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THE BIOLOGY AND MANAGEMENT OF BRUCELLOSIS IN YELLOWSTONE BISON

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THE BIOLOGY AND MANAGEMENT OF BRUCELLOSIS IN YELLOWSTONE
BISON

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

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Lexington, Kentucky

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ABSTRACT OF DISSERTATION

THE BIOLOGY AND MANAGEMENT OF BRUCELLOSIS IN YELLOWSTONE BISON

Disease management along the boundaries of wildlife reserves is a growing conservation problem worldwide, as infected wildlife can migrate outside protected areas and pose a threat to livestock and human health. The bison *Bison bison* population in Yellowstone National Park has long been infected with *Brucella abortus*, the bacterium causing bovine brucellosis. Concern over migratory bison transmitting *B. abortus* to cattle herds on lands adjacent to Yellowstone has led to proposals for bison vaccination. Model simulations suggest that vaccination is unlikely to eradicate *B. abortus* from Yellowstone bison but could be an effective tool for reducing the level of infection and eliminating unpopular management practices such as lethal culling.

The culling of Yellowstone bison to reduce the risk of brucellosis transmission to cattle is negatively affecting long-term bison conservation because of difficulties in diagnosing actively infected animals. Age-specific serology and *B. abortus* culture assays from slaughtered bison were used to develop a diagnostic tool to estimate whether particular animals are infective. Findings suggest that active *B. abortus* infection is age-dependent, which allows true infection probabilities to be estimated based on age and quantitative diagnostic tests.

Active brucellosis infection was associated with below-average nutritional condition, with the intensity of *B. abortus* infection being influenced by seasonal reductions in dietary protein and energy. The reproductive strategy of Yellowstone bison is linked with the seasonal availability of food, which increases bison fitness but may have consequences for *B. abortus* infection. Seasonal food restriction may also influence the ability of vaccinated bison to recall protective immune responses when later exposed to *B. abortus*. The rate of fat metabolism was an important factor influencing cell-mediated responses. Thus, individual variation and the seasonal availability of food may reduce vaccine efficacy when vaccination is applied at the population level. Consequently, effective management practices will require a diverse range of integrated methods, which include maintaining separation of livestock and wildlife, managing habitat to reduce

brucellosis transmission, and reducing disease prevalence in wildlife. The long-term success of these management practices will depend on sound science and support of the stakeholders involved.

KEYWORDS: Bison, Brucellosis, Nutritional Condition, Persistent Pathogens, Yellowstone National Park

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I dedicate this dissertation to my co-advisor and friend Dr. David S. Maehr, who died unexpectedly on June 20, 2008.

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Chapter 1 – Introduction

In recent years, we have witnessed a global rise in emerging and re-emerging infectious diseases, which are increasingly being shared between humans, livestock, and wildlife (Daszak *et al.* 2000). The rapid development of land by humans has reduced the amount of habitat available for wildlife (Cleaveland, Laurenson & Taylor 2001). Much of the wildlife habitat that does remain is often fragmented or found within wildlife reserves, such as national parks. Human development along the boundaries of these preserves has also increased the proximity of humans, domestic animals, and wildlife. Consequently, the risk of infectious disease spread from wildlife to livestock and humans is a legitimate concern that is challenging wildlife conservation along the boundaries of protected areas (Newmark *et al.* 2008). Because wildlife and their diseases do not recognize management or political boundaries, conservation efforts become complicated when risks to human health arise. This has long been the case with brucellosis management in the greater Yellowstone ecosystem.

Brucellosis in Yellowstone bison is a contagious disease caused by the bacterium *Brucella abortus* that can induce abortions or the birth of non-viable calves in livestock and wildlife (Rhyan *et al.* 2009). The bacterium is believed to have been introduced by European livestock to Yellowstone bison (*Bison bison*) and elk (*Cervus elaphus*) before 1930 (Meagher & Meyer 1994). In wildlife and cattle, infection typically occurs through contact with infectious reproductive tissues shed during an abortion or live birth (Thorne 2001). Though rare in the United States, human brucellosis can occur if bacteria are

ingested or enter through the eyes or open wounds. *Brucella abortus* infection is rarely fatal in humans, with human-to-human transmission being insignificant (Godfroid *et al.* 2005). However, if not treated early, human brucellosis can cause recurring, severe, fever-like symptoms (Vassalos *et al.* 2009).

To minimize effects to humans, a nationwide program to eradicate brucellosis from cattle has been in place since 1934. The program has successfully eliminated *B. abortus* from most of the United States with the exception of free-ranging wildlife within the greater Yellowstone ecosystem. Over the past decade, all three states bordering Yellowstone National Park (Idaho, Montana, and Wyoming) have experienced multiple brucellosis outbreaks in cattle herds as a result of contact with infected wildlife, which has caused additional economic expenses for the state livestock industries. Thus, concerns over the risk of brucellosis transmission to cattle have led to decades of conflict regarding management of bison and elk in the greater Yellowstone area. Traditionally, brucellosis management has focused on elk in the southern greater Yellowstone area and bison in the northern portion. This management strategy has been supported by most livestock and natural resources personnel who view supplementally fed Wyoming elk and migrating Yellowstone bison as the primary sources for brucellosis transmission to cattle (Bienen & Tabor 2006). Elk, which are tolerated on state lands, often mingle with cattle. In contrast, resource agencies prevent bison from mingling with cattle through active management practices

The risk of brucellosis transmission to livestock or wildlife is influenced by the amount of infectious material shed onto the landscape and its ability to persist long enough to establish infection when contacted. Environmental factors that increase stress

and concentrate animals, such as deep snow and the presence of predators, may increase the likelihood of exposure to shed infectious tissues. However, expelled tissues are sometimes quickly removed by scavengers (Aune *et al.* 2012). Additionally, the behavior of elk and bison during calving may limit the transmission risk to cattle. Yellowstone bison exhibit synchronous calving, with 80 % of births occurring from late April to late May (Jones *et al.* 2010). Birthing females often consume shed birth tissues within two hours after calving. This behavior reduces the risk of brucellosis transmission to cattle and bison that later encounter the birth site. However, the potential for exposure is higher for bison that are more closely associated with a pregnant animal that has shed infectious tissues (Treanor *et al.* 2010; Treanor *et al.* 2011). During 2004 to 2007, at least one bison was observed making contact with potentially infectious birth tissues in 30 percent of observed bison births (Jones, Treanor & Wallen 2009). Thus, brucellosis transmission requires a source of infection and relies on the behaviors of potential hosts (Cheville, McCullough & Paulson 1998).

The role of elk in the maintenance of brucellosis in the northern portion of the greater Yellowstone ecosystem has traditionally been viewed as less important than that of bison. Unlike most bison, however, female elk segregate themselves from other herd members while giving birth (Johnson 1951). Elk birth sites are dispersed and well cleaned, with the likelihood of other elk encountering infectious birth tissues being low. But transmission risk may be higher during late winter and early spring when elk form large aggregations on low-elevation winter ranges (Hamlin & Cunningham 2008). Brucella-induced abortions under these conditions could expose many susceptible elk to infectious material.

The high seroprevalence (40-60 %) of brucellosis in Yellowstone bison implies they are a likely infection source for Yellowstone elk. However, recent data suggest that transmission between bison and elk is rare (Proffitt *et al.* 2010b). The peak bison calving period, when most the bacteria are expected to be shed, occurs approximately one month earlier for bison than for elk, with little overlap in distribution during this time period. On wintering ranges where elk do mingle with bison, such as the Madison headwaters area in Yellowstone, elk have much lower seroprevalence rates for brucellosis (3%) than do Yellowstone bison or elk associated with feeding programs in Wyoming. Proffitt *et al.* (2010) found that brucellosis transmission risk from bison to elk was low in Yellowstone's Madison headwaters area, despite a high degree of spatial overlap when *B. abortus* is typically shed. Predation risk associated with wolves increased elk and bison spatial overlap temporarily, but these behavioral responses by elk did not have important disease implications. Also, DNA analyses indicate that *B. abortus* sampled from bison and elk is quite different, which suggests that this bacterium is not extensively exchanged between these species (Beja-Pereira *et al.* 2009; Higgins *et al.* 2012). It appears that brucellosis in the greater Yellowstone area is a disease sustained by multiple hosts, and managing the risk of transmission to cattle will require control measures addressing bison, elk, and the factors sustaining infection.

Management of Yellowstone bison and the brucellosis transmission risk they pose to cattle outside the park has a long, contentious history between wildlife managers, livestock producers, and the concerned public. After intensively managing bison numbers for 60 years through husbandry and consistent culling, the National Park Service instituted a moratorium on culling in the park in 1969, which allowed bison numbers to

fluctuate in response to environmental and ecological factors (Cole 1971). Bison abundance increased rapidly, with large winter migrations out of the park beginning in the late 1980s (Meagher 1989a,b). These migrations led to a series of conflicts with stock growers and the state of Montana, largely because of the risk of brucellosis transmission to cattle. As a result, in 2000, the federal government and the state of Montana agreed to a court-mediated Interagency Bison Management Plan (IBMP) that established guidelines for (1) cooperatively managing the risk of brucellosis transmission from bison to cattle and (2) preserving the bison population and allowing some bison to occupy winter ranges on Montana's public lands. The IBMP uses intensive management, such as hazing and hunting of bison migrating outside the park, to maintain separation between bison and cattle. In general, the agencies have successfully maintained spatial and temporal separation between bison and cattle with no transmission of brucellosis (White *et al.* 2011). However, this intensive management is expensive, logistically difficult, and controversial.

The enduring debate over Yellowstone bison management has largely concentrated on the culling of animals that roam outside park boundaries during the winter. In recent decades, large numbers of bison migrating into Montana have been captured by federal and state agencies, when separation between bison and cattle could not be maintained. Many of these bison were tested for brucellosis, with animals testing positive for exposure being shipped to domestic slaughter facilities. Approximately 3,200 bison were shipped to slaughter during 2001 through 2011, to mitigate the risk of brucellosis transmission to cattle (White *et al.* 2011). Despite these actions, brucellosis prevalence in Yellowstone bison has not decreased (Hobbs *et al.* 2009). Intensifying

bison removals to a level that may be effective at reducing brucellosis infection within the herd would be extremely expensive, unacceptable to the public, and counter to the National Park Service policy to maintain ecosystem integrity (Bienen & Tabor 2006).

In this dissertation, I focus on advancing our understanding of brucellosis in Yellowstone bison for the purpose of reducing the level of infection while promoting long-term conservation of this important wildlife resource. In chapter 2, I provide a review of intracellular pathogens known to establish long-term infections and cause abortions in mammalian hosts. Persistence by these disease agents relies on periods of host immune suppression during pregnancy. This review highlights the common strategy used by these pathogens and provides a broad perspective of how these disease agents may be sustained in wild ungulate populations. In chapter 3, I present an individual-based model to evaluate how different vaccination strategies might reduce the level of brucellosis infection in Yellowstone bison. Model simulations suggest that eradication of brucellosis is unlikely with the currently available vaccine and delivery options. However, reducing the level of infection may be achievable but would require a long-term investment.

Chapter 4 addresses the association of brucellosis infection with age in Yellowstone bison. Bison age was found to be an important predictor of active brucellosis infection, with infection increasing in juvenile ages and peaking at sexual maturity. A management tool was developed to estimate the probability of active infection in live bison. This tool, when used in conjunction with bison vaccination, could provide managers with an effective way to reduce brucellosis infection without culling practices that negatively affect bison conservation. In chapter 5, I assess how the

endemicity of brucellosis infection might be influenced by the timing of food restriction with increasing reproductive demands. Seasonal food restriction was found to reduce nutritional condition during late gestation, with the probability of active brucellosis infection being highest for bison in below average condition. This suggests that nutrition may play an important role in the maintenance of brucellosis in Yellowstone bison.

In chapter 6, I compare the immunologic responses to vaccination in captive and free-ranging bison. Immune responses were similar between both study groups, though a single vaccination may offer protection in approximately half of vaccinated yearling female bison. A comparison of bison immune responses with nutritional indicators suggests that bison nutrition during the *B. abortus* transmission period may influence protective immune responses following vaccination. Additionally, vaccinated wild bison released back into the park demonstrated incomplete protection in animals naturally exposed to *B. abortus*. Finally, conclusions and future directions are presented in chapter 7. Here I emphasize future research needs to further understand how brucellosis is maintained in Yellowstone bison and improve disease management practices.

Chapter 2 - Persistent pathogens of wild ungulates: the association of intracellular parasites and immunocompromised hosts in a seasonal environment

Introduction

Pathogen persistence, the ability to survive and multiply within hosts, is an important life history trait influencing the fitness of parasites (Frank 1996; Perlman 2009). The growth of pathogens within their hosts increases the likelihood of transmission, but pathogen growth can negatively affect host survival and thereby decrease transmissibility to new hosts. This trade-off between higher virulence associated with pathogen growth and the increase in host mortality caused by infection has been a focal subject in parasite biology (Anderson & May 1979; Alizon *et al* 2009). Since death of the host reduces transmissibility, pathogens with the highest fitness have been identified as those with an intermediate level of virulence, which allows them to maximize the duration of infection and rate of transmission while keeping their host alive (Mackinnon, Gandon & Read 2008). However, increasing the duration of infection depends on the pathogen's ability to evade protective immune responses of the host.

The primary function of the immune system is to defend against pathogen invasion by generating a protective response against the invader with an accelerated immune response following re-exposure to the same pathogen (Ahmed, Lanier & Pamer 2002). Many disease organisms, especially those directly transmitted between hosts (e.g. viruses of upper respiratory tract), replicate quickly to increase transmission potential before an adaptive immune response is developed by the host (Janeway *et al.* 2005).

However, some pathogens are capable of maintaining infections in mammalian hosts in the presence of a robust adaptive immune response, thereby establishing persistent infections (Monack, Mueller & Falkow 2004). Many of these persistent pathogens are microparasites (i.e. viruses, bacteria, and protozoa), which infect a diversity of host species and have been notoriously difficult to eradicate from humans, domestic animals, and infected wildlife (Godfroid *et al.* 2005; Ruiz-Fons *et al.* 2008; Cross *et al.* 2009).

Microparasites only require a single host species to complete their development and can generally persist in a wide range of environmental conditions; for this reason, microparasites are responsible for the majority of wildlife epidemics (Dobson and Foufopoulos 2001). These pathogens are also the subject of intensive study because of their frequently severe clinical effects in people and the complexities associated with treatment and control (Polley 2005). Microparasites can be classified as extracellular or intracellular based on where they replicate within their hosts; extracellular pathogens are controlled by humoral immune responses and intracellular pathogens are controlled primarily by cell-mediated responses (Bogdan 2008). Intracellular microparasites can replicate within a diversity of host cells and include all viruses, many bacteria (e.g. *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Brucella spp.*, *Salmonella spp.*, *Shigella spp.*, *Coxiella burnetii*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*), certain protozoa (e.g. *Leishmania spp.*, *Toxoplasma gondii*, *Trypanosoma cruzi*) and fungi (e.g. *Histoplasma capsulatum*). Intracellular parasitism is an effective strategy that allows pathogens to persist within their hosts by hiding from host immune defenses.

