population and target high risk animals for vaccination.

Methods: We propose to estimate the proportion of seropositive and seronegative bison that are actively infected with *B. abortus* (i.e., culture positive). We will determine the relationship between age-specific seroprevalence and positive *B. abortus* culture by sampling bison that are captured and consigned to slaughter. At the receiving slaughterhouses, we will collect blood for brucellosis diagnosis using the fluorescent polarization assay, record the sex, and estimate age from tooth eruption patterns (i.e., bison with deciduous teeth). We will collect first incisors from all bison with a full set of permanent teeth for aging based on cementum annuli. The following tissues based on bison sex will be collected for *Brucella* culture: supra mammary lymph nodes, section of mammary gland, retropharyngeal lymph nodes, internal iliac lymph nodes, and superficial inguinal lymph nodes. Tissues will be frozen and shipped to the National Veterinary Services Laboratory in Ames, Iowa for *Brucella* culture. Culture results will be compared with serology tests from the same animals to better understand seroprevalence and the state of infection across bison sex and age classes. We will use a Bayesian statistical approach to estimate the proportion of actively infected bison based on population seroprevalence levels. For each sero-status, the number of culture positive bison is a binomial random variable and we will use informative priors from existing studies that investigated the relationship between serology and culture data (Roffe et al. 1999).

<u>Sampling objective 5b: Obtain estimates of the proportion of seropositive bison that express cross reactive antibodies on standard *Brucella* serologic tests within 95% credible intervals using informative priors in a Bayesian statistical approach</u>.

Justification: Serologic diagnostic tests are confounded by cross reacting antibodies from other organisms that result in false positive reactions on *Brucella* tests. The most common cross reactor is *Yersinia enterocolitica* O:9. *Yersinia* is a bacterial pathogen that has been cultured from birth tissues of Yellowstone bison, but the level of *Y. enterocolitica* in bison is unknown. For diagnostic testing, the molecular structure of the lipopolysaccharide O-antigen of *B. abortus* and *Y. enterocolitica* serotype O:9 are nearly identical, which explains the cross reactivity on conventional serologic tests. By estimating the proportion of seroprevalence that may be attributed to cross reactive *Y. enterocolitica*, we will better understand the level of *B. abortus* infection in Yellowstone bison. Tests have been developed to aid in distinguishing between the two bacteria (Nielsen et al. 2006) and these tests may become important as *B. abortus*

seroprevalence is reduced in bison through vaccination. Elk are also susceptible to *Yersinia* infection and the relatively low levels of assumed *Brucella* seroprevalence in northern Yellowstone elk might be a result of *Yersinia* infection rather than *Brucella*. Monitoring the level of cross reactive agents such as *Y. enterocolitica* in Yellowstone bison and elk will improve our understanding of brucellosis infection and transmission dynamics across the greater Yellowstone area.

Methods: We propose to estimate the proportion of false positive bison and elk for brucellosis infection resulting from cross reacting antibodies for *Yersinia enterocolitica* O:9. *Yersinia* in bison and elk is most likely transmitted through contact with contaminated feces. We will collect fecal samples from bison and elk and culture them for *Y. enterocolitica.* These data will increase our understanding of *Yersinia* epidemiology in Yellowstone bison and elk. We will collect sera from bison (tested, slaughtered, and hunted) and elk (captured, hunted) and test for specific anti-*Yersinia* antibodies using the western blotting technique (Al Dahouk et al. 2005). The *Yersinia* western blot technique has proved to be highly sensitive and specific for the detection of anti-*Yersinia* antibodies (Heeseman et al. 1987). We will use a Bayesian statistical approach to estimate the proportion of *Yersinia* cross reactivity in seropositive bison and elk. The level of seroprevalence resulting from *Y. enterocolitica* infection will be estimated from serum identifying only anti-*Yersinia* antibodies.

<u>Sampling objective 6: Obtain estimates of the number, age, and sex of bison removed at boundary capture pens and during hunter harvests based on knowledge of bison movements and the presence of radio-collared animals</u>.

Justification: Non-random removals of cows and their calves are a consequence of bison management in Yellowstone (Halbert 2003) because calves generally remain with their mothers through the first year of life (Berger and Cunningham 1994) and cow-calf pairs may be opportunistically culled as they exit park boundaries. Gross et al. (2006) used data from Halbert (2003) to estimate that 24% of the removals in Yellowstone were calf-cow pairs, which is about 50% higher than predicted to be removed through random selection of bison. Simulations with high levels (50%) of calf-cow removals suggest the short-term genetic effects of matrilineal removals may be small (Gross et al. 2006). However, the long-term demographic, genetic, and ecological effects of removing bison in this manner are unknown. Thus, further research into the potential effects of non-random culling of matrilineal groups is necessary.

Methods: The Record of Decision for the IBMP indicates that the NPS will operate the Stephens Creek capture facility in the north boundary area if hazing operations are unsuccessful at preventing bison movement north onto private and Gallatin National Forest lands in the Reese Creek area during steps 1 and 2. We will use aerial and ground surveys to track bison movements and the locations of radio-collared bison to estimate the timing, number, age, and sex of bison from each herd captured during boundary management operations. The average relatedness of bison captured together will be compared against a random sample of bison from the associated subpopulation (Halbert 2003). This will require blood to be sampled from all captured bison, regardless of whether they are eventually culled.

Sampling objective 7: Document the commingling of bison and cattle during the third trimester of pregnancy (March-May) for bison.

Justification: Cattle graze on some private and public lands (national forest) adjacent to Yellowstone. Thus, the risk of transmission of brucellosis from bison to cattle increases when bison migrate outside the park during winter, and transmission could occur without agency actions to minimize the risk. The Record of Decision for the IBMP indicates the signatory agencies will control the risk of transmission to cattle outside the park by limiting the number of bison in specified management zones near the northern and western boundary areas.

Methods: We will use aerial and ground surveys during November through May to periodically monitor bison abundance, distribution, and movements north onto private and Gallatin National Forest lands in the Reese Creek area, and west onto private and Gallatin National Forest lands in the Horse Butte area. We will prevent, if possible, and document instances where bison mix with cattle, including the date, number and composition of bison, number and ownership of cattle, location of mixing, actions taken to separate the bison from cattle, and recommendations to abate future mixing.

Sampling objective 8: Document the movements of bison fitted with radio collars after hazing and release from boundary capture facilities.

Justification: Park staff and key stakeholders have expressed concerns that hazing or capture and release operations near the park boundary may exacerbate the mixing of the central and northern bison herds or lead to possible habituation of bison from feeding during captivity.

Methods: We will deploy and monitor GPS and VHF radio collars on bison from the central and northern breeding herds. The movements of these radio-collared bison will be monitored during and after hazing. Also, we will document the length of captivity for radio-collared bison held in the capture facility for spring release, and their movements following release and during subsequent winters.

Sampling objective 9: Assess the strength of specific indicators of cell mediated immune function over time in calf, yearling, and adult female bison induced by syringe injection of the vaccine SRB51.

Justification: The effectiveness of a brucellosis vaccination program in Yellowstone bison will depend on the vaccine's ability to induce a long-lived protective immune response. Protection relies on vaccinated bison being able to re-call this response following exposure to field strain *Brucella*. Parenteral vaccination of bison calves with SRB51was found to be efficacious in protecting against *B. abortus* infection when the same animals were challenged with virulent *B. abortus* during the middle of gestation (Olsen et al. 2003). The effectiveness of SRB51 in bison might be attributed to its ability to induce a significant cell-mediated immune response (γ-interferon production) when compared with non-vaccinated bison (Olsen et al. 2006*a*). These results are in contrast with what has been observed in elk vaccinated with both SRB51 and S19. Olsen et al. (2006*b*) found that brucellosis vaccination of elk did not induce robust and persistent cellular immunologic responses even after booster vaccination with either

vaccine. The lack of strong, long-term, cell-mediated, immune responses in elk following vaccination may be associated with the reduced protection provided by these vaccines. Calf-hood vaccination of elk with S19 produced low protection against abortion, no protection from infection, and was unlikely to lead to a significant reduction of brucellosis in feed-ground elk (Roffe et al. 2004). The ability of SRB51 to induce significant, cell-mediated, immune responses in bison is positively correlated with the vaccine's ability to provide protection against *Brucella* infection. This makes SRB51 a potential candidate for vaccinating Yellowstone bison. However, the enormity of Yellowstone, combined with harsh winter conditions, will make it difficult to re-evaluate the strength of SRB51-induced immune responses following vaccination in free-ranging bison. Bison age and condition may also play an important role in the longevity of protective immune responses. Identifying whether free-ranging bison demonstrate levels of immune protection similar to those observed in experimental studies is necessary for evaluating the level of vaccine protection across the population. By monitoring the strength of the immune response across age classes in vaccinated female bison, we will identify the duration of protective immunity for each class, the conditions influencing declining responses, and the frequency of booster vaccination needed to maintain protective levels of immunity.

Methods: We propose to quantify the strength of the immune response following vaccination using lymphocyte proliferation assays and measuring the gene expression of a specified cytokine profile. These indicators provide insight into protection from *B. abortus* infection (Baldwin 2002) and we will monitor them before and after vaccination to determine the effect vaccination has on immune function. We will quantify the rate of decrease in protective immune responses in the post-vaccination period, as well as the effect vaccine boostering has on the longevity of measurable immune function. This information could be gathered by collecting repeated blood samples from Yellowstone bison calves placed in the quarantine program and bison sampled on private ranches.