Wild ungulates are hosts to several zoonotic intracellular pathogens—that is, pathogens transmissible from animals to humans—which are commonly associated with

livestock and humans (Table 1.1). Since humans are mainly exposed to wildlife pathogens through infected livestock, human tolerance and social behavior make wild ungulates an important host and reservoir for persistent zoonotic diseases (Böhm *et al.* 2008). Supplemental feeding in the southern Greater Yellowstone Ecosystem, for example, is believed to maintain elevated levels of brucellosis among elk (Cross *et al.* 2007), a transmission source for cattle (Bien and Tabor 2006). Deer in northeastern Michigan reach high densities through access to bait and feed piles (Schmitt *et al.* 1997), thus increasing the opportunity for tuberculosis (TB) to spread via infected saliva (Dorn & Mertig 2005). The ability of intracellular pathogens to persist within wildlife hosts frequently leads to endemically infected wildlife populations, which pose an infection risk to humans and their domestic animals (Michel *et al.* 2006; Schumaker, Peck & Kauffman 2012).

Infectious intracellular pathogens causing disease in humans and livestock commonly exploit their hosts during immunocompromised periods, such as pregnancy. Colonization of the reproductive tract during pregnancy often results in abortions that facilitate transmission of the pathogen (Carvalho Neta 2010; Dubey & Schares 2011). In wild ungulates, these abortive disease agents may influence population dynamics and compromise the conservation of threatened populations (Joly & Messier 2005; Pioz *et al.* 2008). Effective tools and strategies for managing or eradicating persistent diseases in wildlife are largely unavailable because we have only a rudimentary understanding of disease dynamics in the wildlife populations. In contrast to domestic livestock, wild ungulates experience seasonal food restriction coinciding with periods of high nutritional demands for reproduction. Consequently, immune function may be suppressed during

these periods, thereby creating transmission and infection opportunities for persistent pathogens (Figure 1.1).

The focus of this review is to identify how intracellular pathogen maintenance strategies are linked to seasonal and life history changes in host immune function. In particular, the mechanisms influencing chronic infection of abortive disease agents in wild ungulates will be evaluated. First, I describe how this group of intracellular parasites is able to evade immune defenses in pregnant hosts. Second, I review the common approach used by these pathogens to establish infection in domestic ruminants and humans. Third, I propose how seasonal factors influencing the nutritional condition of wild ungulates may increase susceptibility to persistent pathogens. Finally, I suggest methods and research needs that may help control persistent diseases of wild ungulates.

Biology of persistence

Pathogens have evolved a variety of strategies to evade host recognition and extend the duration of infection (Schmid-Hempel 2009). The intracellular strategy allows a small number (i.e. low infectious dose) of microparasites to establish infection, persist at low numbers and grow to large numbers when host conditions increase the likelihood of transmission. For these pathogens, acute infection is merely a prelude to a more long-lasting association with the host (Rhen *et al.* 2003). For example, the protozoan *Neospora caninum* persists within its host by using a dormant life stage to establish chronic infection in immunocompetent hosts and then switching to a fast-replicating life stage to multiply in immunocompromised hosts during pregnancy (Eastick & Elisheika 2010). The ability of intracellular bacterial pathogens to modify cellular processes is a common feature that favors persistence and evasion of host immune responses (Monack, Mueller

& Falkow 2004). For example, the Brucellae are intracellular bacteria capable of modulating cellular functions, allowing them to survive within host cells (macrophages), awaiting the opportunity to infect the reproductive tract during pregnancy (Spera *et al.* 2006; Carvalho Neta 2010). The inability of the host to clear or control persistent pathogens can result in reactivation of infection, especially during periods of immune suppression (Bogdan 2008). Thus, chronic infection of wild ungulates populations may result from the effectiveness of these persistence strategies during periods when immune defenses are naturally down-regulated.

In mammals, cell-mediated immune responses, which provide protection from intracellular pathogens, are naturally suppressed during pregnancy (Clemens, Siiteri & Stites 1979; Weinberg 1987). Consequently, the success of many intracellular pathogens is linked to modifications of cell-mediated immune function to protect the developing fetus (Entrican 2002). During a typical infection, antigens from the invading pathogens induce the proliferation of T-cells toward either a T helper cell 1 (Th1) or T helper cell 2 (Th2) immune response. Th1 responses defend against intracellular pathogens, while Th2 responses are more effective against extracellular parasites. Both responses result in the release of cytokines, proteins which coordinate the protective immune response, but the cytokine profiles for Th1 and Th2 responses are antagonistic (Mosmann & Fowell 2002). The production of Th1 cytokines reduces the induction of Th2 cytokines and vice versa. Th1 cytokine profiles can have profound detrimental effects on pregnancy because the developing fetus contains paternal antigens which can be attacked by specific immune cells induced via the maternal Th1 response (Innes *et al.* 2005). These immune cells are capable of inducing an abortion or reabsorption of the fetus (Raghupathy 1997). The

placenta serves as the interface between the mother and developing fetus and induces the production of large amounts of Th2 cytokines during pregnancy. This Th2 profile may be the result of the high levels of the pregnancy hormone progesterone, which promotes Th2 proliferation (Piccinni *et al.* 2000). This inhibits the detrimental effects of Th1 responses on the developing fetus (Miller *et al.* 1996, Miller & Hunt 1998). As a result, the bias toward Th2 cytokine production increases the potential for infection by intracellular pathogens during pregnancy (Quinn, Ellis & Smith 2002).

Immune protection from intracellular pathogens during pregnancy presents a dilemma for the mammalian host. During late gestation, the induction of a strong Th1 cytokine response may compromise the pregnancy while a weak response may compromise the resistance to infection that helps prevent fetal loss. Many intracellular parasites (e.g. *Coxiella burnetii*, *Brucella abortus*, *Toxoplasma gondii*) cause abortions in domestic and wild ruminants (Marreros *et al.* 2011). Since wild ungulates commonly share pastures with livestock, these abortive disease agents may be shared between wildlife and livestock through ingestion of infected feces, urine, or birth tissues. We must expand our understanding of how persistent disease agents are maintained in wildlife to develop and implement control measures that reduce transmission risk to livestock and humans.

Persistent abortive diseases in humans and domestic animals

The protozoan *Toxoplasma gondii* can infect all warm-blooded animals; approximately one-third of the world's population is seropositive for this parasite (Miller *et al.* 2009). The resulting disease, toxoplasmosis, is a major cause of abortion in sheep and goats and causes disease in the developing human fetus or in immunocompromised

individuals (Innes *et al.* 2007). Exposure during pregnancy allows *T. gondii* to infect placental cells and spread to the developing fetus (Buxton & Finlayson 1986). The natural immunomodulation that protects the fetus in pregnant sheep reduces Th1 immune responses (Entrican & Wheelhouse 2006) resulting in vulnerability of the placenta to *T. gondii* infection (Innes *et al.* 2007). Domestic cats (*Felis catus*) consuming infected rodents and birds shed the pathogen in their feces (Dubey & Beattie 1988). Associations have been made between the presence of cats on farms and exposure of sheep to *T. gondii* (Skjerve *et al.* 1998), though infection in sheep has also been linked to contaminated food (Faull, Clarkson & Winter 1986). Infection of pregnant sheep can result in abortion, weak lambs, or clinically normal lambs that may be infected carriers (Buxton 1998).

Bovine neosporosis, caused by the protozoan *Neospora caninum*, is an important, worldwide cause of bovine abortion (Innes *et al.* 2007). Similar to *T. gondii* infection in sheep, cattle may become infected with *N. caninum* through the consumption of contaminated food containing infected dog feces or through vertical transmission from dam to calf during pregnancy (Dubey, Buxton & Wouda 2006). *N. caninum* infection largely results in calves which remain persistently infected and capable of transmitting *N. caninum* to their offspring (Andrianarivo *et al.* 2005). The high rate of vertical transmission resulting in congenitally infected calves suggests a suppression of cell-mediated immune responses during fetal development. This form of transmission is a highly successful strategy, as naturally infected cattle will transmit the parasite over several generations without developing effective immunity (Innes *et al.* 2005). As with *T. gondii*, regulation of Th1 cytokines during pregnancy, may encourage recrudescence of a persistent *Neospora* infection previously kept in check by pro-inflammatory Th-1

immune responses (Innes *et al.* 2007). In dairy cattle naturally infected with *N. caninum*, production of the Th-1 cytokine interferon- γ (IFN- γ) protects against abortion in *Neospora*-infected cows (Lopez-Gatius *et al.* 2007).

Several species of intracellular bacteria have developed effective methods to exploit their hosts during pregnancy. Q fever is a zoonosis with worldwide distribution caused by the obligate intracellular bacterium *Coxiella burnetii* and affects a wide range of domestic and free living mammals, birds, reptiles and fish (Berri *et al.* 2007). Pregnant ruminants are highly susceptible to infection and shed *C. burnetii* into the environment through infected birth tissues discharged at parturition or during an abortion, as well as in urine, milk, and feces (Lang 1990; Woldhiwet 2004). Goats are the most common source of *C. burnetii* infection in humans, which can cause long-term health disorders. (Maurin & Raoult 1999; Hatchette, Hudson & Schezch 2001). In chronically infected goats, multiplication of the pathogen may be reactivated during subsequent pregnancies, with the bacteria excreted in the milk during lactation (Berri *et al.* 2007). The mechanisms influencing recrudescence are not well understood, but immunomodulation during pregnancy may be responsible for persistent infection of the placenta (Polydourou 1981; Ben Amara 2010).

Chlamydiae are obligate intracellular bacteria with a wide host range and cause a variety of diseases. *Chlamydia abortus* infects the placenta of sheep causing abortions and has a major economic impact on agricultural industries worldwide (Kerr *et al.* 2005). Humans are also susceptible to infection, especially pregnant women (Longbottom & Coulter 2003). *C. abortus* is transmitted by ingesting infected birth tissues shed during an abortion or at lambing, inhalation of aerosols from the environment, or transplacentally

from ewe to developing fetus (Buxton *et al.*, 1990, 2002). Lambs born to infected mothers may be latently infected without clinical disease symptoms until their first pregnancy (Wilsmore *et al.* 1990). In sheep, the Th1 cytokine IFN- γ controls the growth of *C. abortus* in the placenta, but a high concentration of IFN- γ can impair survival of the fetus (Brown & Entrican 1996; Kerr *et al.* 2005)

Brucellosis, caused by bacteria in the *Brucella* genus, is a zoonotic disease of concern throughout the world, generating at least a half million new cases annually (Vassalos *et al.* 2009). The Brucellae are intracellular bacteria known to infect a diversity of mammalian hosts. *Brucella abortus*, the bacteria causing bovine brucellosis, is transmitted primarily through the ingestion of infectious tissues (e.g. fetal membranes and uterine discharges) shed following an abortion or at parturition (Samartino & Entright 1993). *B. abortus* appears to exploit the regulatory processes that control immune responsiveness during pregnancy (Kim *et al.* 2005). Replication of *B. abortus* in placental cells is strongly influenced by the stage of gestation, with higher replication rates during late gestation when the cells actively secrete steroid hormones, such as progesterone (Carvalho Neta 2010). As with other intracellular pathogens, immunity to *B. abortus* depends on the activation of white blood cells (macrophages), with the Th-1 cytokine IFN- γ responsible for macrophage activation (Gorvel & Moreno 2002).

Though these bacterial and protozoan parasites use different methods to live within their hosts, they share a common strategy of intracellular persistence followed by within-host multiplication during the immunocompromised state of pregnancy. Modification of cell-mediated immune function protects the developing fetus but increases susceptibility to infection. Persistent pathogens appear to receive endocrine

signals from the host communicating periods of immune suppression. The anti-abortive effects of progesterone favors the production of Th2 cytokines which suppresses protective Th1 responses, while cortisol, a stress hormone elevated during late gestation, further reduces cell mediated immunity (Bouyou-Akotet *et al.* 2005). The resulting decline in immune function is known to influence disease transmission and is well documented in domestic animals. However, the mechanisms sustaining persistent diseases in wild ungulate population are poorly documented (Marreros *et al.* 2011). For free-ranging ungulates, seasonal factors may further impair immune defenses. Simultaneous investment in immune defense and reproduction may not be an option if both food and internal resources are limited.

Seasonal food restriction and immune function in wild ungulates

Wild ungulates experience periods of nutritional restriction, which can influence the maintenance and transmission of infectious disease (Barboza, Parker, & Hume 2009). Nutrition is defined as the rate of ingestion of assimilable energy and nutrients, while nutritional condition is the varying state of an animal's fat reserves and muscle mass influencing future fitness (Cook *et al.* 2001; Stephenson *et al.* 2002). Poor nutrition alters virtually every aspect of the immune response, including vulnerability to attack and reactivation of chronic infections (Jolly and Fernandes 2000). The nutritional condition of mammalian herbivores is driven by seasonal forage availability and quality. Early plant growth stages generally have high nutrition in terms of energy and protein (Van Soest 1994). Since immune defense is fueled by protein and energy (Bueler, Tieleman & Piersma 2010), periods of food restriction may increase susceptibility to persistent pathogens. At these times, intracellular pathogens, which exploit their hosts during

pregnancy, may face less resistance from immune defenses if hosts are in poor nutritional condition (Beldomenico & Begon 2009).

For ungulates at temperate and northern latitudes, parturition usually coincides with the onset of the growing season (Parker, Barboza & Stephenson 2005; Rutberg 1987), but this reproductive schedule may reduce defenses against persistent pathogens. Births synchronized with the early stages of plant growth influence offspring growth rate and, consequently, survival (Guinness *et al.* 1978; Festa-Bianchet 1988). Fancy and Whitten (1991) found that selection of calving sites with newly emergent forage plants by female caribou influenced the survival of neonatal calves. In temperate Africa, the pattern of births is less clear and may be a combination of the phenology of food supply plus antipredator adaptations (Sinclair *et al.* 2000). For wild ungulates at temperate and northern latitudes, timing parturition to coincide with the availability of high quality forage increases fitness but also guarantees that pregnant animals will be in a state of reduced nutritional condition near parturition.

The food required to meet reproductive demands may be supported by a combination of food intake (income) and body stores (capital) (Barboza, Parker, & Hume 2009). Capital breeders, such as northern ungulates, rely on body reserves during periods of food restriction for successful reproduction (Jönsson 1997). If internal resources are limiting, it is reasonable to assume that investment of resources in reproductive demands might reduce the resources available to invest in immune defense (Sheldon & Verhulst 1996; Martin, Weil & Nelson 2008). Wild ungulates experience increasing reproductive demands during late gestation when food is limited and body reserves are depleted, and thus nutrient resources allocated to fetal growth and early lactation may lead to a loss of

immune protection near parturition (DelGiudice *et al.* 2001; Houdijk, Jessop & Kyriazakis 2001; Nyman *et al.* 2008).

These seasonal dietary deficiencies can decrease a host's ability to counteract and contain parasites (Valderrabano, Gomez-Rincón, & Uriate 2006). Various aspects of the immune system are condition-dependent, with immune responsiveness positively associated with nutritional condition and the availability of dietary nutrients (Hoi-Leitner *et al.* 2001; Demas *et al.* 2003; Ezenwa 2004; Xu & Wang 2010). Experimental studies with mice indicate that even mild energy limitations can alter protective immunity against nematode infection (Koski, Su, & Scott 1999). In ewes, resistance to parasitic infection near parturition was enhanced when diets were supplemented with protein and energy during late pregnancy and early lactation (Donaldson *et al.* 1998, Houdijk *et al.* 2001). Further, a negative correlation has been observed between prevalence of bovine tuberculosis in African buffalo (*Syncerus caffer*) and body condition (Caron *et al.* 2003). Because dietary demands are elevated during gestation and lactation (Gallagher 1981), metabolic adjustments to nutritional deficiencies may be more evident in reproductive than in nonreproductive animals (Lochmiller *et al.* 1988). Therefore, limitation of resources for the competing physiological demands, such as reproduction and immune defense, may help explain how chronic diseases are maintained in wild ungulate populations.

In seasonal environments, mounting and maintaining an immune response is a nutritionally expensive process involving tradeoffs between competing demands for reproduction and immunity (Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000; French, Moore & Demas 2009). For wild ruminants, the dietary protein and energy

needed to fuel immune responses are largely reduced in the months before the emergence of spring vegetation (Parker, Barboza & Gillingham 2009). At this time, temperate ungulates have depleted energy reserves (fat) which can inhibit an effective immune response when combined with low dietary protein (Nelson 2004; Gustine *et al.* 2011). Protein scarcity seems to affect cellular immunity to a much larger extent than it affects antibody-mediated immunity (Calder and Jackson 2000). Thus, intracellular strategies are used by many pathogens in malnourished hosts, indicating that the cellular immune system is affected (Cunningham-Rundles 2002). Protein and energy deficiencies decrease the production of several cytokines, particularly IL-2 and INF- γ (Chandra 1992), which are essential for controlling intracellular infection (Monack, Mueller & Falkow 2004). For this reasons, pregnant ungulates in temperate and northern regions may experience an increase in susceptibility to persistent pathogens, which may lead to endemically infected wildlife populations.

Combating persistent pathogens

The discovery of new infectious agents and diseases transmissible to humans has raised concerns regarding free-ranging wildlife as a source of emerging human pathogens (Daszak, Cunningham & Hyatt 2000). By sharing pathogens with humans and domestic animals, wildlife can serve as a reservoir for diseases that threaten human health.

Approximately 60% of human pathogens are zoonotic, with the interface between livestock and wildlife being the most important factor in disease transmission (Bengis *et al.* 2002; Bengis *et al.* 2004). In particular, pathogens that are able to evade the immune defenses of their wildlife hosts and establish persistent infections are especially problematic (Collins 2001). The inability of some wildlife hosts to recover from

persistent infectious diseases such as tuberculosis and brucellosis leads to chronic infection and a long-term wildlife disease reservoir. For example, tuberculosis is the leading cause of death in adult humans worldwide, with domestic livestock and wildlife acting as important transmission sources (Alexander *et al.* 2002; Cross *et al.* 2009; Jolles *et al.* 2005; Gortázar *et al.* 2008; Nishi, Shury & Elkin 2006). Similarly, brucellosis is regarded by the World Health Organization (WHO) as the world's most widespread zoonosis (Godfroid *et al.* 2005), with wild ungulates serving as reservoir hosts (Muñoz *et al.* 2010; White *et al.* 2011). Thus, wild ungulates are an important source of persistent pathogens where their distributions overlap with domestic livestock.

Managing the risk of disease transmission from wildlife to domestic animals and humans traditionally has resulted in control strategies that negatively impact wildlife. Traditional test-and-slaughter programs have been effective for managing diseased livestock, but these practices may not be effective, realistic, socially acceptable, or ethical for wildlife (Nishi Shury & Elkin 2006; Gortázar *et al.* 2007; White *et al.* 2011). The ability of persistent pathogens to establish latent and chronic infections creates difficulties for identifying infected animals. Consequently, animals that react positively on antibody tests that detect exposure but cannot distinguish active from inactive infection are frequently culled from the population (Treanor *et al.* 2011). Culling is rarely appropriate for controlling wildlife diseases and may increase disease prevalence under certain conditions (Choisy & Rohani 2006; Woodroffe *et al.* 2009; Beeton & McCallum 2011).

Wildlife vaccination has been proposed as an alternative to culling and has been successful in some situations in reducing infectious disease (Rupprecht, Hanlon & Slate

2004; Tomkins *et al.* 2009). Though vaccination has been effectively used to control infectious disease in humans and livestock, delivering vaccines to free-ranging wildlife poses significant challenges (Plumb *et al.* 2007). Additionally, vaccines that generate long-lived cellular responses for protection against intracellular diseases such as HIV, malaria, and tuberculosis have not provided consistent protection against these agents (Seder and Hill 2000). Because immune responses against intracellular pathogens have high nutritional costs, the efficacy of vaccines tested under experimental conditions may be reduced in wild ungulates. The nutritional demands needed to induce protective immune responses following vaccination against intracellular parasites may not be available during late gestation for wild ungulates. Further research is needed to compare vaccine efficacy under experimental and natural conditions to assess how food restriction during pregnancy influences the effectiveness of vaccines.

The ability of persistent pathogens to establish long-term infection within their hosts suggests that they will remain within infected wildlife populations without disease reduction efforts. Deciding on appropriate disease management practices for wildlife frequently leads to disagreements between stakeholders, agency managers, and the concerned public. Disease eradication is usually the option preferred by livestock producers (Schumaker Peck & Kauffman 2012), but this may not be possible for persistent diseases in wildlife. Managing the risk of disease transmission from infected wildlife to livestock typically involves practices to maintain spatial separation between wild ungulates and livestock. Though maintaining spatial separation will help protect livestock from infected wildlife, it does not reduce disease prevalence in wild ungulates and requires a continuous investment in management and surveillance efforts. Reducing

the prevalence of persistent pathogens in wild ungulates may be a compromise between eradication and risk management, but will require improved tools, such as efficacious vaccines, realistic delivery methods, accurate diagnostic tests, and an effective monitoring program.

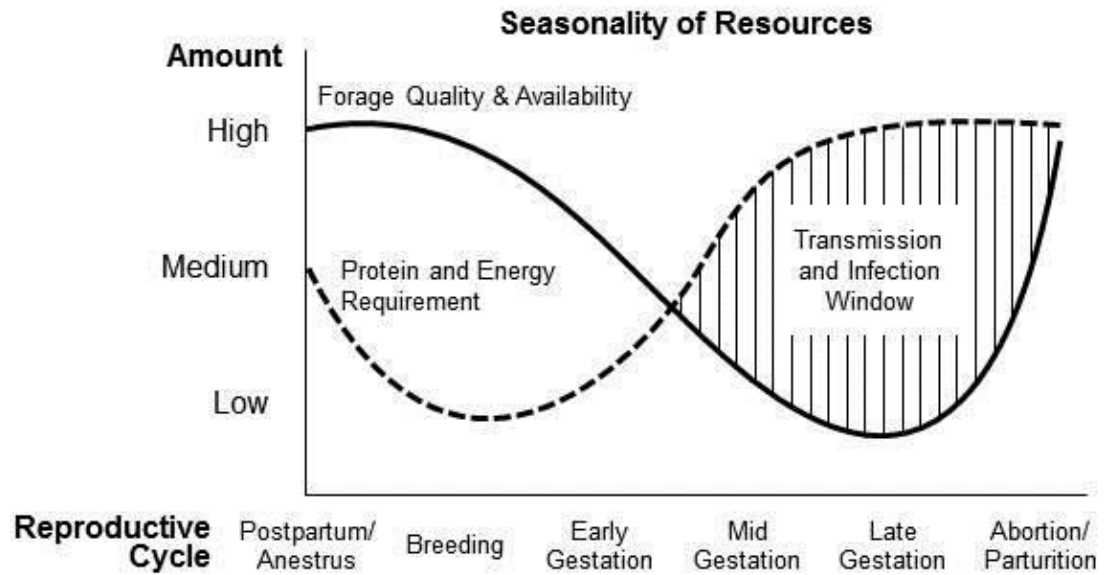
Here I have reviewed the intracellular pathogens known to cause abortive disease in pregnant ruminants in an attempt to further understand the factors sustaining these disease agents in wildlife populations. Though these diseases have been extensively studied in domestic animals, much less is known about their epidemiology in free-ranging wildlife. In wildlife populations, the breakdown of immunity during immunocompromised periods may be better understood through life history trade-offs involving resource allocation. Further research is needed on how competing resource needs (reproduction and immune defense) in wild ungulates influence the maintenance of persistent pathogens. For example, experimental studies could assess the effect of dietary restriction (e.g. diets varying in protein and energy content) on the induction of protective immune responses and ultimately transmission potential (i.e. shedding of the pathogen). These studies would advance understanding of how environmental conditions influence disease incidence rates and overall prevalence in endemically infected wildlife populations. From a management perspective, it will be important to distinguish whether a short-term reduction in disease prevalence resulted from management suppression efforts rather than environmental factors (e.g. mild winters, food availability) that improve host resistance during the critical period. Otherwise, public support for disease reduction programs may be short-lived if infection levels spike despite consistent disease reduction efforts. Managing persistent diseases of free-ranging wildlife will be a

tremendous challenge requiring innovative management approaches. Identifying the ecological factors that influence immune suppression will help initiate control measures that benefit human health and promote wildlife conservation.

Disease/Agent	Taxonomy	Host	Zoonotic	Transmission\Persistence Strategy	Immune Response
Bovine Neosporosis (<i>Neospora caninum</i>)	Protozoal	Mainly Cattle	No Support	Consumption of contaminated feed and transplacental infection of calf via recrudescing cow	Cell-mediated
Ovine Toxoplasmosis (<i>Toxoplasma gondii</i>)	Protozoal	Mainly sheep	Yes	Consumption of contaminated feed and congenitally infected lambs via transplacental infection with re-infection occurring in subsequent pregnancies	Cell-mediated
Malaria (<i>Plasmodium falciparum</i>)	Protozoal	Humans	Yes	Through mosquitoes during pregnancy, emerging new variants are able to evade acquired immune response	Cell-mediated
Brucellosis (<i>Brucella abortus</i>)	Bacterial	Cattle, Wild Ungulates	Yes	Through birth fluids associated with aborted fetus or during normal delivery. Vertical transmission occurs from cow to calf. Chronic infection can result in infection and transmission during future pregnancies	Cell-mediated
Q Fever (<i>Coxiella burnetii</i>)	Bacterial	Goats, sheep, and cattle	Yes	Through birth fluids, organs and fetal membranes shed during normal delivery or during abortion, as well as via the urine, milk and feces. Latent persistence results in reactivation of infection in pregnant ruminants	Cell-mediated
Chlamydia (<i>Chlamydia abortus</i>)	Bacterial	Sheep	Yes	Infection of the placenta induces abortions and transmission occurs through consumption of infected birth fluids and membranes. Persistence results from latent infection that is reactivated during pregnancy.	Cell-mediated
Listeriosis (<i>Listeria monocytogenes</i>)	Bacterial	Domestic Animals, Wildlife	Yes	Infection of the fetus via the placenta frequently results in abortion in sheep and cattle. Can multiply both extracellularly and intracellularly and grow at low temperatures outside host.	Cell-mediated
Bovine Viral Diarrhea Virus	Viral	Cattle, Wild Ungulates	NO	Infections of the persistent form of the virus occur in the developing fetus prior to immune system development. Transmission occurs through the consumption of infected feces, urine, or birth tissues	Cell-mediated

Table 2.1. List of persistent intracellular pathogens with their hosts, taxonomic affiliation, maintenance strategy and required protective immune response.

Figure 2.1. Conceptual framework describing changes in forage quality and availability in relation to protein and energy requirements over the reproductive cycle in wild female ungulates. Increasing reproductive demands during periods of reduced food availability create transmission and infection opportunities for persistent pathogens during late gestation.



Chapter 3 - Vaccination strategies for managing brucellosis in Yellowstone bison

Introduction

The discovery of new infectious agents and diseases transmissible to humans has raised concerns regarding free-ranging wildlife as a source of emerging human pathogens (Daszack *et al.* 2000; Bengis *et al.* 2004). Humans are often indirectly exposed to wildlife pathogens through infected livestock. The crowding and mixing of wildlife with domestic livestock can increase disease prevalence and transmission potential (Dorn & Mertig 2005; Cross *et al.* 2007) thereby, increasing exposure to humans. Disease transmission risk from wildlife to domestic animals and humans traditionally has resulted in control strategies that negatively impact wildlife populations. Traditional test-and-slaughter programs have been effective for managing diseased livestock but these practices may not be realistic or socially acceptable for wildlife (Bienin & Tabor 2006; Nishi *et al.* 2006). An approach to wildlife disease management is needed that addresses both public health concerns and long-term wildlife conservation. Vaccination is commonly used for disease control in veterinary medicine and wildlife vaccination may offer a promising solution (Plumb *et al.* 2007). The success of a vaccination program is influenced by vaccine efficacy and the proportion of the population inoculated. Our ability to deliver efficacious vaccines and monitor their effectiveness is restricted in free-ranging wildlife. Additionally, we seldom have all the information necessary to predict the effectiveness of a wildlife vaccination program, but management actions will need to move forward despite these uncertainties.