Age-specific seroprevalence rates of Yellowstone bison indicate that approximately 50% of bison are exposed within their first 20 months of life (Treanor et al. 2007*b*, Rhyan et al. 2009). Early exposure to *B. abortus* may facilitate the shedding of the bacteria during the first pregnancy in female bison (Rhyan et al. 1994). Thus, vaccinating bison as calves may allow immature bison to develop adaptive immunity to brucellosis infection. To assess vaccine-induced immune responses in calves, we will sample female Yellowstone bison calves that have been placed in the quarantine study during 2008. All bison were test negative as calves and will be vaccinated with SRB51. We will collect blood from each animal prior to vaccination and then periodically (approximately 10 times) until parturition following their first pregnancy. Also, we will booster vaccinate a select number of bison (20 animals) one year following initial vaccination. We will compare immune responses in these bison with responses in single vaccinates to identify the effect of booster vaccinations. Bison peripheral blood mononuclear cells (PBMC) will be stimulated *in vitro* with heat killed *B. abortus*, incubated, and analyzed for lymphocyte proliferation and the gene expression of select cytokines. We will assess nutritional condition using ultrasonography (mm of back fat) and body condition scoring (palpation). These indicators of condition will serve as covariates in a repeated measures analysis.

Private bison ranches typically vaccinate their bison with SRB51 as calves and do not re-vaccinate. Record keeping and marking of bison in private herds provides exact age information

and the time since calf-hood vaccination. We could use the annual roundup and bleeding of these bison to identify differences in immune re-call responses using techniques described above. Nutritional condition and pregnancy status could be used as covariates in the analysis.

Sampling objective 10: Identify the difference in SRB51 induced cell-mediated immune responses in bison calves vaccinated using bio-bullet delivery versus parenteral vaccination.

Justification: A vaccination program for Yellowstone bison is unlikely to be successful without an effective remote delivery component (Treanor et al. 2007*a*, 2008, 2010). Currently, vaccine delivery via bio-bullets is the only option available for remotely vaccinating bison. Controlled experiments have demonstrated that bio-bullet delivery of SRB51can induce immune responses in bison similar to parenteral delivery (Olsen et al. 2006*a*), but recent efforts to reproduce these results have been inconsistent. For example, studies of the immunologic responses of bison to bio-bullet vaccination with hydrogel SRB51 during 2007 indicated poor proliferative and γ-interferon responses compared to parenteral vaccination (S. Olsen, Agricultural Research Service, unpublished data). A Yellowstone bison vaccination program would involve both parenteral vaccination of bison captured at park boundaries and remote vaccination of bison within the park. Predicting the potential decrease in brucellosis infection resulting from vaccination requires understanding the level of protection provided by each delivery method. By evaluating how each method influences protective immune responses, we will better understand the limitations of each approach. For this sampling objective, we will identify the difference in bison immune response following vaccination using parenteral and remote delivery methods.

Methods: We will consult with the Animal and Plant Health Inspection Service and request they initiate or facilitate research to assess and improve the vaccine for bison and attain high efficacy via bio-bullet delivery. Once the vaccine and hydrogel is improved, we propose to initiate research that rigorously quantifies the strength of the immune response following bio-bullet vaccination via lymphocyte proliferation assays and measuring the gene expression of a specified cytokine profile. These indicators will be used to compare both vaccine delivery methods, but the spatial scale and free-ranging conditions of Yellowstone pose a problem for making such a comparison. Thus, private bison herds near Yellowstone, which are required to vaccinate animals with SRB51, may be more appropriate for assessing the effectiveness of remote vaccination. To compare the immune responses induced by the two delivery methods, we will assign 30 non-vaccinated bison calves to one of three treatments in a completely randomized design: 1) SRB51 vaccination via parenteral delivery; 2) SRB51 vaccination via remote (bio-bullet) delivery; and 3) control group (i.e., saline or select media via parenteral or remote delivery). There will be an equal number (n = 10) of individual bison receiving each treatment (i.e., 10 replicates per treatment class). We will measure and use body mass and rump fat (measured using ultrasonography) as covariates that may influence immune responses to treatments. We will draw blood from all bison prior to treatments (3 sampling periods) and after treatment (2, 6, 12, 18, and 24 weeks). Pre-treatment samples will identify variability in immune responses prior to treatment that will be used to evaluate treatment effects (vaccination delivery methods). We will assay the proliferation of T-lymphocyte subpopulations using flow cytometry and quantify the gene expression of select cytokines using RT-PCR. These indicators will serve

as the response variables for assessing the effects of vaccination. We will compare results between delivery methods.

Sampling objective 11a: Decrease the seroprevalence of brucellosis in Yellowstone bison by approximately 30% during the first 10 years of the remote vaccination program, 50% within 20 years, and 90% within 30 years through boundary management and remote vaccination.

Justification: Seroprevalence is the most accessible indicator of brucellosis infection in Yellowstone bison. Seropositive bison can be reliably and quickly estimated from bison handled during boundary management operations without killing animals. However, seroprevalence is an indicator of exposure rather than active infection, which makes it an insensitive indicator for monitoring decreasing infection through vaccination. Antibodies (i.e., responses to *B. abortus* exposure) can be long-lived and false positive diagnoses can result from other cross reactive agents. Therefore, monitoring decreases in brucellosis infection resulting from vaccination will require monitoring multiple indicators. We will use the following indicators to measure the effectiveness of the vaccination program: 1) population seroprevalence; 2) ratio of seropositive to culture positive bison; 3) the proportion of vaccinated bison in the population; and 4) the incidence rates of *B. abortus* infection (Treanor et al. 2008, 2010).

Based on currently available tools (i.e., vaccine, delivery method, and diagnostics), the success of effectively reducing seroprevalence levels in Yellowstone bison will require remote vaccination of bison within the park. Model simulations suggest the largest decrease in population seroprevalence can be best achieved by combining boundary management (i.e., test, remove reactors, vaccinate nonreactors) with remote vaccination of all female bison (Treanor et al. 2007*a*, 2008, 2010). Under this scenario, model simulations demonstrate a decrease in seroprevalence of approximately 66% (0.47 to 0.16) over a 30-year period.

Methods: To track vaccination progress in reducing the level of brucellosis infection in Yellowstone bison, we will use annual seroprevalence rates as an indicator of the state of brucellosis infection. We will estimate population seroprevalence from Yellowstone bison that are handled at each of the boundary management facilities. Captured bison will be restrained in a squeeze chute while blood is collected via venipuncture. We will collect blood and use serum to diagnose sero-status using the fluorescent polarization assay (FPA) and card tests. We will compare results to previous years to identify the rate of decrease.

Sampling objective 11b: Increase the proportion of vaccinated bison in the Yellowstone population to at least 50% of all female bison across all age classes and sustain this level of vaccinated animals for the duration of the park-wide vaccination program.

Justification: Decreases in brucellosis infection will be inversely related to the proportion of vaccine-protected bison in the population. Focusing vaccination efforts on all female bison increases the likelihood that bison in all age classes will be protected from infection. However, identifying bison remotely vaccinated with SRB51 cannot be done through standard serological tests (Stevens et al. 1994) and will require alternate methods. Adone et al. (2002) combined SRB51 antigen with *B. abortus* smooth strain 99 (S99) to detect cattle vaccinated with SRB51. The RB51/S99-based complement fixation test was sensitive and specific for rapid detection of

both *B. abortus* infection and SRB51 vaccination. Validation of this technique in bison would aid in identifying bison remotely vaccinated with SRB51 when tested at the boundary facilities. Also, remotely vaccinated bison that are subsequently exposed to field strain *B. abortus* could be identified with this technique. Otherwise, these bison would appear as seropositive on standard serologic tests and would most likely be removed from the population. The ability to identify bison captured at the boundary that have been remotely vaccinated with SRB51 will allow us to select these animals for identifying protective immune responses to brucellosis infection. We can evaluate if the vaccination program is reducing brucellosis infection by identifying whether vaccinated bison are demonstrating strong, long-term, cell-mediated, immune responses and maintaining 50% of female bison in this state.

Methods: Monitoring for identifying the proportion of vaccine-protected bison in the population will involve testing bison captured at the boundary, identifying vaccinated animals, and quantifying the level of protective cell-mediated immune responses to *B. abortus* infection. We will identify bison vaccinated with SRB51 using the complement fixation test to detect antibodies to SRB51 vaccine. We will assess the immune response of these vaccinated bison using lymphocyte proliferation and cytokine assays. The levels of these indicators will be compared to protective levels identified in experimental challenge studies.

<u>Sampling objective 11c: Decrease the incidence rate (i.e., the rate at which seronegative animals become seropositive) for brucellosis over the duration of the vaccination program</u>.

Justification: The proportion of bison infected with brucellosis is expected to be positively related to the rate of seroconversion in the population. The probability a susceptible host will be exposed increases with the frequency of *Brucella* shedding. Monitoring the incidence rate (i.e., the increase in new cases of brucellosis infection over time) will provide information on the effect vaccination has on the disruption of brucellosis transmission. Incidence rates can also help with the interpretation of seroprevalence data because population seroprevalence is expected to decrease as the frequency of new cases of brucellosis infection decreases. Since nearly 50% of bison are exposed to *B. abortus* early in life (<2 years old), monitoring incidence rates in young bison will provide the most valuable information on the effectiveness of the vaccination program. Newborns represent a continuous cohort of susceptible animals and their seroconversion rate identifies the rapidity at which susceptible bison are becoming infected. Thus, we will focus our monitoring of *B. abortus* incidence primarily on this young age class.