Yellowstone National Park of the western United States was created in 1872, and encompasses 9018 km² in portions of Idaho, Montana, and Wyoming, but only about 3175 km² of this area currently serves as principal bison habitat (Fig. 2.1). The successful conservation of bison (*Bison bison*) from a low of 23 animals in 1901 to a high near 5000 animals in 2005 has led to an enduring series of societal conflicts and disagreements among various publics and management agencies regarding the potential transmission of *Brucella abortus* to domestic livestock. *B. abortus*, the bacteria causing the disease bovine brucellosis, was introduced to Yellowstone bison by cattle before 1917 and approximately 40-60% of the Yellowstone bison population has been exposed (Cheville *et al.* 2008). Since that time, successful conservation increased the abundance of Yellowstone bison from approximately 400 to >4700 in 2007 (Fuller *et al.* 2007). A portion of the Yellowstone bison population periodically moves between habitats in the park and adjacent lands in Montana during winter (Gates *et al.* 2005), resulting in a risk of brucellosis transmission from bison to cattle on overlapping ranges adjacent to the park (Plumb & Aune 2002). Humans are also susceptible to infection, though brucellosis is no longer a widespread health threat in North America due to the use of sanitary procedures (e.g., pasteurization) in milk processing (Young & Corbel 1989). When livestock are infected, brucellosis results in economic loss from slaughtering infected cattle herds and imposed trade restrictions (Godfroid 2002). More than \$3.5 billion were spent since 1934 to eradicate brucellosis in domestic livestock across the United States (Cheville *et al.* 1998), however the disease remains endemic in bison and elk (*Cervus elaphus*) in the greater Yellowstone ecosystem (Gates *et al.* 2005). Many livestock

producers and cattle regulatory agencies contend that any risk of brucellosis transmission is unacceptable for economic and public health reasons.

To manage the risk of brucellosis transmission from Yellowstone bison to livestock, the federal government and State of Montana agreed to the Interagency Bison Management Plan (U.S. Department of the Interior 2000^{a,b}). This plan established guidelines for implementing hazing, test-and-slaughter, hunting, and other actions affecting bison abundance and distribution near the park boundary, where bison could potentially co-mingle with livestock. The plan also indicates that the National Park Service will conduct a remote delivery vaccination program of vaccination-eligible bison within the park to increase tolerance of untested bison on winter range lands outside the park. The National Park Service is currently considering the implementation of such a program to reduce brucellosis infection in the bison herd. Much remains to be learned about brucellosis epidemiology in bison and how effective vaccination may be, but it will be necessary to make decisions and proceed despite uncertainty. Simulation models can be effective tools for informing this decision-making process by evaluating the effectiveness of different management strategies. Thus, I developed an individual-based model to evaluate alternate vaccination strategies and how brucellosis infection in Yellowstone bison might respond under different approaches.

Model context

B. abortus infection

Brucellae are facultative intracellular pathogens, which evade the host's immune system by replicating within the host's white blood cells (e.g., macrophages) (Dormand *et al.* 2002). During middle to late gestation, *Brucellae* that have infected the uterus

undergo massive replication in placental cells. The extensive replication causes a rupture compromising placental integrity by allowing the bacteria direct access to the fetus (Bellaire *et al.* 2003). The resulting abortions and premature calves are highly infectious due to the large number of Brucellae on the fetus, placenta and birth fluids. Following this acute phase of infection, some bison are unable to clear the bacteria and remain infected. The pathogen's ability to establish persistent infections in some animals results in a class of latent carriers. The relapsing of latently infected animals to the infectious state during future pregnancies, is a concern with Yellowstone bison.

Intracellular protection and replication are crucial components of incubation, latency, and chronic infection of Yellowstone bison (Nicoletti & Gilsdorf 1997). Thus, I modeled these aspects of brucellosis infection in bison by including an incubation period in the model. This allowed for deciding whether a pregnant, susceptible bison that was recently exposed would have adequate time to shed *B. abortus*. Also, I addressed latent infection in the model by assuming bison never truly recover from brucellosis and that adult females can potentially shed *B. abortus* throughout their reproductive lives.

Brucella abortus transmission

Transmission of *B. abortus* in Yellowstone bison is believed to occur primarily through contact with an aborted fetus or infected birth tissues shed during a live birth. The number of exposures that occur during these infectious events depends on the behavior of the bison cow at the time of parturition. Bison tend to give birth in close proximity to other group members, which increases the likelihood of transmission. *B. abortus* is also known to cause mammary gland infections (Bevins *et al.* 1996) and can be transmitted through infected milk (Nicoletti & Gilsdorf 1997; Olsen & Holland 2003;

Olsen *et al.* 2003). Though bacterial numbers in milk are lower than in an infected placenta, they are typically high enough to present a serious risk of transmission (Cheville *et al.* 1998). The role vertical transmission (transmission from cow to newborn calf) plays in the maintenance of *B. abortus* in Yellowstone bison is unclear, but may help explain the low frequency of observed abortions, high seroprevalence rates among young animals, and latent infection.

I modeled *B. abortus* transmission via infectious events (i.e., abortions and infectious live births) and vertical transmission to calves. I assumed that a proportion of latently infected adult cows will recrudescence in any given year and have an infectious live birth. Also, a proportion of calves born from these infectious births will become infected through vertical transmission.

A key component of brucellosis transmission is the number of exposures that occur during an infectious event. Thus, the ability of the *Brucella* pathogen to spread could be influenced by group size, composition, cohesion, and the infection status of associates (Gudelj *et al.* 2004). Yellowstone bison appear to have a dynamic social structure with fluid movements between groups (McHugh 1958; Lott & Minta 1983; Rutberg 1984a; Lott 1991; Aune *et al.* 1998). The fundamental social unit is the cow-calf association, which persists for approximately 9 months in male calves and 14 months in female calves (Green *et al.* 1989; Lott 1991). There is little evidence that groups of related females form lifelong associations (McHugh 1958), but cows with calves tend to be found more often in groups with other cow-calf pairs (Rutberg 1984b). Also, group sizes tend to get larger as habitat becomes more open and generally increase during the spring calving season (Rutberg 1984a).

I did not assume that every individual in the population is equally likely to become exposed to *B. abortus*. If the association among cows is not random, an individual's chance of being exposed is influenced by the infection status of its associates. I modeled the bison social group as a fluid unit where infectious events occur and the cow-calf pair as the focal unit of exposure

B. abortus detection in bison

Identifying the state of brucellosis infection within the Yellowstone bison population relies on diagnostic tests performed on a segment of the bison population captured at the park boundary. Brucellosis infection is diagnosed in bison through serologic tests and bacterial cultures. For serologic tests, the fluorescent polarization assay (FPA) is the diagnostic test of choice for detecting brucellosis in bison because of its high sensitivity (94.5%), specificity (99.5%), and adaptability to field use (Gall *et al.* 2000; Gall & Nielsen 2001; Nielsen & Gall 2001). Serologic tests provide indirect evidence of infection because they detect antibodies (i.e., responses to infection) rather than living bacteria and can result in both false positive and false negative diagnoses. Thus, it is unlikely that the probability of identifying truly infectious individuals can be accomplished by serology alone (Cheville *et al.* 1998). Combining serologic testing with tissue culture identified that nearly half (46%) of slaughtered seropositive bison were also culture positive (Roffe *et al.* 1999). Based on this work, I estimated that 46% of seropositive bison were culture positive animals and considered to be actively infected. I assumed, based on the use of the FPA as a diagnostic tool, that all actively infectious bison and a high proportion of latent infected animals could be diagnosed as positive under boundary capture scenarios.

Vaccination of Yellowstone bison

The objective of bison vaccination is to stimulate an acquired immune response to *B. abortus* thereby increasing herd immunity and reducing the potential for transmission. The live *B. abortus* strain RB51 (SRB51) is the official brucellosis vaccine for cattle in the United States, but has the potential to induce abortions in pregnant bison vaccinated in mid-gestation (Palmer *et al.* 1996). However, bison calves vaccinated with SRB51 may be safely booster-vaccinated during their first pregnancy, with early gestation being a potentially safe period for adult pregnant bison (Olsen & Holland 2003). Based on these findings I developed vaccination strategies that would limit the potential for vaccine induced abortions by focusing on reproductively immature bison and adult females during early gestation.

There is uncertainty about the level of protection (i.e., efficacy) SRB51 will provide Yellowstone bison based on experimental studies. Vaccination of bison calves provided protection from abortions and placental infection when challenged with virulent *B. abortus* during their first pregnancy (Olsen *et al.* 2003). However, SRB51 was found to have little efficacy in adult and calf bison despite repeated vaccinations (Davis & Elzer 1999; Davis & Elzer 2002). Thus, the duration of protection provided by a single dose of SRB51 is unknown and older cows may need to be booster-vaccinated to extend the protection of the vaccine (Olsen & Holland 2003). A key feature of SRB51 is that vaccinated bison remain seronegative when tested with standard serologic tests (Olsen *et al.* 1998) which prevents the removal of tested vaccinated animals. Delivery of vaccine poses a problem with free-ranging bison and, currently, the most feasible method of remote vaccine delivery is via biodegradable projectiles (i.e., “bio-bullets”). Ballistic

vaccination has been used to inoculate free-ranging elk on feedgrounds in Wyoming (Herriges *et al.* 1991) and tested experimentally with bison. Ballistic inoculation of bison with photopolymerized SRB51 packaged into bio-bullets induced a significant cell-mediated immune response that was similar to syringe delivery of the vaccine (i.e., parenteral vaccination) (Olsen *et al.* 2006). I assume that remote delivery of SRB51 to free-ranging bison would provide protection equal to bison given syringe vaccinations when handled at the boundary. I also addressed waning immune protection in the years following vaccination and included an increase in protection with booster vaccination.

The individual-based model

Model development

I developed an individual-based model (IBM) using MATLAB 7 (The MathWorks, Natick, MA, USA) to evaluate the effectiveness of vaccination at reducing brucellosis infection in Yellowstone bison under the following three vaccination alternatives: 1) vaccination of female calves and yearlings captured during boundary management operations, 2) combining remote vaccination using bio-bullet delivery with boundary vaccination of female calves and yearlings, and 3) vaccinating all female bison during boundary operations and as targets for remote delivery. Under each alternative, I assumed bison captured at the park boundary were all tested and positive reactors removed.

The IBM tracked information on each female bison born into the population (Fig. 3.2A). The model used a yearly time step to simulate population level processes and daily time steps to simulate exposure routes during the transmission period (Fig 3.2B). The yearly time step components involved mating, natural mortality, exposure to *B.*

abortus via elk, and effects of management operations (testing and subsequent removal of seropositive bison at park boundaries). The daily time step detailed the processes (*Brucella* induced abortions and infectious live births) leading to shedding and transmission of *B. abortus* among Yellowstone bison. Male bison were included in yearly outputs, but were not a focal component of the model because their role in maintenance and transmission is expected to be minimal (Robison *et al.* 1998). Age, sex, disease status, reproductive status, and vaccination status were recorded for each female bison modeled.

Modeled bison were initially assigned a disease status (susceptible, infected, or latent) based on estimates derived from Yellowstone bison seroprevalence data. Bison that had never been exposed to *B. abortus* were classified as susceptible. Infected bison were viewed as actively infectious and modeled to shed *B. abortus* at a high probability during their next pregnancy. These infected bison then entered a latent class with a low probability of shedding *B. abortus* during future pregnancies. Changes in the disease classes of individuals were used to predict the disease status for the overall population with population seroprevalence being the sum of infected and latent bison. Individuals changed their disease class based on events (i.e., exposure, vaccination) and rules associated with their current state (i.e., disease class, pregnancy status, vaccination status).

The model included two types of infectious events for simulating horizontal transmission: *Brucella* induced abortions and infectious live births. I assumed that both events had equal transmission potential. I also assumed that infected bison did not fully recover from the disease. These animals had a low probability of shedding the bacteria in

future pregnancies while remaining latently infected. In situations where latent cows recrudesced and shed *B. abortus* during an infectious live birth, their calves became infected through vertical transmission (consuming infected milk) at a specified probability.

In the model, vaccinated, susceptible bison were classified as vaccine-protected based on the assigned efficacy of the vaccine. These bison remained vaccine-protected when exposed to the field strain at specified probabilities corresponding to vaccine efficacy. When field exposure overwhelmed the protection of the vaccine, the bison became infectious (i.e., entered the infected disease class). A vaccine delivery parameter was used for alternatives involving remote vaccination. This represented the proportion of targeted bison in the population that were likely to receive the vaccine. Once the vaccine was delivered, bison entered the vaccine-protected class based on the level of vaccine efficacy.

Model processes

Model parameters (Table 3.1) were initialized prior to running the model. Management options were set to simulate desired vaccination alternatives under specified levels of vaccine effectiveness. Each bison was assigned to a social group during initialization. Bison were provided with demographic information (i.e., age, sex) and assigned to a disease class based on estimates derived from seroprevalence data. Age was assigned using estimates of bison population age structure (1-15 years) and sex was assigned assuming an equal sex ratio. Bison social groups were then subdivided into maternal units, with calves assigned to mothers (i.e., cow-calf unit).

The annual time step began with bison becoming pregnant based on estimates of age-specific pregnancy rates. Pregnant bison were given either a pregnancy date or an abortion date depending on the individual's disease class. The abortion period included the last trimester (90 days) of gestation (287 days) before the live birth period (61 days). Depending on their disease status, pregnant bison had a non-infectious live birth (i.e., *Brucella* not shed; calves classified as susceptible), an infectious live birth (i.e., *Brucella* shed; calves classified as susceptible (0.34) or infected (0.66)), or a brucellosis-induced abortion. I treated infectious material from abortions and infectious live births equally with regard to disease transmission. Susceptible bison had a non-infectious live birth unless exposed to *B. abortus* during pregnancy and there was sufficient incubation time (35 days) for *B. abortus* to be shed. If there was insufficient incubation time (<35 days) before parturition, the female did not abort or have an infectious birth. However, the female's newborn calf was infected via vertical transmission with a set probability (0.66). Bison infected with greater than 35 days of incubation prior to parturition aborted their pregnancy at a specified probability (0.96) or infected their newborn calves via vertical transmission (0.66). Pregnant, latent cows had a non-infectious birth unless they relapsed to the infectious state. I assumed 5% of latently infected adult females relapsed in a given year and shed *B. abortus* through infectious live births and infected their calves through vertical transmission.

Based on field observations of bison group members interacting with new born calves and birth tissues, I assumed cow-calf pairs approached parturition sites and were exposed to *B. abortus* together. Thus, transmission was modeled using maternal units, which were either cows and their newly born calves or single female bison (≥ 1 -year old),

that were exposed to *B. abortus* during an infectious event (i.e., abortion and infectious live birth). Maternal units in the susceptible class became infected when exposed, while the disease status of already infected and latent class bison remained unchanged. The number of maternal units exposed per infectious event was decided by drawing from a Poisson distribution fitting field observations of Yellowstone bison licking newborns or expelled birth tissues. Contact with birth material was treated as a discrete random variable and a Poisson distribution was fit to the frequency of contacts by group members. The rate parameter that best fit the field data ($\lambda = 1.42$) was adjusted ($\lambda = 1.0$) to fit the historical population seroprevalence estimates (Fig. 3.3).

Long-term group size information for Yellowstone bison (McHugh 1958) was used to divide the population into groups of cows and their calves. Social groups of 24-48 females and calves were assumed to have greater contact with each other than with bison outside their group. Thus, the probability of exposure following an infectious event is expected to be higher within groups than among groups due to the proximity of individuals to infectious birth tissues. However, the mixing of bison social groups and the ability of *B. abortus* to persist on the landscape (Aune *et al.* 2007; Aune *et al.* 2012) suggests there is transmission potential to bison outside the social group experiencing the infectious event. The specific maternal units exposed were determined using a biased draw from the population, with parameter β biasing exposures in favor of bison maternal units within the social group where the infectious event occurred. The probability that an exposure will occur in any group, other than the group containing the infectious event,

was expressed using Equation 1:

$$\text{Probability of outside group transmission} = \frac{N_i}{\beta (N_k - 1) + \sum_{\substack{j=1 \\ j \neq k}}^n N_j} \quad (1)$$

where N_i is the number of bison maternal units in a social group where infectious material was not shed, $(N_k - 1)$ is the number of maternal units in the social group experiencing the infectious event less the shedding maternal unit, $\sum N_j$ is the total number of maternal units in all social groups not experiencing the infectious event and beta (β) is a constant. The constant β was used to increase the probability of exposures occurring within the social group experiencing the infectious event and was expressed using Equation 2:

$$\text{Probability of within group transmission} = \frac{\beta (N_k - 1)}{\beta (N_k - 1) + \sum_{\substack{j=1 \\ j \neq k}}^n N_j} \quad (2)$$

Following the daily processes influencing transmission and exposure, the remaining annual processes were simulated. Social groups and their maternal units were reestablished based on group size criteria. Bison were subjected to natural mortality based on estimated age-specific death rates. Management operations (i.e., test, remove, vaccinate) were modeled for each of the three vaccination alternatives. The portion of the Yellowstone bison moving beyond the park boundary was modeled based on the past 20 years of capture operations. I used a frequency distribution of the portion of the population captured (< 0.1 , $0.1-0.2$, and $0.2-0.3$) at the park boundary each winter during

1985-2005 (Table 3.2) to estimate the number of bison that might be tested in a given year.

Seropositive bison were removed from the model to simulate management operations based on the sensitivity and specificity of the FPA serologic test. I assumed that infected and latent bison could be correctly diagnosed as seropositive during 100% and 95% of the tests, respectively. The remaining seronegative bison were vaccinated and assigned vaccine-protected status based on the specified efficacy of the vaccine. These vaccinated bison retained their vaccine-protected status if exposed to *B. abortus* based on the level of vaccine efficacy. Also, I assumed no abortions or mortality occurred due to vaccination itself. Simulations were run over a range of vaccine efficacy values under each management alternative. Vaccine-protected bison that were subsequently exposed to *B. abortus* were expected to react positively on serologic tests and, consequently, be removed during management operations. I recorded the proportion of these seropositive-vaccinates in the model under each alternative. Bison previously exposed to *B. abortus* (i.e., infected and latent bison) remained in their original states if vaccinated. I included a duration-of-protection component to vaccine efficacy, which modeled a decreasing level of vaccine protection in years following vaccination to identify the effect of waning immune protection.

Elk populations in the greater Yellowstone ecosystem are also infected by *B. abortus* and have been implicated as the source of brucellosis infection to cattle herds in Idaho, Montana, and Wyoming (Galey *et al.* 2005; Higgins *et al.* 2012). The pathology of the disease in elk is believed to be similar to bison and cattle. I included elk as a

potential source of brucellosis infection for bison and modeled exposure from elk to bison at a low probability (0.01).

The annual processes concluded by outputting all relevant information for each year. The data were then analyzed over a 30-year period and comparisons were made between the three vaccination alternatives. The rate of decrease in population seroprevalence and the corresponding proportion of the population vaccinated were used to assess the effectiveness of each vaccination alternative. Each vaccination alternative was evaluated by running multiple model simulations over a range of vaccine efficacy and delivery parameters.

Results

I conducted 10 simulations at intermediate levels of vaccine efficacy (0.5) for each of the three vaccination alternatives: 1) boundary vaccination of female calves and yearlings; 2) combination of boundary and remote vaccination of female calves and yearlings; and 3) boundary and remote vaccination of all females. Under Alternative 1, seroprevalence decreased by 24% from 0.46 to 0.35 over the 30-year period, with 1% of the population vaccinated. Under Alternative 2, seroprevalence decreased by 40% from 0.47 to 0.28 over the 30-year period, with 10% of the population vaccinated. Under Alternative 3, seroprevalence decreased by 66% from 0.47 to 0.16 over the 30-year period, with 29% of the population vaccinated. Thus, combining boundary and remote vaccination of all female bison (Alternative 3) resulted in the greatest seroprevalence decreases over the 30-year simulation period (Fig. 3.4A).

Alternative 3 resulted in a larger proportion of vaccine-protected bison compared to the other two alternatives (Fig. 3.4B), and the relationship between seroprevalence and

the proportion of the bison population vaccinated over the 30-year period was $y = 2.4x + 0.85$ ($R = 0.92$). Boundary removals resulting from migrations out of the park were stochastic, but there was a reduction of seropositive bison removed at the boundary as the level of vaccine-protected bison increased in the population. The proportion of seropositive-vaccinates (i.e., vaccinated bison that were subsequently exposed to *B. abortus*) was larger under Alternative 3 than Alternatives 1 and 2. Population growth rates increased from $\lambda = 1.02$ (Alternative 1) to $\lambda = 1.05$ (Alternative 3) with greater vaccination effort.

Simulations indicated the effect of decreasing levels of vaccine efficacy (0.10, 0.20, and 0.30 per year) on seroprevalence had the most pronounced effect on Alternative 3 (i.e., the alternative with the most remote vaccination effort, Fig. 3.5), while model trajectories were more variable in the other two alternatives with less vaccination effort. Exploratory simulations to better understand the response of infection under a short-term (10 years) implementation of Alternative 3, after which all vaccination and management activities ceased, indicated seroprevalence returned to pre-vaccination levels and the rate of return was more sensitive to the level of vaccine efficacy (0.10, 0.30, 0.50, and 0.70) for alternatives with greater vaccination effort (Fig. 3.6A). The level of vaccinated animals decreased toward zero as individuals were removed based on natural mortality rates (Fig. 3.6B).

Discussion

Vaccinated bison exposed to field strain *B. abortus* are less likely to become infectious and transmit the bacteria to other herd members. Model simulations suggest that syringe vaccination of females captured at the park boundary will provide only a

small decrease in brucellosis infection due to low vaccination rates that rely on out-of-the-park migrations. Remote delivery vaccination extends the reach of management and allows for considerably more bison to be protected from infection. Thus, the greatest potential for reducing brucellosis infection could be achieved by combining vaccination at boundary capture pens with the remote delivery of vaccine throughout the park to all bison believed to be important in the maintenance of the disease. The projected reduction in seroprevalence results from disrupting the transmission cycle of *B. abortus* by reducing the quantity of *Brucella* bacteria shed onto the landscape and decreasing the exposure rate of susceptible bison. Thus, fewer animals are exposed and the number of seropositive bison removed during boundary capture operations decreases. Model simulations demonstrated that the interconnectedness of these variables was dependent on vaccine efficacy and vaccination effort. The sensitivity of vaccine efficacy was more pronounced in the alternatives involving remote vaccination due to the greater opportunities to vaccinate bison. However, improving the efficacy of a vaccine against *B. abortus* may take some time and increasing vaccination effort may compensate for less than desirable vaccine efficacy in the short term.

The current vaccine, SRB51, is not expected to provide lifetime protection and female bison may need booster vaccinations (Olsen & Holland 2003). Thus, targeting only young animals for remote vaccination (Alternative 2) would increase the variability in seroprevalence declines because the level of vaccine protection would likely decrease as animals age. However, SRB51 is safe for multiple immunizations (Davis & Elzer 1999), which would reduce the uncertainty of protection in years following vaccination. Targeting all female bison (Alternative 3) allows animals to receive multiple vaccinations

that extend the duration of vaccine protection and reduce the potential for latently infected bison to relapse into an infectious state.

The difficulty in monitoring the level of brucellosis infection within the population underscores the need for multiple indicators to evaluate the effectiveness of a vaccination program. Seroprevalence is an attractive indicator of infection because serum is easily obtained, diagnoses are quick and simple, and sampling does not involve killing the animal. However, seroprevalence indicates a history of exposure (i.e., antibody responses) and does not provide a complete picture of how bison may be responding to vaccination because rates of active infection are likely to be much lower than indicated by seroprevalence (Roffe *et al.* 1999). Thus, testing bison at boundary capture facilities should combine serologic tests with tissue culture on the seropositive bison that are shipped to slaughter. Because the antibody responses to *B. abortus* are long-lived, the proportion of actively infected bison would be expected to decrease faster in response to vaccination than population seroprevalence. Also, vaccinated bison that are subsequently exposed to field strain *Brucella* will react positively on serologic tests even though they may be protected from further transmission. These bison would be removed during boundary operations, thereby impeding the reduction of brucellosis infection. These bison play an important role in herd immunity by reducing the number of exposures of susceptible bison during an infectious event. Thus, a delay in seroprevalence decrease is expected in the first 10 years of initiating a vaccination program because of high population seroprevalence, long-lived antibodies, and the removal of vaccinated, seropositive bison.

Model simulations demonstrated an increase in seroprevalence as vaccinated bison were removed through natural mortality under short-term vaccination scenarios. Even under high levels of vaccine efficacy, investment in short-term vaccination efforts will not reach long-term goals of reducing brucellosis infection in bison. Thus, a consistent long-term investment in vaccination will be required to meet the objective of the Interagency Bison Management Plan for reducing brucellosis transmission risk to cattle by reducing infection within Yellowstone bison. The precise level of acceptable risk has not been articulated, but model simulations indicate that brucellosis infection, as indexed by seroprevalence, can be substantially reduced with a vaccine of intermediate efficacy and realistic remote vaccination effort. Vaccination is likely to be a constant, long-term investment with the tools (i.e., vaccine, delivery method, and diagnostics) currently available. Reductions in the level of infection can be achieved, but will require a strong surveillance program to validate the corresponding decrease in infection with vaccination effort.

There is still much to be learned before remote delivery vaccination becomes operationally feasible. The efficacy of vaccine SRB51 has not been tested under field conditions and research is needed to estimate its efficacy within the Yellowstone system. Also, the duration of vaccine protection offered by SRB51 is unknown, but undoubtedly plays an important role in reducing infection and transmission. Yellowstone bison experience strong seasonal changes that cause stress and a reduction in nutritional condition. How bison respond to vaccination under these conditions will be important for estimating responses to exposure after vaccination. Also, the bio-bullet delivery method has been validated under experimental conditions, but its effectiveness has not been

evaluated in Yellowstone bison. In addition, realistic group responses of bison to vaccination are largely unknown, and disturbances from remote vaccination may make bison difficult to vaccinate with this method over the long term. Remote vaccination effort will be unable to compensate for vaccine efficacy if bison are difficult to vaccinate.

The large proportion (0.5) of young, immature bison in Yellowstone that are seropositive indicates that exposure to *B. abortus* occurs early in life. However, little is known about transmission through infected milk or trans-placental transmission in bison. The risk of this route of exposure increases the need to vaccinate reproductively mature cows to reduce mammary gland and placental infection. A greater understanding of this potentially important route of transmission will lead to improved surveillance methods and parameterizing more detailed transmission models. Also, latent carriers of *B. abortus* are well documented, but the causes of recrudescence are speculative. Thus, all the potential transmission routes and female age classes contributing to transmission require further investigation.

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Table 3.1 – Default parameter values for an individual-based model predicting how brucellosis infection in Yellowstone bison might respond under alternate vaccination methods.

Parameter/Variable	Value	Source
Pregnancy rate (Pr)		National Park Service
2 year olds	0.71	
3 year olds	0.79	
4 year olds	0.76	
Adults (5 yrs+)	0.89	
Calving rate (Cr)	0.71	National Park Service
Birth period (Bdays)	61 days	Berger and Cain 1999
Abortion period (Adays)	90 days	Dobson and Meagher 1996
Death rate (Dr)		
0-2 years	0.2	
3-13 years	0.1	
14 years	0.2	
15 years	1.0	
Social group size		National Park Service
Minimum	24	
Maximum	48	
Susceptible (S)	0.53	Department of Livestock
Infected (I)	0.22	
Adult Latent (L)	0.25	
Rate of recrudescence	0.05	Review of latency literature
Exposures / infectious event	Poisson ($\lambda=1$)	National Park Service
Vertical transmission	0.66	Gross, Miller & Kreeger 1998
Minimum incubation time	35 days	Gross, Miller & Kreeger 1998
Social transmission factor (β)	1.5	Fitted parameter
Bison captures at Park boundary per year		
0-10% of population	0.84	
10-20% of population	0.11	
20-40% of population	0.05	
Bison removals at capture facility		
Removal of infected class	1.0	Nielsen & Gall 2001
Removal of latent class	0.94	
Vaccine Efficacy		Modeled over a range of values

Table 3.2 – Annual proportions of bison captured at the boundary of Yellowstone National Park during winters 1985-2005.

Winter	Bison captured	Population count	Proportion captured
1985	88	2114	0.041
1986	57	2291	0.024
1987	6	2433	0.002
1988	35	2644	0.013
1989	569	3159	0.180
1990	4	2606	0.001
1991	14	3178	0.004
1992	271	3426	0.079
1993	79	3304	0.023
1994	5	3551	0.001
1995	427	3956	0.107
1996	433	3398	0.127
1997	1084	3436	0.315
1998	11	2105	0.005
1999	94	2239	0.041
2000	0	2444	0.000
2001	6	2800	0.002
2002	265	3286	0.080
2003	252	3880	0.064
2004	488	3824	0.127
2005	184	4239	0.043

Figure 3.1 Map of the distribution of bison within Yellowstone National Park and location of boundary capture areas for migrating bison. The northern and western boundary capture areas include facilities where bison are tested and vaccinated for brucellosis.