Methods: We will estimate incidence rates by fitting seronegative bison calves captured during boundary management operations with an ear tag or invisible identifiable marker (i.e., pit tag) and releasing them back into the park. We will calculate incidence rates from the number of recaptured bison that seroconverted during the specific time interval. We will supplement these estimates of incidence rates with data from repeated captures of radio-collared bison that demonstrate seroconversion between capture years.

IMPLEMENTATION AND EXPECTED OUTCOMES

To accomplish this suite of monitoring activities, we will work with the other IBMP agencies (Animal and Plant Health Inspection Service, Confederated Salish and Kootenai Tribes,

Intertribal Buffalo Council, Montana Department of Livestock, Montana Department of Fish, Wildlife, and Parks, Nez Perce Tribe, U.S. Forest Service), the Yellowstone Wildlife Health Program, and other scientists and stakeholders to implement field, controlled, and laboratory studies to collect empirical data for evaluating progress. The data will be used to develop and parameterize models that will serve as analytical tools for evaluating how bison and brucellosis may respond to management actions within specified confidence bounds. The IBMP agencies produce an annual report that describes monitoring activities, the status of Yellowstone bison, and relevant brucellosis management issues. This report is disseminated at <www.ibmp.info>. The Wildlife Biologist leading the Bison Ecology and Management Program at Yellowstone will be responsible for managing the monitoring system and producing the monitoring portion of the annual IBMP report. This monitoring plan, analyses, and inferences in reports will be subject to interdisciplinary, scientific peer review by other scientists from the NPS, agency partners, and/or anonymous reviewers selected by editors of scientific journals. Pursuant to Bulletin M-05-03 issued by the Office of Management and Budget on December 16, 2004, the intensity of peer review will be commensurate with the significance of the information being disseminated.

Success in adaptive management ultimately depends on effectively linking monitoring and assessment to objective-driven decision making (Williams et al. 2007). Though different philosophies exist regarding how adaptive management should be implemented, certain characteristics transcend them, including: 1) linkages among key steps such as identifying objectives, implementing monitoring, and adjusting management actions based on what is learned; 2) collaborating with agency partners; and 3) communicating with and engaging key stakeholders (U.S. Government Accountability Office 2008). This monitoring program will provide timely and useful information to help develop adaptive management adjustments needed to conserve Yellowstone bison, reduce the risk of brucellosis transmission from bison to cattle, and reduce the prevalence of brucellosis in the bison population. It will also allow IBMP managers to track system responses to these management actions through continuation of monitoring. Examples of actions by the NPS that monitoring may trigger based on the information collected include:

- Deciding whether or not to implement remote vaccination based on vaccine efficacy (i.e., stimulation of cellular immunity) and the development of adequate delivery options to obtain the desired reductions in seroprevalence and infection;
- Discontinuing vaccination in its implemented form if there is no indication of progress over a reasonable period;
- Implementing conservation measures to decrease mortality and increase the growth rate of the population if estimated bison abundance decreases towards 2,500;
- Altering culling or harvest strategies if significant and biologically important effects to age, genetics, and/or sex structure are detected; and
- Discontinuing disease containment or suppression actions if estimated bison abundance decreases below 2,500 and agency partners do not strictly implement conservation measures to abate further reductions in abundance.

MONITORING SYSTEM EVALUATION

The monitoring program will be considered successful if it provides data to: 1) evaluate progress towards achieving objectives; 2) determine resource status to identify appropriate management

actions and adjust management decisions; 3) increase understanding of resource dynamics via the comparison of predictions against survey data; and 4) enhance and develop models of resource dynamics as needed and appropriate. The following questions were adapted from Williams et al. (2007) and will be considered throughout the duration of this plan to increase the likelihood of successful monitoring and evaluate progress in achieving objectives:

- Stakeholder Involvement: Are stakeholders committed to and involved in the monitoring and assessment program? Is the monitoring process able to adapt to changes in stakeholder and public viewpoints?
- Objectives: Are the monitoring objectives achievable and sustainable? Is the monitoring program providing information that can be used to track progress in meeting management objectives and better understand trade-offs among objectives?
- Management Actions: Does the monitoring plan provide information that can be used to assess the potential effects and effectiveness of the range of feasible management actions (e.g., no action, vaccination, selective culling, quarantine) and trade-offs among them? Is progress being made towards achieving management objectives? Has the set of management alternatives or implemented actions been adjusted over time based on information obtained during monitoring?
- Models: Are the hypotheses underlying the strategies for resource management expressed as testable models? Have explicit links between management actions and resource dynamics been incorporated into the models? Has the monitoring plan contributed to a better understanding of the ecological processes that drive resource dynamics? Are the relevant environmental factors incorporated into the models? Are the models calibrated with available monitoring information?
- Monitoring Plan: Does the plan support the testing of alternate models and measurement of progress towards accomplishing management objectives? Does the plan monitor the metrics necessary to estimate relevant resource and disease attributes? Have the necessary levels of accuracy been attained? Have commitments among managers, scientists, and other stakeholders been sustained during the monitoring program? Does the plan provide meaningful and useful data and information within timeframes that allow for adaptive decision making?
- Decision Making: Are decisions based on the understanding and status of the resource derived from monitoring data? Are decisions being guided by management objectives and monitoring information regarding these objectives? Are stakeholders informed and given the opportunity to comment before decisions are made or changed?
- Follow-up Monitoring: Are analysis needs understood and being met? Is monitoring conducted on a timely basis? Is monitoring targeted to system attributes that are useful for evaluation and learning? Are monitoring data collected and managed so they are available and easy to access? Can the monitoring data be used to update measures of model confidence?
- Assessment: Have monitoring data been used to evaluate the expected effects of alternate management strategies and update predictions? Have changes in management been implemented when monitoring data indicate management objectives are or are not being met?
- Iteration: Are management actions and decisions reviewed frequently based on monitoring and assessment information? Have resource management alternatives been revisited or

modified over time? Has uncertainty related to resource and disease dynamics and the effects of management actions been reduced through monitoring and learning over time? Are the monitoring objectives likely to be achieved within specified or reasonable timeframes?

Each year through regularly scheduled IBMP meetings and the completion of the annual IBMP report, we will solicit review, comment, and discussion by our agency partners and key stakeholders in the refinement of objectives, design of monitoring, and assessment to build support for the legitimate process and provide a foundation for learning-based resource management. Public information staff will share the results of monitoring activities with key stakeholder groups through timely press releases and web-mails, and reports and articles will be made available on-line at the website for the IBMP agencies (<www.ibmp.info>).

MONITORING FINDINGS

The following paragraphs summarize findings of monitoring and research since the monitoring plan was initiated in 2008. These findings were reported at IBMP meetings and considered by the IBMP agencies in developing the annual reports and recommendations for adaptive management adjustments (White et al. 2009, Zaluski et al. 2010).

Conservation (Preserve a Functional, Free-Ranging Bison Population)

1. Estimate the abundance, demography, and limiting factors for the overall bison population and two primary subpopulations (i.e., central and northern breeding herds).

- NPS staff collaborated with colleagues at Montana State University to estimate demographic rates from 80 adult female bison in the central herd during 1995-2006 (Geremia et al. 2009).
    - Animals testing positive for exposure to brucellosis had significantly lower pregnancy rates across all age classes compared to seronegative bison.
    - Birth rates were high and consistent for seronegative animals, but lower for younger, seropositive bison. Seronegative bison that converted to seropositive while pregnant were likely to abort their 1st and 2nd pregnancies.
    - There was a pronounced decrease in survival for animals >12 years old. Also, brucellosis exposure indirectly lowered bison survival because more bison were culled over concerns about transmission to cattle when bison attempted to move to lower-elevation areas outside the park.
    - There was a significant decrease in adult female survival when the number of bison in the central herd exceeded 2,000-2,500 animals, which was exacerbated during winters with severe snow pack because more bison moved outside the park. Except during 1996-97, the vast majority of radio-marked bison culled at the north and west boundaries during 1995-2006 came from the central herd.
    - The effects of brucellosis on survival, pregnancy, and birth rates lowered the growth rate in the central herd. Population growth rates will likely increase by more than 15% if vaccination plans are implemented and successful.

- NPS staff collaborated with colleagues at Colorado State University to synthesize available information and interpreted results of a spatially explicit model (Coughenour 2005) of the Yellowstone system (Plumb et al. 2009).
  - Bison abundance has not exceeded the theoretical food-limited carrying capacity of 6,200 in Yellowstone.
  - More bison migrate earlier to lower-elevation winter ranges as numbers increase and climatic factors interact with density to limit nutritional intake and foraging efficiency.
  - A gradual expansion of the winter range as bison numbers increased enabled relatively constant population growth and increased food-limited carrying capacity.
  - Current management actions should attempt to preserve bison migration to essential winter range areas within and adjacent to the park, while actively preventing dispersal and range expansion to outlying areas via hazing, translocations, and culls.
  - A population of 2,500-4,500 bison should satisfy collective interests concerning the park's forage base, bison movement ecology, retention of genetic diversity, brucellosis risk management, and prevailing social conditions.