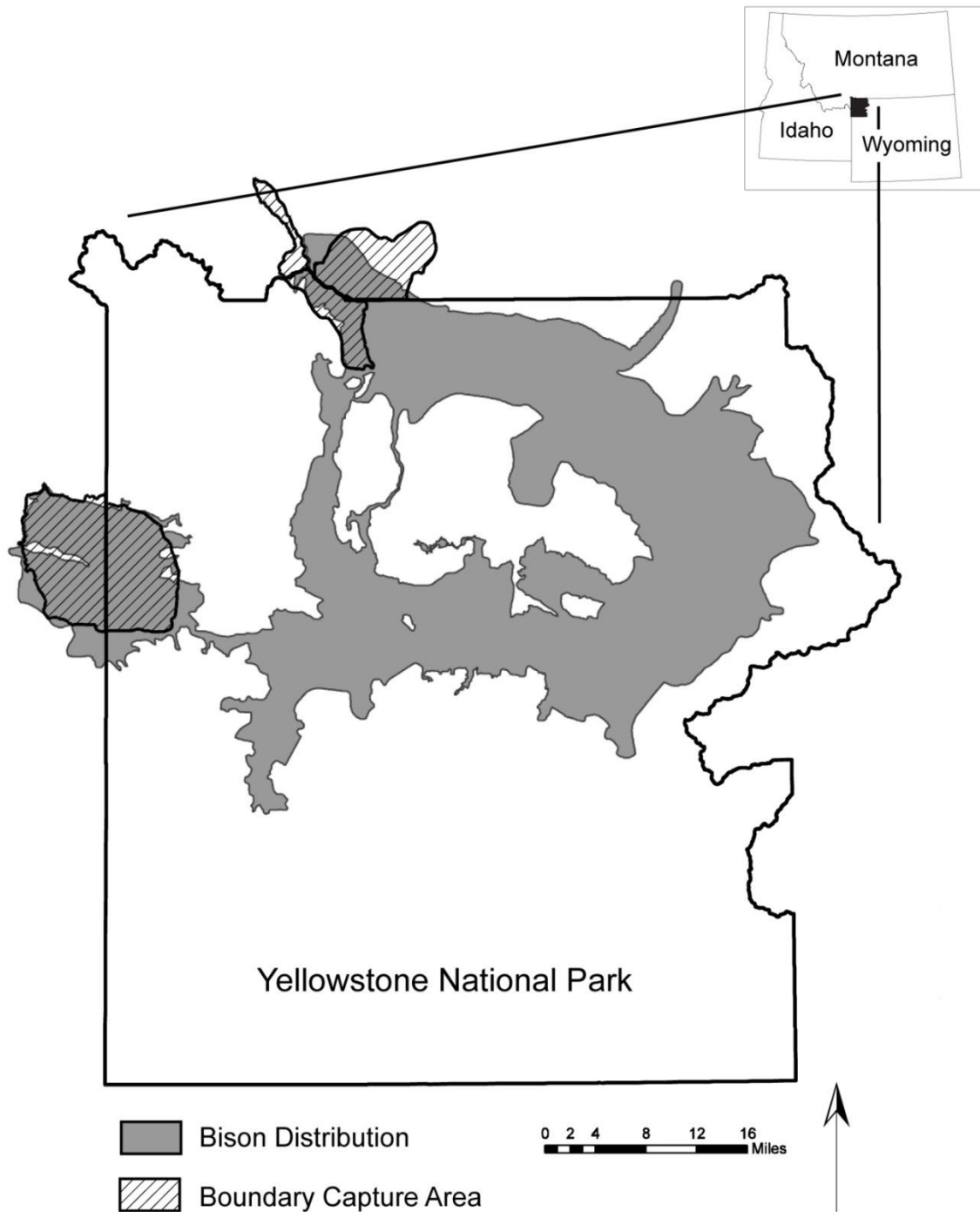


Figure 3.2 Flow diagram of individual-based model processes influencing the state of *B. abortus* infection in Yellowstone bison. The sequences simulated (panel A) for the three vaccination alternatives were run for a 30-year period with yearly processes controlling population demographics and vaccination status and daily processes detailing *B. abortus* exposure and transmission. Changes in disease state and vaccination status (panel B) were based on rules of exposure and vaccine efficacy.

A

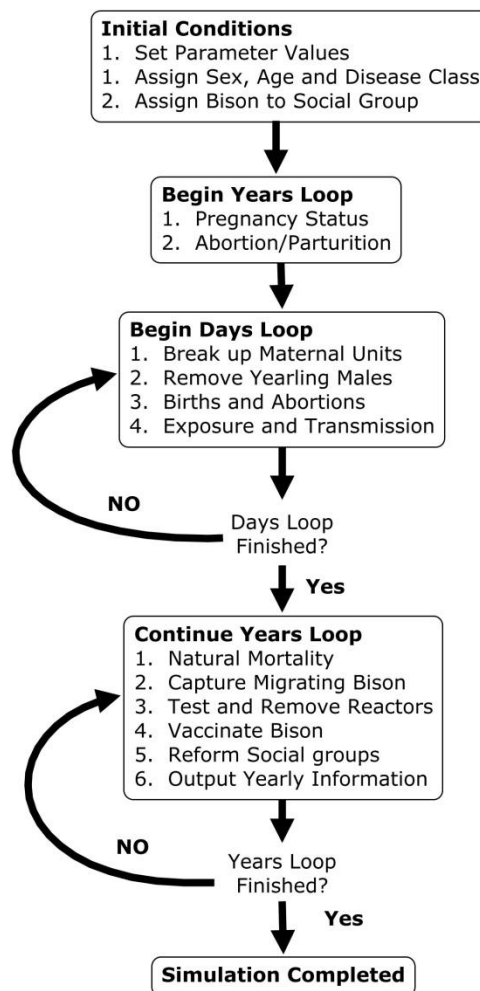


Figure 3.2 Continued

B

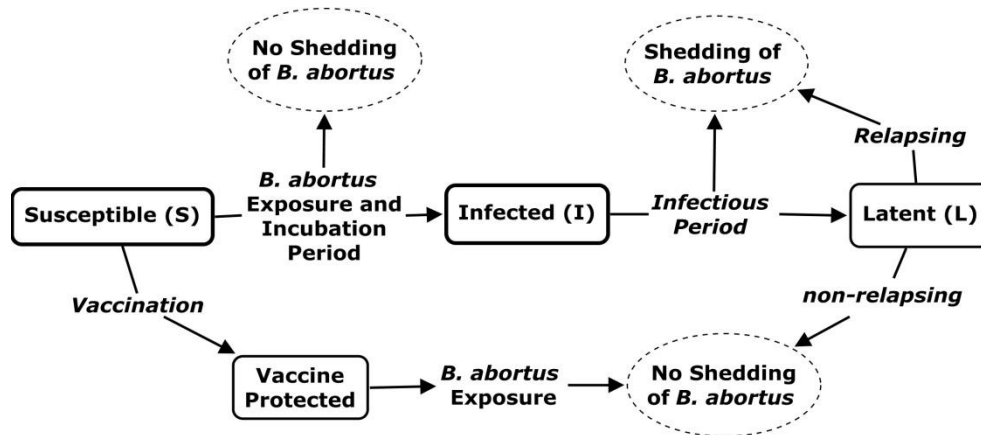
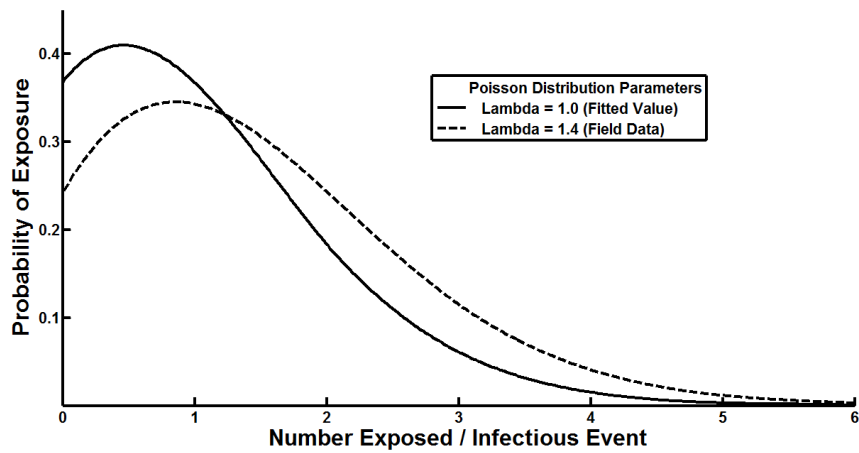


Figure 3.3 Estimate of *B. abortus* transmission following an infectious event (abortion or live birth). The number of bison exposed was estimated using a probability (Poisson) distribution fit to field observations of bison making contact with newborns and expelled birth tissues (panel A). The rate parameter (lambda) for the probability of exposure was adjusted to simulate historic seroprevalence ranges (40-60%) for Yellowstone bison (panel B).

A



B

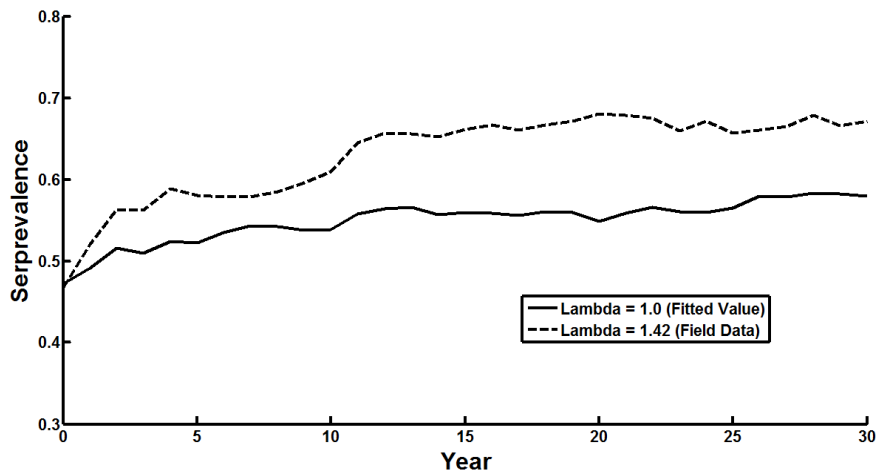
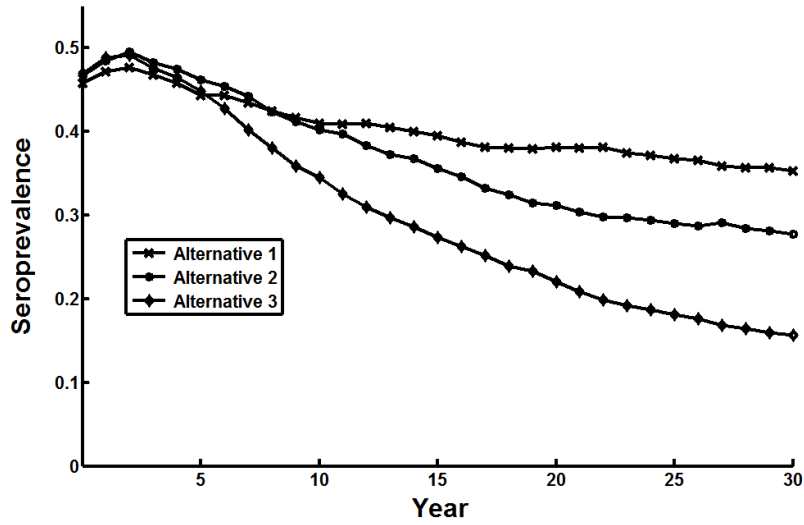


Figure 3.4 Simulated declines in brucellosis seroprevalence (panel A) and the proportion of the bison population vaccinated (panel B) for each of the vaccination alternatives.

A



B

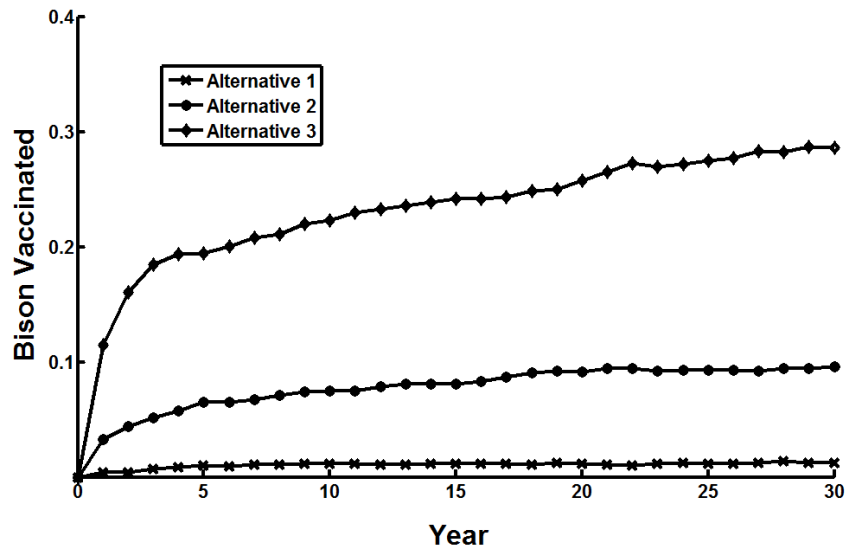


Figure 3.5 Simulated declines in seroprevalence for alternative 3 with waning vaccine protection. Line markers correspond to decreasing vaccine protection (based on the initial vaccine efficacy of 0.5) each year. The initial level of protection was restored if bison were re-vaccinated.

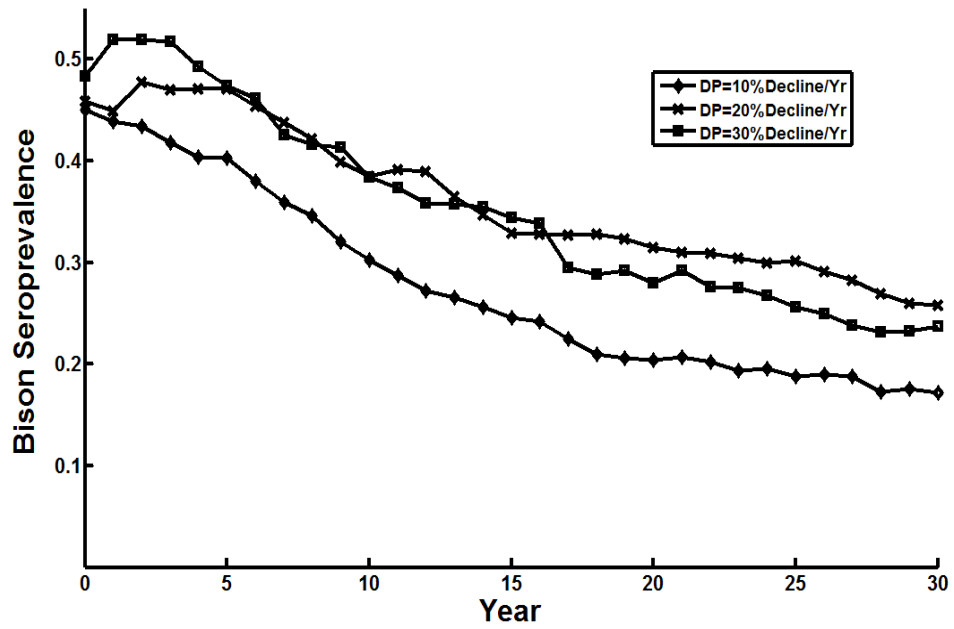
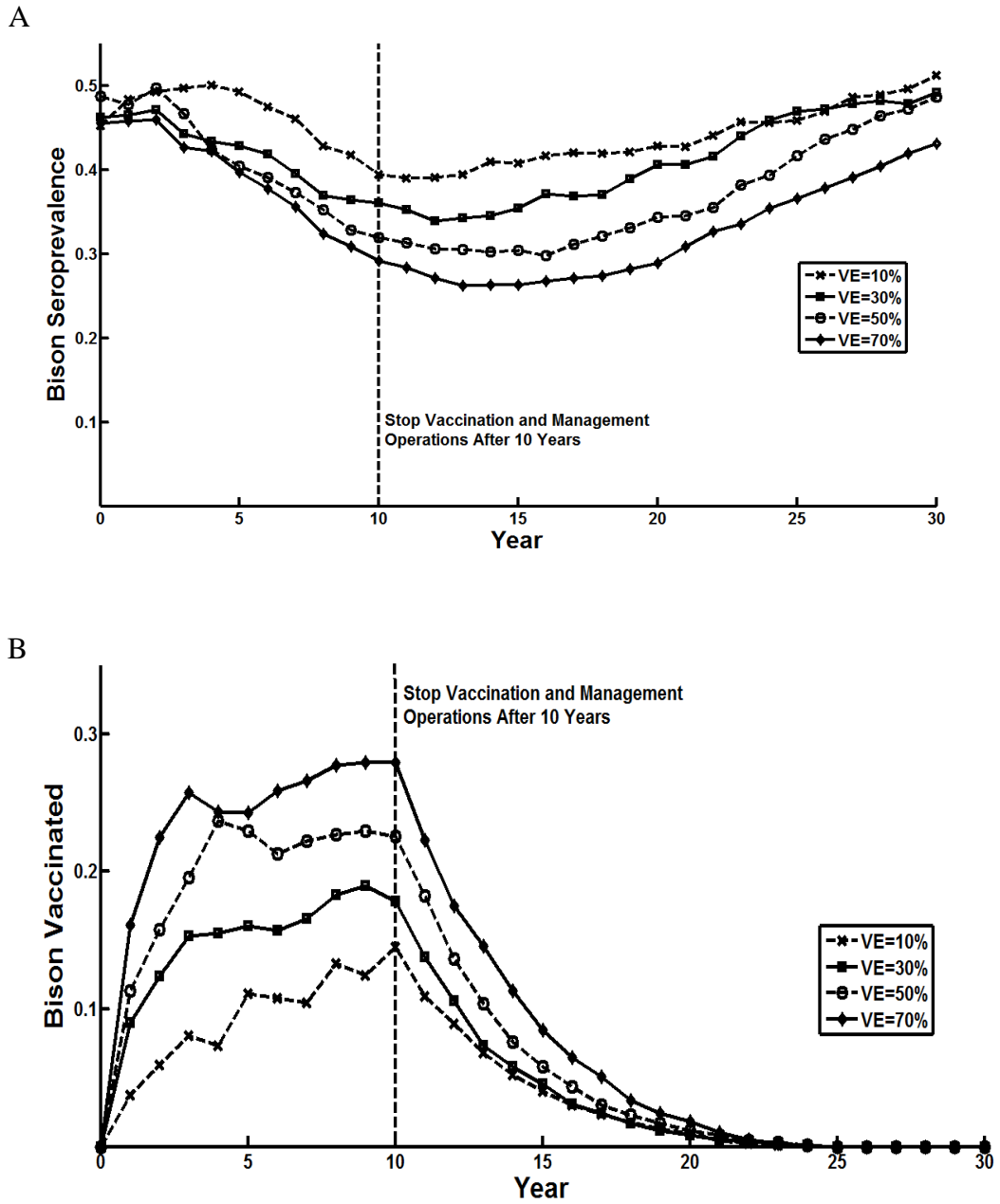


Figure 3.6 Simulations of short-term (10 years) vaccination and boundary management for alternative 3. Line markers correspond to the level of vaccine efficacy influencing seroprevalence declines (panel A) and the proportion of the bison population vaccinated (panel B).



Chapter 4 -Estimating probabilities of active brucellosis infection in Yellowstone bison through quantitative serology and tissue culture

Introduction

The increasing number of wildlife diseases transmissible to humans has raised worldwide concerns regarding free-ranging wildlife as a source of emerging human pathogens (Daszak, Cunningham & Hyatt 2000). In particular, agents of infectious diseases, such as bovine tuberculosis, brucellosis, and salmonellosis, which can establish persistent infections in wild ungulates, are especially difficult to manage (Renter *et al.* 2006; Cross *et al.* 2009; White *et al.* 2011). The limitation of diagnostic tests to accurately identify infectivity of persistent bacterial diseases in wild ungulates has led to disease management practices that are not aligned with wildlife conservation. Traditional test-and-slaughter programs, which have been effective for livestock management, may not be realistic or socially acceptable for wildlife (Bien & Tabor 2006). However, the sharing of diseases at the human, livestock and wildlife interface is a global problem (Böhm *et al.* 2007), which requires management practices that advance wildlife conservation as well as human and animal health.

During the past century, the livestock industry and wildlife managers in the Greater Yellowstone Area in the western U.S. have been concerned about the infectious disease brucellosis caused by the bacterium *Brucella abortus*. This zoonosis can infect the reproductive organs of several ungulate species during pregnancy, and can lead to the induction of late-term abortions of infected hosts. Aborted fetuses are highly infectious

and can serve as a transmission source for the disease (Thorne 2001). This non-native disease was most probably introduced to Yellowstone bison by European cattle (*Bos* spp.)(Cheville, McCullough & Paulson 1998) nearly a century ago (Mohler 1917). In 1934, a nationwide brucellosis eradication program was initiated and has since resulted in the elimination of *B. abortus* in most of the United States, with the exception of free-ranging wildlife in the three states surrounding Yellowstone National Park (Yellowstone): Montana, Idaho, and Wyoming. Cattle industries in these states have additional economic expense if they lose their brucellosis-free status, as has occurred in all three states due to multiple brucellosis exposures in the past decade (Montana Department of Livestock 2008). As such, this remaining pocket of brucellosis has led to decades of conflict among wildlife managers, environmental groups and the livestock industry over management of Yellowstone bison.

Although brucellosis-infected elk *Cervus elaphus* have been responsible for disease transmissions to cattle (Beja-Pereira *et al.* 2009; Higgins *et al.* 2012), Yellowstone bison have long been the focus of brucellosis management in the northern portion of the Greater Yellowstone Area. Yellowstone bison management operates under an Interagency Bison Management Plan (IBMP) aimed at conserving wild bison while reducing the risk of brucellosis transmission to Montana livestock (USDI and USDA 2000). Bison management practices used to prevent brucellosis transmission to local cattle conflicts with the goal of conserving bison and the processes that sustain them (e.g. migration). Severe winter conditions encourage bison movement to low elevation ranges outside Yellowstone (Geremia *et al.* 2011) where they are not tolerated because of the risk of transmitting brucellosis to cattle. In some years, large numbers of migrating bison

are captured and tested for brucellosis, with seropositive animals being shipped to slaughter. Approximately 3,200 Yellowstone bison were shipped to domestic slaughter facilities between 2001-2010, with 899 shipped during 2006 and 1,434 shipped during 2008.

These large scale bison removals have not been random, because bison social structure and the reproductive demands of pregnancy predispose female bison and their recent offspring (i.e. male and female calves and yearlings) to culling as they move onto low elevation winter ranges outside the park. The effects of several large, non-random culls during the past decade have contributed to a skewed sex ratio in favor of male bison, gaps in the population's age structure, and reduced productivity that, if continued over time, could reduce the potential of Yellowstone bison to respond to future challenges (White *et al.* 2011). Additionally, boundary culling has not contributed to a measurable reduction of brucellosis infection in the bison population. The proportion of seropositive adult female bison has increased slightly since 1985 or remained constant at approximately 60% (Hobbs *et al.* 2009).

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 provide protection to the overall population through the effect of herd immunity (John & Samuel 2000), 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. In bison, *B. abortus* antibodies are long lived (Rhyan *et al.* 2009), thus seroprevalence overestimates the level of active infection (Roffe *et al.* 1999) by failing to

distinguish between infected and recovered animals (i.e. bison that have cleared the bacteria). Though it is highly probable that bison can have serologic titers and not be infected (Cheville *et al.* 1998), all seropositive Yellowstone bison have been treated as actively infected because of potentially chronic or latent forms of the disease.

The ability of *B. abortus* to establish undetectable latent infections in young animals (e.g. heifer syndrome) has been documented in cattle (Lapraik *et al.* 1975; Wilesmith 1978; Catlin & Sheehan 1986), though the occurrence of latency is infrequent (Ray *et al.* 1988; Rhyan *et al.* 2009). Latently infected animals are typically exposed as calves and do not react on serologic tests until they have calved or aborted their pregnancy following infection. The proportion of adult bison that develop chronic infections (i.e. persistent infection of lymphatic tissue) following acute disease (i.e. reproductive tract infections) is unknown. However, the epidemiology and pathogenesis of brucellosis in chronically infected bison is similar to chronically infected cattle (Rhyan *et al.* 2009), and most infected cattle recover by clearing the infection and exhibiting life-long immunity (Ficht 2003).

The epidemiology of brucellosis in Yellowstone bison appears typical of an endemic disease, with a reduced portion of seropositive animals being actively infected and immune protection increasing with age. *B. abortus* has been isolated from 46% of seropositive Yellowstone bison, with young bison and high antibody titered animals predominantly infected (Roffe *et al.* 1999). Thus, the relationship between bison demography and quantitative serologic responses may be an important association for identifying actively infected animals. I integrated age-specific serology and *B. abortus* culture results from Yellowstone bison shipped to slaughter in 2008 to estimate

probabilities of active infection. I then applied this information to brucellosis risk management for the purpose of identifying high risk animals (i.e. those contributing to brucellosis maintenance) and low risk animals (i.e. recovered bison contributing to herd immunity). This approach has important application to the growing problem of disease management along the boundaries of protected reserves (Newmark 2008). The ability to target specific individuals that disproportionately contribute to the maintenance of infectious disease can improve the effectiveness of disease management programs while supporting long-term conservation efforts.

Methods

Study area

Yellowstone bison comprise the largest (3,000-5,000) wild population of plains bison in North America. Two semi-distinct breeding herds migrate and disperse across an extensive landscape (>90,000 ha). The central herd occupies the central plateau, which extends from the Pelican and Hayden valleys with a maximum elevation of 2,400 m in the east to the lower-elevation and geothermally-influenced Madison headwaters area in the west. Winters are often severe, with snow water equivalents (i.e. mean water content of a column of snow) averaging 35 cm and temperatures reaching -42 C. The northern herd occupies the northern portion of Yellowstone where elevation decreases from 2,200-1,600 m over approximately 90 km between Cooke City and Gardiner, Montana. The northern range is drier and warmer than the rest of the park, with mean snow water equivalents decreasing from 30 to 2 cm along the east-west elevation gradient. In both breeding herds, bison tend to migrate to lower-elevation ranges in and

outside the park as bison density and climatic factors (i.e. snow, drought) interact to limit food availability (Geremia *et al.* 2011).

Data collection

During the winter and spring (February-April) of 2008, 1,805 migrating bison were captured and held at bison management facilities on the northern and western boundaries of Yellowstone. A total of 1,434 bison were consigned to slaughter by the IBMP partner agencies. All bison captured at the west boundary ($n = 158$) were shipped untested to slaughter. The large number of bison exiting the park's northern boundary ($n = 1,647$) resulted in bison shipped untested to slaughter ($n = 860$) between 11 February 2008 and 19 March 2008. Bison were transported in trailers from Yellowstone to slaughter houses in Montana and Idaho where blood was collected by state or federal inspectors and transferred to state diagnostic laboratories for serologic testing.

After 19 March 2008, many of the bison ($n = 191$) shipped to slaughter from the park's northern boundary were animals that tested positive on standard serologic tests conducted at the boundary facility. Test-negative bison were held at the northern boundary facility and released into the park in May 2008. For bison that were tested prior to being shipped to slaughter, whole blood was collected into vacutainer blood tubes, immediately centrifuged, and serum was tested for *B. abortus* antibodies using the standard card test and Fluorescent Polarization Assay (FPA). The quantitative FPA is the diagnostic test of choice for bovine brucellosis because of its high sensitivity (94.5%) and specificity (99.5%), quick diagnosis, and ease of use (Gall & Neilsen 2001). FPA is a homogenous assay that measures the rotation time of labeled antigen molecules through a

specified angle of polarized light, where slower molecular rotation times indicate larger molecule size because of the binding of *B. abortus* antibodies (Muma *et al.* 2006). Measured rotational times are converted to milli-polarisations (mP) where seropositivity is determined by comparisons to mP values of positive and negative controls. FPA results were obtained from state laboratories for bison that were shipped untested to slaughter and from Yellowstone's northern boundary capture facility for tested bison using the Sentry Fluorescence Polarisation Analyser (Diachemix Sentry TM 100, single tube reader, Diachemix LLC, Wisc.USAFPM-1).

I collected tissue samples from 402 slaughtered bison for culture of *B. abortus*. At receiving slaughter houses, bison were killed by gunshot, and tissues were collected immediately after death. Blood was collected to test for presence of *B. abortus* antibodies using FPA. Depending on bison sex and age, I collected a section of mammary gland and pairs of the following lymph nodes which have been identified as the priority tissues for isolating *B. abortus* from Yellowstone bison (Rhyan *et al.* 2001): 1) retropharyngeal, 2) supramammary, 3) superficial inguinal, and 4) internal iliac. Tissues were collected into sterile Whirl-Paks (Nasco, Fort Atkinson, Wisconsin) and stored frozen (-20°C) until sent to the National Veterinary Services Laboratories (Ames, Iowa, USA). Ages of young bison (<5 years old) were determined by incisor eruption patterns (Fuller 1959) and by cementum annuli analysis of the first incisor for older bison (≥ 5 years old) with all permanent teeth.

The long held gold standard for brucellosis diagnosis is isolation of the bacteria. Though molecular diagnostic techniques are promising (e.g. polymerase chain reaction (PCR) assays), much work still needs to be done before they can be used in routine

brucellosis testing (Yu & Nielsen 2010). Consequently, application of the PCR assay for *B. abortus* testing in bison blood samples have not been accurate, as results have largely been negative in culture positive animals (Roberto & Newby 2007). Similarly, culture assays may not be sensitive enough to identify low level infections in all animals sampled without culturing an unrealistic amount of tissue. Based on the large sample size in this study, I chose a subset of tissues demonstrating no statistical difference in isolating *B. abortus* from Yellowstone bison compared to a much larger comprehensive set of tissues (Schumaker *et al.* 2010).