- NPS staff contributed to a chapter on conservation guidelines for population, genetic, and disease management of American bison for the IUCN (Gates et al. 2010).
  - Overarching principles for conserving bison were to (1) maximize the number of bison in a population (i.e., 'maximum sustainable' rather than a 'minimum viable' population size) to better retain natural variation and provide more resiliency to 'surprises' or catastrophic events, (2) support and promote 'wild' conditions and behaviours in an environment where bison are integral to community and ecosystem processes, exposed to natural selection, and active management interventions are minimized, (3) preserve genetic integrity and health by maintaining bison lineages and carefully evaluating all movements of bison between populations, and (4) conducting routine monitoring and evaluation of demographic processes, herd composition, habitat, and associated ecological processes that are central to evaluating herd health and management efficacy.

- NPS staff developed a population model using data collected from Yellowstone bison during 1970-2011 and estimated the abundance, composition, and trends of each breeding herd to evaluate the relative impacts of harvests and other types of management removals (Geremia et al. 2011a).
  - Demographic estimates were integrated with a model of bison migration (Geremia et al. (2011b) to predict the numbers of bison moving to the park boundary each winter. These tools combined long-term monitoring data with information gained from radio-collared bison to draw conclusions about future conditions of Yellowstone bison.
  - A decision-making process was developed to advise the management of population abundance and trans-boundary movements of bison. During June and early July, NPS staff conducted population counts and age and gender classifications of each breeding herd. They then used long-term weather forecasts and the models described above to predict herd abundances and compositions at the end of the upcoming winter, and the magnitude of numbers of bison migrating to park boundaries.
  - NPS staff established annual removal objectives for bison based on abundance, disease, distribution, and demographic (age, herd, sex) goals to reduce bison numbers

towards an end-of-winter target of 3,000, while progressing towards equal abundance in each herd and sex ratios of 50% adult males and 50% adult females in each herd.
    - A variety of management tools were considered for reducing bison numbers including (1) public and treaty harvests in Montana, (2) selective culling (shipment to slaughter) at boundary capture facilities to reduce the proportion of infectious bison, (3) selective culling (shooting, shipment to slaughter) in Montana to prevent brucellosis transmission to nearby livestock or due to human safety or property damage concerns, (4) transfer of bison to American Indian tribes or other organizations for quarantine and eventual release, and (5) transfer bison to research facilities**.**

2. Describe migratory and dispersal movements by bison at a variety of temporal and spatial scales in and outside the park.

- NPS staff collaborated with colleagues at Montana State University to quantify annual variations in the magnitude and timing of migration by central herd bison during 1971-2006 and identify potential factors driving this variation (Bruggeman et al. 2009c).
    - Bison from the central herd were partially migratory, with a portion of the animals migrating to the lower-elevation Madison headwaters area during winter while some remained year-round in or near the Hayden and Pelican valleys.
    - There was significant bison migration to the Madison headwaters area before the Hayden and Pelican valleys were fully occupied and abundance approached the food-limiting carrying capacity of these valleys.
    - After the central herd exceeded 2,350 animals, however, the number of bison wintering in the Hayden and Pelican valleys appeared to stabilize, while bison continued to migrate to the Madison headwaters area. Also, more bison migrated earlier as density increased.
    - Some bison migrated outside the west-central portion of the park between the summer and winter counts each year when the central herd exceeded 2,350 bison, perhaps relocating to northern range.
    - The timing and magnitude of bison migration were accentuated during years of severe snow pack that limited access to food.

- NPS staff collaborated with colleagues at Montana State University to quantify how snow, topography, habitat attributes, and roads influenced the travel patterns and non-traveling activities of 30 radio-marked, adult, female bison from the central herd during three winters (Bruggeman et al. 2009a, b).
    - Bison were less likely to use a point on the landscape for traveling or feeding as snow pack increased. However, bison used local areas with deeper snow as the overall snow pack increased on the landscape.
    - Distance to stream was the most influential habitat covariate, with the spatial travel network of bison being largely defined by streams connecting foraging areas. Distances to foraging areas and streams also significantly influenced non-traveling activities, being negatively correlated with the odds of bison foraging or resting.
    - Topography significantly affected bison travel patterns, with the probability of travel being higher in areas of variable topography that constrained movements (e.g., canyons). Distance to road had a significant, negative effect on bison travel, but was nine times less influential compared to the impact of streams.

- Road grooming has a minimal influence on bison travel and habitat use given the importance of natural dynamic and static landscape characteristics such as snow pack, topography, and habitat attributes on bison choice of travel routes and habitat use for foraging and resting.

- NPS staff collaborated with staff from Colorado State University to analyze the relationships between bison population size, winter severity, and the number of bison removed near the boundary of Yellowstone during 1990-2010 (Geremia et al. 2011b).
  - Migration differed at the scale of herds, but a single unifying exponential model was useful for predicting migrations by both herds.
  - Migration beyond the northern park boundary was affected by herd size, accumulated snow water equivalent, and aboveground dry biomass. Migration beyond the western park boundary was less influenced by these predictors, and model predictions since 2006 suggest additional drivers (e.g., learning) of migration were not in the model.
  - Simulations of migrations over the next decade suggest that a strategy of sliding tolerance where more bison are allowed beyond park boundaries during severe climate conditions may be the only means of avoiding episodic, large-scale reductions to the Yellowstone bison population in the foreseeable future.

3. Estimate the existing heterozygosity, allelic diversity, and long-term probabilities of genetic conservation for the overall bison population and identified subpopulations.

- NPS staff collaborated with colleagues at the University of Montana to test the hypothesis that bison from the central and northern breeding herds would be genetically differentiated based on mitochondrial and microsatellite DNA from fecal samples.
  - Based on mitochondrial DNA analyses, there was significant genetic differentiation between bison sampled from the northern and central breeding herds, likely due to strong female fidelity to breeding areas (Gardipee 2007).
  - However, analyses using nuclear microsatellite markers indicated genetic differences between herds were much less, suggesting that male movements between the two breeding herds are likely substantial and resulting in significant gene flow throughout the population (F. Gardipee, unpublished data).

- NPS staff collaborated with colleagues at the University of Montana and to conduct a mathematical modeling assessment that provided predictive estimates of the probability of preserving 90 and 95% of the current level of genetic diversity values (both heterozygosity and allele diversity) in Yellowstone bison (Pérez-Figueroa et al. 2010).
  - Findings suggested that variation in male reproductive success had the strongest influence on the loss of genetic variation, while the number of alleles per locus also had a strong influence on the loss of allelic diversity.
  - Fluctuations in population size did not substantially increase the loss of genetic variation when there were more than 3,000 bison in the population. Conservation of 95% of the current level of allelic diversity was likely during the first 100 years under most scenarios considered in the model, including moderate-to-high variations in male reproductive success, population sizes greater than 2,000 bison, and approximately five alleles per locus, regardless of whether culling strategies resulted in high or low fluctuations in abundance.

- However, a stable population abundance of about 2,000 bison was not likely to maintain 95% of initial allele diversity over 200 years, even with only moderate variation in male reproductive success. Rather, maintenance of 95% of allelic diversity is likely to be achieved with a fluctuating population size that increases to greater than 3,500 bison and averages around 3,000 bison.

- NPS staff provided information to the Department of Interior for review by scientists from government agencies and non-governmental organizations with professional population geneticists and the development of guidance for the genetic management of federal bison populations (Dratch and Gogan 2010).
  - Parks and refuges that currently have bison populations, with the exception of Yellowstone National Park, do not have enough land to support a population of more than 1,000 bison (i.e., minimum target to preserve genetic variation over centuries).
  - Yellowstone bison have relatively high allelic richness and heterozygosity compared to other populations managed by the Department of Interior.
  - Yellowstone bison are the only population with no molecular evidence (i.e., microsatellite markers) or suggestion (i.e., SNPs) of potential cattle ancestry (i.e., introgression of cattle genes). Thus, this population constitutes a genetic resource that must be protected from inadvertent introgression.
  - The Yellowstone and Wind Cave bison populations are genetically unique and the lineages are not represented elsewhere within populations managed by the Department of Interior. Thus, high priority should be given to replicating these significant lineages via satellite herd establishment.

- The NPS reviewed a study by Pringle (2011) that concluded that some Yellowstone bison have deleterious genetic mutations and, as a result, “are predicted significantly impaired in aerobic capacity, disrupting highly evolved cold tolerance, winter feeding behaviors, escape from predators and competition for breeding."
  - Bison with haplotype 6 in their mitochondrial genome carry a double mutation that affects two genes: cytochrome b and ATP6. These bison are primarily found in the central breeding herd based on recent genetic sampling. This inherited mutation could affect their production of energy (i.e., ATP produced by mitochondrial oxidative phosphorylation). Bison with haplotype 8 in their mitochondrial genome do not carry the double mutation and are primarily found in the northern breeding herd.
  - Even if the genetic sequences and analyses reported by Pringle (2011) are correct, genetic mutation does not automatically equal genetic disease. There are multiple compensating mechanisms in biological systems that combine to overcome theoretical metabolic deficiencies.
  - Also, there is direct evidence that even if Yellowstone bison have some sort of genetic deficiency, it has not been manifested through any biologically significant effect on their ability to survive. Estimated annual survival rates and birth rates for adult female bison were quite high during 1995-2006; especially given the severe, prolonged, high-elevation winter conditions and predator-rich environment in and near Yellowstone National Park.
  - The NPS is taking steps to follow-up on Dr. Pringle's work and recommendations.

Risk Management (Prevent Brucellosis Transmission from Bison to Livestock)

4. Estimate the probabilities (i.e., risks) of brucellosis transmission among bison, cattle, and elk, and the elk feed grounds in Wyoming and northern Yellowstone.

- NPS staff collaborated with colleagues at the Agricultural Research Service and University of Montana to genotype 10 variable number of tandem repeat DNA loci in 58 *B. abortus* isolates from bison, elk, and cattle and test which wildlife species was the likely origin of recent outbreaks of brucellosis in cattle in the greater Yellowstone area (Beja-Pereira et al. 2009).
  - Findings suggested that isolates from cattle and elk were nearly identical, but highly divergent from bison isolates. Thus, elk, not bison, were the reservoir species of origin for these cattle infections.

- NPS staff collaborated with colleagues at the U.S. Geological Survey and other agencies and universities to assess several plausible hypotheses for observed increases in the seroprevalence of brucellosis in several free-ranging elk populations of Wyoming (Cross et al. 2009).
  - Free-ranging elk appear to be a maintenance host for *B. abortus* in some areas.
  - Brucellosis seroprevalence in free-ranging elk increased from 0-7% in 1991-1992 to 8-20% in 2006-2007 in four herd units not associated with feed grounds.
  - These seroprevalence levels, which are comparable to units where elk are aggregated on feed grounds, are unlikely to be sustained by dispersal of elk from feeding areas with high seroprevalence or an older age structure.
  - The rate of seroprevalence increase was related to the population size and density of each herd unit. Enhanced elk-to-elk transmission in free-ranging populations may be occurring due to larger winter elk aggregations.
  - Elk populations inside and outside of the greater Yellowstone area that traditionally did not maintain brucellosis may now be at-risk due to recent population increases. In particular, some neighboring populations of Montana elk were 5-9 times larger in 2007 than in the 1970's with some aggregations comparable to the Wyoming feed ground populations.

- NPS staff collaborated with colleagues at Colorado State University to develop a Bayesian state space model to guide adaptive management of the Yellowstone bison population by assimilating from ongoing population monitoring and designed studies of population processes (Hobbs et al. 2009).
  - There was 6.8 times more support in the data for frequency-dependent (i.e., population expands as bison numbers increase) than density-dependent brucellosis transmission (i.e., area used by a population is fixed).
  - The average continuous rate of brucellosis transmission was 3.9 per year (95% CI = 2.6, 5.3), and the basic reproductive ratio (i.e., number of new infectious individuals created by a single infectious individual) averaged 2.5 (95% CI = 1.9, 3.7).
  - Forty percent (95% CI = 27, 55) of yearling and adult females would need to be susceptible for the disease to establish in the population.

- Estimation of population growth in the presence of brucellosis $\lambda = 1.06$ (95% CI = 1.01, 1.10) reflected the depressing effect of the disease on recruitment. In the absence of brucellosis, the population was predicted to grow at a rate of $\lambda = 1.11$ (95% CI = 1.06, 1.15).
- Seroprevalence of adult females varied between 40 and 60% during the last two decades, but only 10% of adult females were infectious.
- The probability that a susceptible bison would become infected via horizontal transmission varied annually between 10 and 20%. The probability of vertical transmission was 0.22 (95% CI = 0.03, 0.50), but accounted for fewer than 12% of transmission events.
- The annual removal of 200-300 sero-positive females was likely to maintain the population below 3,500 animals five years into the future and had a high probability (0.72-0.94) of reducing sero-prevalence below 10%. In contrast, the traditional practice of episodic removals of large numbers of untested animals was unlikely to maintain abundance <3,500 bison five years into the future and would fail to reduce seroprevalence. However, annual removals of 200-300 females created substantial risks of reducing the population to <2,500 bison and increasing the adult and yearling male proportion of the population to >40%.

- The NPS reviewed and provided comments on a draft of the Kilpatrick et al. (2009) article that used a model to integrate epidemiological and ecological data to quantify and assess the spatiotemporal relative risk of transmission of *Brucella* from bison to cattle outside Yellowstone under different scenarios.
  - The risk of transmission of brucellosis from bison to cattle is likely to be a relatively rare event, even under a 'no plan' (no management of bison) strategy.
  - The risk of transmission of brucellosis from bison to cattle will increase with increasing bison numbers and severe snow fall or thawing and freezing events.
  - As the area bison occupy outside Yellowstone in the winter encompasses new area and additional cattle grazing (as is presently occurring), the risk of transmission will increase. Thus, adaptive management will be most effective.
  - Risk of transmission could be effectively managed with lower costs, but land use issues and the larger question of bison population management and movement outside the park might hinder the prospect of solutions that will please all stakeholders.

- NPS staff estimated the timing and location of parturition events that may have shed tissues infected by *B. abortus* during April to mid-June, 2004-2007 (Jones et al. 2010).
  - Observed abortions occurred from January through 19 May, while peak calving (80% of births) occurred from 25 April to 26 May, and calving was finished by 5 June.
  - Observed parturition events occurred in the park and on the Horse Butte peninsula in Montana, where cattle were not present at any time of the year.
  - Allowing bison to occupy public lands outside the park where cattle are never present (e.g. Horse Butte peninsula) until most bison calving is completed (late May or early June) is not expected to significantly increase the risk of brucellosis transmission from bison to cattle because: 1) bison parturition is essentially completed weeks before cattle occupy nearby ranges, 2) female bison meticulously consume birthing tissues, 3) ultraviolet light and heat degrade *B. abortus* on tissues, vegetation and soil,

4) scavengers remove fetuses and remaining birth tissues and 5) management maintains separation between bison and cattle on nearby ranges.
    - Allowing bison to occupy public lands outside the park through their calving season will help conserve bison migratory behavior and reduce stress on pregnant females and their newborn calves, while still minimizing the risk of brucellosis transmission to cattle.

- NPS staff collaborated with colleagues at Montana State University to analyze conditions facilitating contact between bison and elk on a shared winter range in the Madison headwaters area of Yellowstone during 1991 through 2006 (Proffitt et al. 2010).
    - Spatial overlap between bison and elk increased through winter as snow pack increased and peaked when late-term abortion events and parturition occurred for bison. Wolves contributed to immediate, short-term responses by elk that increased spatial overlap with bison, but longer-term responses to wolves resulted in elk distributions that reduced spatial overlap with bison.
    - Despite this relatively high risk of transmission, levels of elk exposure to *B. abortus* (2-4%) were similar to those in free-ranging elk populations that do not commingle with bison (1-3%), suggesting that *B. abortus* transmission from bison-to-elk under natural conditions is rare.
    - Management of brucellosis in elk populations could focus on reducing elk-to-elk transmission risk and, to the extent feasible, curtailing practices that increase elk density and group sizes during the potential abortion period.

- NPS staff collaborated with colleagues at Colorado State University to develop Bayesian models to estimate rates of incidence and routes of transmission of *B. abortus* bacteria among Yellowstone bison during 1995-2010 and assessed the reproductive costs (C. Geremia, unpublished data).
    - The median probabilities of horizontal (from unrelated bison) and vertical (from mother) exposure to calves were 0.10 (95% credible interval = 0.03, 0.22) and 0.10 (0.00, 0.28), respectively; though the distribution for vertical transmission was skewed left with most of the probability closer to zero.
    - Probabilities that adult bison were exposed to brucellosis since the preceding parturition season varied from 0.03-0.37 and snow pack severity exacerbated incidence.
    - We detected a measureable probability (0.01, 0.12) of bison recrudescing from a latent to an infectious state.
    - There was a reproductive cost of diminished birth rates following brucellosis infection, with only 59% of seropositive and recently seroconverting females with calves compared to 79% of seronegative females with calves.
    - These results suggest brucellosis is maintained through mixed transmission modes and the duration of infection may extend beyond the acute phase.

- NPS and Animal Plant and Health Inspection Service staff collaborated with colleagues at the University of California-Davis on a spatially-explicit assessment of brucellosis transmission risk among bison, elk, and cattle in the northern portion of the greater Yellowstone area (Schumaker et al. 2010).

- Population size and winter severity were major determinants influencing bison movements to lower elevation winter grazing areas, overlapping with federally-regulated domestic cattle grazing allotments. Increasing population size resulted in higher herd densities and increased bacterial shedding.
- Median total risk to cattle from elk and bison was 3.6 cattle-exposure event-days (95% P.I. 0.1-36.6). The estimated percentage of cattle exposure risk from the Yellowstone bison herd was small (0.0-0.3% of total risk) compared with elk which contributed 99.7-100% of the total risk.
- Natural herd migration and boundary management operations were important in minimizing the contribution of bison to cattle exposure risk, which supports continued boundary management operations for separation between bison and cattle.
- Transmission risks to elk from elk in other populations or from bison were very small. Minimal opportunity exists for *B. abortus* transmission from bison to elk under current natural conditions in the northern greater Yellowstone area.
- Management alternatives that reduce bison seroprevalence are unlikely to substantially reduce transmission risk from elk to cattle. Strategies that decrease elk herd densities and group sizes and reduce elk-to-elk transmission could reduce the overall risk to cattle grazing in the northern portion of the greater Yellowstone area.
- Efforts should be taken to reduce the comingling of cattle and elk, especially during the late gestation period for elk, when spontaneous elk abortions pose a risk for interspecies disease transmission.
- Bison vaccination did not meaningfully reduce *B. abortus* transmission risk to cattle. Effective strategies included delaying the turn-on date to cattle grazing allotments, reducing elk seroprevalence, reducing the number of cattle at-risk, or prohibiting the comingling of elk and cattle on individual premises.

5. Estimate age-specific rates of bison testing seropositive and seronegative for brucellosis that are also culture positive and the portion of seropositive bison that react positively on serologic tests due to exposure to cross-reactive agents other than *B. abortus* (e.g., *Yersinia*).

- NPS staff collaborated with colleagues at the University of Montana to investigate if *Y. enterocolitica* serotype *O:9* caused false-positive reactions in brucellosis serological tests for bison using culturing techniques and multiplex PCR (See et al. 2010).
  - *Y. enterocolitica* was not detected in samples of feces collected from 53 Yellowstone bison culled from the population and 113 free-roaming bison from throughout the greater Yellowstone ecosystem.
  - These findings suggest *Y. enterocolitica O:9* cross-reactivity with *B. abortus* antigens is unlikely to cause false positive serology tests in bison, and that *Y. enterocolitica* prevalence is low in these bison.

- NPS and Animal and Plant Health Inspection Service staff sampled more than 400 bison that were consigned to slaughter during winter 2007-08 and collected blood and tissues to estimate the proportion of seropositive and seronegative bison that were actively infected with *B. abortus* (i.e., culture positive; Treanor et al. 2011).
  - Removing brucellosis-infected bison is expected to reduce the level of population infection, but test and slaughter practices may instead be removing mainly recovered bison. Recovered animals could provide protection to the overall population through

the effect of herd immunity, thereby reducing the spread of disease. Identifying recovered bison is difficult because serologic tests (i.e., blood tests) detect the presence of antibodies, indicating exposure, but cannot distinguish active from inactive infection.

- Age-specific serology and *B. abortus* culture results from slaughtered bison were integrated to estimate probabilities of active brucellosis infection using a Bayesian framework. Infection probabilities were associated with age in young bison (0-5 years old) and with elevated antibody levels in older bison (>5 years old). Results indicate that Yellowstone bison acquire *B. abortus* infection early in life but typically they recover as they grow older.
- A tool was developed to allow bison management to better reflect the probability that particular animals are infective, with the aim of conserving Yellowstone bison while reducing the risk of brucellosis transmission to cattle. Fluorescent polarization assay (FPA) values were higher in seropositive bison that were culture positive compared to seropositive bison that were culture negative, supporting that active infection is associated with increased antibody production.
- The two covariates (age and FPA) have management application to identify the probability of active infection within specified credible intervals. This would allow for removing bison that most likely contribute to brucellosis maintenance in the population, while keeping bison that contribute to herd immunity which reduces brucellosis transmission.
- Estimation of true infection probabilities can replace culling practices (such as the slaughter of all seropositive individuals) that conflict with bison conservation. Combining selective removal of infectious bison with additional management practices, such as vaccination, has the potential to advance an effective brucellosis reduction program.

6. Estimate the timing and portion of removals from the central and northern herds each winter, including the portion of removals from each age and sex class and calf-cow pairs.

- NPS staff retrospectively evaluated if reality met expectations by comparing assumptions and predictions for the alternative selected from the Final Environmental Impact Statement and described in the Record of Decision for the IBMP (USDI and USDA, 2000a, b) with observed impacts and changes since implementation of the plan began in 2001 (White et al. 2011).
  - Intensive management near conservation area boundaries maintained separation between bison and cattle, with no transmission of brucellosis.
  - However, brucellosis prevalence in the bison population was not reduced and the management plan underestimated bison abundance, distribution, and migration, which contributed to larger risk management culls (total >3,000 bison) than anticipated.
  - Culls differentially affected breeding herds and altered gender structure, created reduced female cohorts, and temporarily dampened productivity.
  - This assessment was used to develop adaptive management adjustments to the IBMP in 2008 and similar future assessments will be essential for effective management to

conserve the largest free-ranging population of this iconic native species, while reducing brucellosis transmission risk to cattle.

7. Document bison use of risk management zones outside the northern and western boundaries of Yellowstone and commingling with livestock during the likely brucellosis-induced abortion period for bison each spring.

- Bison migrated outside the northern and the western boundaries of Yellowstone National Park during November 2010 to May 2011. Bison use of cattle-occupied pastures was observed numerous times in the northern management area, but not in the western area. Bison often crossed the Yellowstone River from the vicinity of Stephens Creek and entered a pasture (Stermitz property) where it was unfenced along the river. Groups ranging from 1 to 39 bison were observed in this pasture on at least 10 occasions, with some mixed-gender groups of bison mingling with cattle during the brucellosis-induced abortion period (primarily from February to mid-May; Jones et al. 2010). However, no bison parturition events were observed or reported. A few male bison were also observed in the Rate/Thomas pastures (located approximately 12 miles north of Stephens Creek) on less than five occasions. However, no commingling with cattle occurred because cattle were kept in a different pasture.

Northern Management Area Chronology

By late December 2010, about 35 to 50 bison had accumulated in the Gardiner basin (Table 1) and about half of these bison were consistently moving north into the Zone 2 area along the Yellowstone River. At this time, additional bison were moving into the Gardiner basin every few days.

Sixty-eight bison were captured at the Stephens Creek facility during January 4-8 to select a group of 25 bison testing negative for brucellosis for a study of bison habitat use north of Yellowstone National Park. During this time, hazing operations pushed other small groups of bulls gathered along the northern park boundary south towards Gardiner, Montana (within the park). When the 25 test-negative bison were released north of the park on January 19, hazing operations continued to keep untested bison from moving north to interact with the test group. By January 28, all but one of the 25 animals in the study group had returned to the park on their own or through capture and return to the park. One bison stayed north near Cutler Lake until May, when it was returned to the park during the end-of-winter hazing operations.

By January 31, about 320 bison had accumulated in the Gardiner basin and the NPS was capturing of groups that routinely moved north of Stephens Creek into the risk management zone. A flight on February 14 documented less than 50 bison in the Gardiner basin outside the Stephens Creek facility. The agencies managed bison distribution by hazing groups of up to 42 animals from private lands north of Reese Creek to the Stephens Creek area within the park. Few bison were migrating into the Gardiner basin during the latter half of February.

On March 23, a group of 6 male bison were prevented from moving north through Yankee Jim Canyon and were hazed back to Cutler Meadow where they were observed daily for the remainder of the month. Groups of female bison moving north of Reese Creek became common after March 25 and hazing operations were conducted on most days. On March 29, more than 50 bison were observed north of Corwin Springs and more than 100 bison were observed north of

Reese Creek. The primary goal of hazing at this time was to keep groups with adult females from mingling with cattle.

During late March and early April, about 550 additional bison migrated into Gardiner basin, resulting in more than 300 bison north of the park boundary during April 7-19. On April 11, a thorough ground count detected 300 bison north of Reese Creek and another 450 bison south of Reese Creek. Hazing operations were conducted 4-6 days per week from April 8 through the end of the month. State personnel conducted operations to move bison south to the park, while park staff conducted operations to move bison south away from the Reese Creek boundary and, on occasion, south of the town of Gardiner to make space for other bison being brought back from north of the park boundary.

A major effort was conducted on April 19-20 to move a large number of bison near Yankee Jim Canyon south and to redistribute bison more to the south end of the Gardiner basin. This effort was successful and on April 21 only 28 bison were observed north of Reese Creek. Thereafter, the number of bison north of Reese Creek was generally less than 30 as hazing operations gathered groups of bison that arrived at Reese Creek and turned them south and back into the park.

During the third week in April, the sizes of bison groups hazed in the vicinity of the park boundary decreased from over 200 to less than 50 as many bison moved south and east out of the basin to points upstream along the Yellowstone River. On April 27 and 29, about 100 bison were moved south towards Gardiner (within the park). Due to persistent snow fall throughout April, some bison would drift back to the north following hazing operations and be gathered up in subsequent events.

Table 1. Number of bison observed during aerial counts conducted over the northern management area during November 2010 to May 2011.

	Nov 1	Dec 21	Jan 26	Feb 14	Mar 23	Apr 23	May 15
Zone 1	0	27	114	7	235	318	236
Zone 2	0	0	4	26	34	30	0
Eagle Creek	0	11	57	13	10	53	15
Hellroaring Creek	24	330	67	20	6	230	41
Blacktail Deer Plateau	172	1,253	1,230	1,125	830	300	536
Swan Lake	11	no data	53	29	40	4	5
Total	207	1,621	1,525	1,220	1,155	935	833
Number held in Stephens Creek facility	0	0	62	524	632	664	386
Number in the Gardiner basin	0	38	237	570	911	1,065	637

Hazing operations during April reduced the number of bison in the Gardiner Basin from 750 on April 11 to 470 on April 19 and 250 on April 21. During the last week of April, the majority of bison in the Gardiner basin were located at the eastern (upstream) end, setting the stage for systematic release of bison that were held in the two fenced facilities (Stephens Creek and Corwin Springs).

Ground counts of bison in the Gardiner basin during the first week of May indicated a decrease in bison abundance from 270 to less than 50 on May 6. As a result of the general southward and eastward movements of bison, management operations transitioned from hazing to releasing bison from the Stevens Creek and Corwin Springs facilities during May 7 to 25.

Western Management Area Chronology

Bison occupied the western risk management zone outside Yellowstone National Park for most of the autumn, winter, and spring months (Table 2). There were 34 inches of snow at the West Yellowstone Snotel site on May 1, compared to zero inches during other recent years. This Snotel site registered zero inches of snow on May 16.

Bison operations began on May 1 when MDOL located 25 bison in Zone 3/Red Canyon/Highway 287 area. From May 1 to May 11, six separate hazing operations were conduced along Highway 287. A mixed group of 25 adult female and calf bison often occupied the road corridor because the right-of-way was clear of trees and presented the first grazing opportunities of the season. Areas outside the road corridor were snow covered with limited grazing opportunities.

On May 16, eight bison were hazed from the Denny Creek Road/Zone 3 to the old burn on the Madison Arm Road/Zone 2. On May 23, more than 20 bison were observed in Zone 3/Denny Creek Road during an aerial survey by MDOL. These bison remained in Zone 3 until May 25 when a mixed group of 38 adult female and calf bison were hazed from the Denny Creek Road/Zone 3 to Barns Hole/Zone 2. On May 18, eight bull bison (1 bull from Zone 3) were hazed east along Highway 287 to an area south of the highway that had limited grazing opportunities.

There were 10 separate breaches of Zone 3 by a total of 169 bison. A total of about 99 bison reached the Highway 287/Zone 3 area on seven occasions (May 1, 2, 4, 5, 9, 11, and 18). A total of about 70 bison reached the Denny Creek Road/Zone 3 area on 3 occasions (May 16, 25, and June 1).

June 1 was established as the date to begin moving all bison back into Yellowstone National Park. During June 1 through 3, 969 bison were hazed (24 from Zone 3, 353 from Zone 2, 592 from Zone 1). Mixed bison groups with adult females and calves were easier to haze on June 1 than during spring 2010. Hazing large groups of bison was more successful with assistance from the MDOL contract helicopter.

A second operation was conducted on June 8 and 9, during which 92 bison were hazed from Zone 2. A third operation was conducted on June 14 and 128 bison were hazed (98 from Zone 2, 30 from Zone 1). A fourth operation was conducted June 22 and 23, during which 192 bison were hazed (77 from Zone 2, 115 from Zone 1). On June 29, seven bulls were hazed from north of Duck Creek to Cougar Meadows.

Interagency operations to move bison back into the park during June were successful because approximately 75% of the bison using Zone 2 at the beginning of the operations remained in the park following the first three days of operations.

Cattle arrived in the Hebgen basin on or about June 17. Bison did not commingle with cattle in the Hebgen basin during winter or spring.

Table 2. Number of bison observed during aerial counts conducted over the western management area during November 2010 to May 2011.

	Number of Bison Observed					
	Nov 1	Jan 26	Feb 14	Mar 23	Apr 23	May 22
IBMP Zone 1	8	215	15	62	109	116
IBMP Zone 2	1	36 [1]	44	63	114	319
West of Madison Junction to 7-mile bridge	28	41	26	30	14	33
Total	37	292	85	155	237	468

[1] Approximately 50 bison were harvested by state and tribal hunters (November to January).

Table 3. Number of bison hazed from the Hebgen basin, Montana back into Yellowstone National Park along the west boundary during June 2011.

Date	1	2	3	8	9	14	22	23	29
South Fork	160						71		
Horse Butte	93		45	74		98			
North of Grayling Arm		79			18			6	7
Number hazed each week	377			92		98	77		7

8. Estimate the effects of hazing or temporarily holding bison in capture pens at the boundary of Yellowstone (for spring release back into the park) on subsequent bison movements or possible habituation to feeding.

- Forty bison were captured during winter 2008 at the Stephens Creek capture facility and fitted with radio transmitters prior to their release in spring. The winter movements of these bison (minus mortalities) were monitored during winters 2009 through 2011 to evaluate if the capture and feeding of bison appeared to be influencing future migration tendencies towards the park boundary (Table 4). Results during these winters with snow packs ranging from modest (2010) to severe (2011) snow pack conditions suggest few bison are habituated to hay provided at the Stephens Creek capture facility and most bison do not migrate to lower elevations to seek forage until deep snow accumulates at higher elevations.

Table 4. Winter movements of radio-marked bison following release from the Stephens Creek capture facility in spring of 2008.

	Winter 2009	Winter 2010	Winter 2011
Percent of marked bison returning to the Gardiner basin	13 of 38 = 34%	3 of 37 = 8%	28 of 33 = 85%
Percent of marked bison returning to the Blacktail Deer Plateau, but not migrating as far as the Gardiner basin	14 of 38 = 37%	11 of 37 = 30%	3 of 33 = 9 %
Percent of marked bison that remained on interior ranges of the park	8 of 38 = 21%	19/37 = 51%	1 of 33 = 3%
Percent of marked bison that migrated to the west boundary of the park	3 of 38 = 8%	4/37 = 11%	1 of 33 = 3%

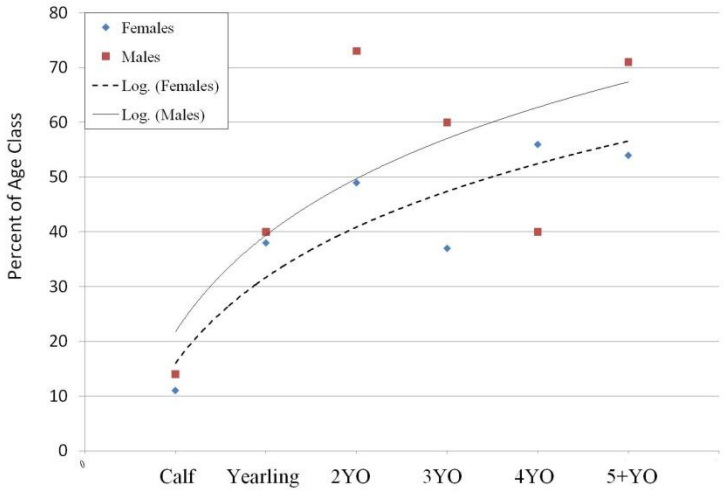
Brucellosis Suppression (Reduce Disease Prevalence)

9. Determine the strength and duration of the immune response in bison following parenteral (e.g. syringe delivery) vaccination for brucellosis.

- NPS staff conducted brucellosis tests (FPA) on 692 bison captured at the Stephens Creek facility during January to May of 2011. Males tended to have higher seropositive rates than females, and young bison had significantly lower seropositive rates than older individuals. Seropositive rates in older age classes tended to asymptote (level off) and not change significantly with increasing age (Treanor et al. 2011). The NPS vaccinated a total of 153 bison in 2011, including 76 calf and 61 yearling females, one seronegative non-pregnant 2-year-old female, and 15 calf and yearling males

Brucellosis test results (FPA) from bison captured at the Stephens Creek facility in Yellowstone National Park during January to May 2011.

	Females	**Males**
Total tested = 692	**479**	**213**
Seropositive rates		
Calves	**12 of 105 = 11%**	**10 of 71 = 14%**
Yearlings	**44 of 115 = 38%**	**28 of 69 = 40%**
2 year-olds	**28 of 57 = 49%**	**28 of38 = 73**
3 year-olds	**10 of 27 = 37%**	**12 of 20 = 60%**
4 year-olds	**5 of 9 = 56%**	**2 of 5 = 40%**
5+ year-olds	**89 of 165 = 54%**	**5 of 7 = 71%**
Vaccinates = 153	**138**	**15**



Age-specific seropositive rates for male and female bison based on serology results from blood tests (FPA) collected at the Stephens Creek facility in Yellowstone National Park during January to May 2011.

- NPS staff are collaborating colleagues from the Animal and Plant Health Inspection Service and Montana State University to measure the cell-mediated immune responses (CMI) induced by SRB51 vaccination in bison (J. Treanor, unpublished data).
  - During winter 2008-09, 12 yearling bison in the quarantine feasibility study were parenterally vaccinated with SRB51. Immune responses were assessed prior to vaccination and at 3, 8, 12, 18, and 21 weeks after vaccination.
  - Additionally, 20 wild, yearling, female bison were captured at the Stephens Creek facility during late winter 2008 and 14 of these bison were parenterally vaccinated with SRB51, while six served as non-vaccinated controls. The CMI response of the vaccinated bison was analyzed at 2 and 6 weeks post vaccination. Thereafter, all 20 bison were released back into the wild during May 2008. During autumn and winter 2008-2009, 14 of the 20 bison in the study were recaptured to measure cell-mediated immune responses 24+ weeks following vaccination.
  - Both study groups showed strong initial CMI responses (IFN-γ (Ng/Ml) at 2-8 weeks post-vaccination). CMI responses were significantly different from pre-vaccination levels for both study groups at nearly all post-vaccination time points. However, post vaccination CMI responses were more variable between individuals in the free-ranging bison compared to quarantined animals.

- Through the Civilian Research and Development Foundation, the NPS provided cooperative funding to key Russian vaccinologists to develop the first comprehensive review of scientific laboratory and field studies on the primary Russian brucellosis vaccine derived from *B. abortus* strain 82 (SR82), and publish this report in an English language peer-reviewed scientific journal (Olsen et al. 2010, Ivanov et al. 2011).
  - The smooth-rough strain SR82 vaccine combines the desired weak responses on standard tests with high efficacy against brucellosis.
  - In 1974, prior to widespread use of strain SR82 vaccine, 5,300+ cattle herds were known to be infected with *B. abortus* across the former Soviet Union.
  - By January 2008, only 68 cattle herds in 18 regions were known to be infected, and strain SR82 continues to be the most widely and successfully used vaccine in many regions of the Russian Federation.

10. Determine the strength and duration of immune response in bison following remote delivery (e.g. bio-bullet) vaccination for brucellosis.

- Olsen et al. (2006a) reported the ballistic inoculation of bison with biobullets containing photopolymerized, polyethylene glycol-based hydrogels with SRB51 induced a significant cell-mediated immune response similar to syringe injection of the vaccine. However, the immunologic responses of bison to hydrogel vaccination with SRB51 during 2007 indicated poor proliferation and interferon response compared to parenteral injection (S. Olsen, unpublished data). These findings suggest the measured immune responses to vaccination are variable or there may be consistency issues with vaccine hydrogel formulation and/or encapsulation in biobullets.

- During 2003-2005, NPS staff collaborated with the University of Utah and the Agricultural Research Service to develop procedures for vaccine encapsulation and maintaining the structural consistency of projectiles. This effort demonstrated successful proof-of-concept for delivering a degradable ballistic brucellosis live vaccine remotely to bison from a distance of 40 meters using commercial components and a novel hydrogel vaccine carrier (Christie et al. 2006).

- A second vaccination trial on bison conducted using a photopolymerized SRB51 vaccine payload manufactured at the Agricultural Research Service lab in Ames, Iowa during 2007 indicated poor immunologic proliferation and interferon response compared to syringe injection (S. Olsen, Agricultural Research Service, unpublished data). Results also demonstrated biobullet failure with projectiles fracturing or being too soft to penetrate the skin of vaccinates. These inconsistencies between studies regarding the cell-mediated immune responses observed following hydrogel vaccination of bison with SRB51 may have been due to differences in the photopolymerization process used to encapsulate vaccine in projectiles.

- NPS staff collaborated with the University of Utah and the Agricultural Research Service to develop a protocol for pursuing minor enhancements to the vaccine payload performance and the ballistic delivery system under quality controlled production prior to field test on bison. It will also involve (1) negotiating supply agreements with various reagent vendors, (2) developing scientific and technical protocols to facilitate technology transfer to a contractor who can procure and produce the entire vaccine component line, (3) initiation and supervision of a production program for biobullet vaccine formulations under quality systems validation, and (4) final delivery of ready-to-use biobullet vaccine formulations and protocols for field use (Grainger 2011).

11. Document long-term trends in the prevalence of brucellosis in bison, and the underpinning effects of remote and/or parenteral vaccination, other risk management actions (e.g., harvest, culling), and prevailing ecological conditions (e.g. winter-kill, predation) on these trends.

- NPS staff collaborated with colleagues at the U.S. Geological Survey and Montana State University to estimate how much time (years) it takes to detect a change in seroprevalence in bison over time using three analytical approaches: the single year estimate, the 3-year running average, and regression using all years to date (Ebinger and Cross 2008).
    - Capture and sampling of more than 200 bison during a given year would be necessary to detect significant changes in seroprevalence following vaccination, and detection would likely take 5-20 years depending on sample sizes and detection method.
    - The ability to detect a change in seroprevalence is a function of the (1) amount of decrease in seroprevalence, (2) shape of the seroprevalence decrease curve, and (3) the sample sizes used for estimating seroprevalence. The ranges of possibility for the amount of decrease in seroprevalence and for the shape of the decrease curve are relatively unknown.

- The single-year estimate approach consistently showed more variation around the median. The regression model tended to be a more powerful approach, though there was more variation around this estimate for the gentler decreases in prevalence.
- The change in research captures had surprisingly little effect on the year of first detection. The major contribution of increased research captures was in reducing the variation associated with the year of first detection.
- The probability of detecting a difference between the baseline and some future point in time increases as you increase the number of individuals periodically tested. An annual testing increment of fewer than 200 individuals provides a poor probability of detecting a decrease in seroprevalence to below 40%. In addition, sampling at much greater numbers than 250 individuals does not significantly improve the probability of precision in detecting a change in seroprevalence.

- NPS staff collaborated with colleagues at the University of Kentucky to develop an individual-based model to evaluate how brucellosis infection might respond under alternate vaccination strategies, including: 1) vaccination of female calves and yearlings captured at the park boundary when bison move outside the primary conservation area; 2) combining boundary vaccination with the remote delivery of vaccine to female calves and yearlings distributed throughout the park; and 3) vaccinating all female bison (including adults) during boundary capture and throughout the park using remote delivery of vaccine.
  - Simulations suggested Alternative 3 would be most effective, with brucellosis seroprevalence decreasing by 66% (from 0.47 to 0.16) over a 30-year period resulting from 29% of the population receiving protection through vaccination.
  - Under this alternative, bison would receive multiple vaccinations that extend the duration of vaccine protection and defend against recurring infection in latently infected animals.
  - The initial decrease in population seroprevalence will likely be slow due to high initial seroprevalence (40–60%), long-lived antibodies, and the culling of some vaccinated bison that were subsequently exposed to field strain *Brucella* and reacted positively on serologic tests.
  - Vaccination is unlikely to eradicate *B. abortus* from Yellowstone bison, but could be an effective tool for reducing the level of infection.

- NPS staff collaborated with colleagues at the U.S. Geological Survey and Montana State University to use an individually-based epidemiological model to assess the relative efficacies of three management interventions (sterilization, vaccination, and test-and-remove; Ebinger et al. 2011).
  - Sterilization and test-and-remove were most successful at reducing seroprevalence when they were targeted at young seropositive animals, which are the most likely age and sex category to be infectious. Sterilization and test-and-remove, however, also required the most effort to implement. Vaccination was less effective, but also required less effort to implement.
  - For the treatment efforts we explored (50-100 females per year), sterilization had little impact on the bison population growth rate when selectively applied and the

population growth rate usually increased by year 25 due to the reduced number of disease-induced abortions.
  - Initial declines in seroprevalence followed by rapid increases occurred in 3-13% of simulations with sterilization and test-and-remove, but not vaccination. We believe this is due to the interaction of super-spreading events and the loss of herd immunity in the later stages of control efforts.
  - Vaccination reduces seroprevalence while maintaining herd-immunity and minimizing the occurrence of super-spreading events. Sterilization and test-and-remove reduce herd-immunity and super-spreading events become more common as the population becomes more susceptible.
  - Sterilization provided a mechanism for achieving large disease reductions while simultaneously limiting population growth, which may be advantageous in some management scenarios. However, the field effort required to find the small segment of the population that is infectious rather than susceptible or recovered will likely limit the utility of this approach in many free-ranging wildlife populations.

- NPS staff prepared a Draft Environmental Impact Statement to decide whether or not to proceed with implementation of remote delivery vaccination of bison in the park. Three alternatives were included in the document (USDI 2010):
  - The no action alternative describes the current vaccination program that is intermittently implemented at the Stephens Creek capture facility in concert with capture operations. The second alternative would include a combination of the capture program at Stephens Creek and a remote delivery vaccination strategy that would focus exclusively on young, non-pregnant bison of both sexes. Remote delivery vaccination could occur from March to June and mid-September to mid-January through many areas of bison distribution in the park. A third alternative would include all components of the second alternative, as well as the remote vaccination of adult females during autumn. The vaccination program is intended to lower the percentage of bison susceptible to brucellosis infection.
  - The Notice of Availability for the Draft Environmental Impact Statement was published in the Federal Register (75 FR 27579) on May 17, 2010. The comment period was from May 28, 2010 to September 24, 2010. Also, NPS staff conducted three public meetings to gain information from the public on the park's purpose and significance, issues, and alternatives presented in the Draft Environmental Impact Statement. These meetings were held in Bozeman, Montana on June 14, 2010, Helena, Montana on June 15, 2010, and Malta, Montana on June 16, 2010.
  - The NPS received a total of 1,644 correspondences via letters, electronic mail (email), faxes, comments from public meetings, park forms, and web forms. These correspondences were distilled into 9,410 individual comments. From this correspondence, the NPS in collaboration with Weston Solutions, Inc. identified 6,629 substantive comments, which were divided into 26 concern statements.
  - Most respondents associated with conservation constituencies opposed the remote vaccination program and recommended vaccination of cattle rather than bison. Conversely, most respondents associated with livestock groups supported vaccination. Nearly all respondents suggested that the projected cost of park-wide remote vaccination was too expensive to justify the benefits. A few constituency groups initiated letter writing campaigns to suggest re-directing funding to purchase

grazing opportunities from private landowners outside Yellowstone National Park. Many respondents disputed the scientific information presented in the draft Environmental Impact Statement or suggested that inadequate scientific information existed to justify a decision to implement remote vaccination.

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