Brucella abortus isolation from tissue samples was conducted according to established standard operating procedures (USDA-NVSL a). Trained staff minced and then mixed each tissue separately in phosphate buffered saline and plated the macerated tissue suspension onto the agar surface of 5 plates: 1 tryptose agar plate with 5% bovine serum, 1 tryptose agar plate with 5% bovine serum and antibiotics; 1 tryptose agar plate with 5% bovine serum, antibiotics, and ethyl violet; 1 Farrell plate; and 1 Ewalt plate. Inverted plates were incubated at 37°C with 10% CO₂ for a minimum of 10 days. Plates were observed at 5 days and 10 days for the presence of morphologically suspect *Brucella* colonies. Suspect colonies were identified as *B. abortus* using traditional biochemical analysis (USDA-NVSL b).

Bayesian analysis framework

I used a Bayesian approach to estimate the probability of *B. abortus* presence in targeted bison tissues based on bison age and quantitative serology data collected in 2008. I collected at least partial information from 402 bison, including gender (402), age (401), culture results (397), and serologic status (299). Approximately 41% ($n = 165$) of

the slaughtered bison studied were sampled after brucellosis testing began. This may have biased culture results toward seropositive animals that were more likely to be actively infected and not representative of the infection status of the Yellowstone population. I corrected for this by using a two-sample test of proportions and determined that the portion of culture-positive calves was significantly higher once disease screening began ($P < 0.0001$, $n = 49$). Thus, in this analysis I considered calves prior to disease screening and all results for other ages ($n = 374$). Antibody values specific to *B. abortus* were determined using the FPA based on the difference between the observed mP value and the negative control. I centered and scaled the net FPA difference by subtracting the mean from the raw values and dividing the difference by the mean. Centered and scaled values were between -1.25 and 2.25, which facilitated model convergence. Bison age, determined using incisor eruption patterns and cementum annuli analyses for animals >5 years old, varied between <1 and 15 years. Of the 374 bison with culture results, I had 273 results with FPA and age data, and 101 results with only age data. Censoring results when one of multiple predictor values is unknown increases the uncertainty of parameter estimates (Gelman & Hill 2007). The Bayesian framework provides a coherent method for imputation by treating missing values as random variables that arise from the distribution of observed values (Clark 2007), and I imputed missing FPA values within this Monte Carlo Markov chain algorithm.

I hypothesized that Yellowstone bison are exposed to *B. abortus* at a young age and experience acute infection early in their reproductive lives from which they generally recover (i.e. clear *B. abortus*). I expected the probability of active infection to increase rapidly after birth through reproductive maturity followed by a gradual decline with

increasing age. The Ricker function, a common phenomenological model used for ecological variables, starts at zero, increases to a maximal value, and gradually decreases back to zero. This functional form allowed a process model to be developed that could describe exposure early in life, followed by acute infection at reproductive maturity, and gradual recovery with increasing age. In its simplest form, the Ricker model includes the shape parameters a , the initial linear rate of increase, and b , the reciprocal of the maximum response value. I chose a single functional form for this process model with interpretable parameters based on previous brucellosis studies in Yellowstone bison (Pac & Frey 1991; Roffe *et al.* 1999).

My response variable y_i , was active *B. abortus* infection identified by bacterial culture of a single colony forming unit from tissues collected from each individual bison. Thus, bison were either culture positive or culture negative. I treated y_i as a random Bernoulli variable with probability of isolating bacteria equaling the Ricker equation $aA_i e^{-bA_i}$ where A_i was animal age. This basic model was used with age as the single predictor variable to determine if the data supported my hypothesis of early infection and recovery with age. A Bayesian analysis seeks the posterior distribution which is the probability of the parameters conditional on the data. I initially evaluated the posterior distribution as:

$$P(a, b | Y, A) \propto \prod_{i=1}^{374} \text{Bernoulli}(y_i | a, b, A_i) \times \text{gamma}(a | 0.001, 0.001) \times \text{beta}(b | 1, 1)$$

I expected a positive relationship between FPA mP values and active infection status with higher FPA scores relating to higher culture prevalence. However, reproductively immature animals that are responding to active infection may have fairly low FPA values

due to competing protein needs (i.e. antibody production vs. growth and maintenance). Thus, I anticipated that the relationship between FPA and culture status would be more pronounced in older bison that have completed growth and achieved sexual maturity. To account for these effects, I again treated y_i as a random Bernoulli variable, but with a probability of isolating bacteria equaling $aA_i e^{-bA_i + cF_i + dA_i F_i}$ where F_i was individual FPA mP value. Missing FPA values ($F_{m,i}$) were imputed in the Monte Carlo Markov chain algorithm by treating missing values as arising from the distribution of observed values, where $\bar{\mu}$ was the mean and σ was the variance of observed FPA values. In this refined analysis, I evaluated the posterior distribution as: $P(a, b, c, d, F_m | Y, A, F, \bar{\mu}, \sigma) \propto$

$$\prod_{i=1}^{273} \text{Bernoulli} (y_i | a, b, c, d, A_i, F_i) \times \prod_{i=274}^{374} \text{Bernoulli} (y_i | a, b, c, d, A_i, F_{m,i}) \\ \times \prod_{i=274}^{374} \text{normal} (F_{m,i} | \bar{\mu}, \sigma) \times \text{gamma} (a | 0.001, 0.001) \times \text{beta} (b | 1, 1) \\ \times \text{normal} (c | 0, 1000) \times \text{normal} (d | 0, 1000)$$

Existing information on probability models of the parameters, or prior distributions, were unavailable. Thus, I used uninformative probability models illustrating that I knew little about the values of these parameters. I used normal distributions for the prior parameter distributions for FPA ($c \sim \text{NORMAL} (0, 1000)$) and age by FPA interaction ($d \sim \text{NORMAL} (0, 1000)$). I used a gamma distribution to estimate the initial linear rate of increase ($a \sim \text{GAMMA} (0.001, 0.001)$) and beta distribution to estimate the reciprocal of the age of maximum infection probability ($b \sim \text{BETA}(1, 1)$).

Monte Carlo Markov Chain procedures were implemented using the RJAGS package to call JAGS (Plummer 2009) version 2.1.0 from R (R Core Development Team 2010). I ran each of the three models for 25,000 iterations using three different Monte

Carlo Markov chains. The first 5,000 iterations were excluded to allow for burn-in. Convergence was assessed visually and when the potential scale reduction factor was less than 1.1 for all parameters (Gelman & Hill 2007).

Bayesian application

I used the described Bayesian analysis framework to develop a management tool for making probabilistic statements about the true infection status of bison based on age and net positive FPA values. The objective of the tool is to increase the effectiveness of a brucellosis reduction program, such as vaccination by selectively removing bison with a specified probability of being infectious.

Results

Brucellosis seroprevalence in bison shipped untested to slaughter ($n = 237$) increased with age in bison < 6 years old and decreased in older age classes (Table 4.1). Active *B. abortus* infection measured by culture prevalence increased rapidly in bison < 3 years old, closely matching seroprevalence in young bison, and then decreased in age classes following reproductive maturity (Table 4.1). The highest culture prevalence was observed in 2.75 year old bison, just before the age of first parturition. The portion of sampled seropositive bison that were actively infected was highest in calves and decreased with age (Figure 4.1). FPA values in seropositive bison were more variable across ages for culture-positive bison compared to culture-negative animals (Figure 4.2), indicating that seropositive bison that were culture negative may maintain reduced levels of antibodies, while active infection raises this level because of repeated exposure to *B. abortus* antigen.

The magnitude of estimated parameters in a simple form of the Ricker model, which included only bison age as a predictor variable, indicated the probability of active *B. abortus* infection increased in young bison (median $a = 0.29$, 95% credible interval = 0.19-0.43) and peaked at 2.6 years of age (median $1/b = 2.63$, credible interval = 2.08-3.45), which coincided with middle to late gestation during the age of first pregnancy (Figure 4.3). These results suggest that active *B. abortus* infection in Yellowstone bison occurs early in life and peaks during the time when female bison would have their first opportunity to transmit the bacteria.

Posterior distributions from the process model which included covariate values for age and FPA allowed for testing whether higher antibody levels were associated with active infection in older bison. Posterior probabilities indicated that active infection was greatest in young bison while active infection in older bison was associated with higher FPA values (Figure 4.4). The posterior distributions of model parameters suggested that active infection increased with age in young bison (median $a = 0.32$, 95% credible interval = 0.20-0.49) and peaked just before age of first parturition (median $1/b = 2.22$, 95% credible interval = 1.72-3.03). FPA antibody levels had a positive effect on my ability to culture *B. abortus* (median $c = 0.32$, 95% credible interval = -0.04-0.64). There was a high probability (0.82) that the interaction of age and FPA was positively associated with active infection (median $d = 0.05$, 95% credible interval = -0.05-0.15), indicating the probability of active infection increased at higher FPA values in older bison.

I developed a management tool that forecasts the true infection status of bison based on age (0.75 to 14.75 years old) and net FPA values (-25 to 250 in 25 mP

increments). Posterior distributions of the probability of infection for each age and net FPA combination were recovered. I used the empirical cumulative distribution function to determine age and net FPA combinations such that the probability of infection was >95%, >85%, >75%, >65%, and >55% (Table 4.2).

Discussion

The data supported my hypothesis that *B. abortus* in bison behaves much like an endemic disease, with infection occurring primarily in young animals and recovery increasing with age. Bison age was an important predictor of active infection, in which active infection increased rapidly in young bison and peaked during the age of first pregnancy. These findings are in agreement with Rhyan *et al.* (2009), which found seroconversion rates in Yellowstone bison to be highest in calves and juveniles (20%) compared to adult females (10%). Bison social structure may predispose newborns and reproductively immature bison to *B. abortus* exposure. In Yellowstone, pregnant and barren females tend to associate with females in similar states of pregnancy (Rutberg 1984). Young bison in these groups have been observed licking birth tissues shed by calving females (Jones, Treanor & Wallen 2009). The high seroprevalence observed in reproductively immature bison may result from close associations with infectious, pregnant females. Additionally, neonates born to infected mothers may become infected at birth or through *B. abortus* in milk; nursing calves receive maternal antibodies, but these wane after 5-6 months of age (Rhyan *et al.* 2009). In this study, calves were approximately 9 months old when sampled, and antibodies were highly associated with active infection (Table 4.1), suggesting active *B. abortus* infection begins early in life.

The highest probability of active infection was in bison approximately 2.75 years old. Seroprevalence (0.46) and culture prevalence (0.43) were also similar in this age class, which may be caused by systemic infection following reproductive maturity. Ingestion of *B. abortus* into the gastrointestinal tract is the most common route of exposure from where infection spreads to local lymph nodes before colonization of the uterus during pregnancy (Carvalho Neta *et al.* 2010). The proliferation of *Brucella*, necessary for transmission, is influenced by the stage of gestation (Nicoletti 1980), with increasing replication in placental cells during late gestation (Carvalho Neta *et al.* 2010). Female bison in Yellowstone typically experience their first fertile oestrous early (27 months of age) as two year olds. In the present study, all reproductively mature female bison sampled were in middle to late stages of gestation, suggesting that this large, recently infected age group may be the primary source of infection for the overall population.

Seroprevalence tracked culture prevalence in young animals but diverged after reproductive maturity (age 2, Table 4.1). Similarly, the proportion of seropositive bison found to be culture positive decreased as age increased, with low levels of active infection prevalence after age 5 (Figure 4.1). These results suggest that bison exposed to *B. abortus* early in life may begin to recover from acute infection after their first pregnancy following seroconversion. However, *B. abortus* exposure, measured in seroprevalence, increased until bison were older than 5 years of age. Rhyan *et al.* (2009) found the greatest potential for positive tissue culture occurred within 2 years following seroconversion, which suggests a high risk of transmission during this period. In this study, seroconversion began early in life, prior to reproductive maturity, and active

infection peaked at the age of first parturition with a steady decrease in the probability of active infection beyond the age of 3. This suggests that young bison (< 3 years old) may be a vulnerable age class to *B. abortus* infection in comparison to older animals, where a large proportion has experienced *B. abortus* infection earlier in life.

The protective effect of the humoral immune response (i.e. serum antibodies) in brucellosis is mild or questionable (Carvalho Neta *et al.* 2010) because the *Brucellae* are intracellular pathogens able to hide from humoral defenses. Accordingly, the positive association between bacterial isolation and high serologic titers (Roffe *et al.* 1999; Thorne 2001) may indicate reactivation of persistent infection or recent exposure. In the absence of *B. abortus* antigen, antibody levels are expected to decrease. Yellowstone bison identified with low antibody titers have converted back to seronegative status (Rhyan *et al.* 2009). This finding may explain the delayed decline in seroprevalence after the age of 5 in comparison to declines in culture prevalence after the age of 3. If bison are able to clear the infection with age, then one may observe a slower decrease in seroprevalence because of circulating antibodies (i.e., long-lived humoral IgG responses), high test sensitivity, and spikes in antibodies resulting from re-exposure.

I found a positive relationship with FPA values and active infection (Figure 4.2, Figure 4.4), supporting the view that active infection is associated with increased antibody production. Bison that have cleared the infection will still react positive on serologic tests until antibodies have decreased below detectable levels. The FPA is a sensitive test for detecting antibodies specific to *B. abortus*, and the high rates of seroprevalence in older bison include animals that seroconverted at younger ages because

of antibody persistence. These bison may have undergone acute infection early in life and have developed some level of immunity against re-exposure.

Though *B. abortus* antibodies may not be protective, they do play an important role in decreasing the number of bacteria upon subsequent exposure. Opsonized *Brucellae*, which are extracellular bacteria marked for destruction by host antibodies, are less likely to survive and establish intracellular infections (Carvalho Neta et al. 2010). Once *B. abortus* establishes intracellular infection, bacterial clearance requires effective cell-mediated immune responses induced by activation of specialized T-cells (Oliveira, Soeurt & Splitter 2002). Memory T-cells persist after the infection has been cleared and allow for secondary responses to low concentrations of *B. abortus* antigen that are faster and of greater magnitude than the primary response (Bernard & Tough 2002). Effective cell-mediated immune responses may clear the initial infection and quickly respond to secondary infections, thereby reducing antibody levels as a result of eliminating *B. abortus* from bison tissues. Thus, high antibody levels may indicate ineffective cell-mediated immune responses, and, conversely, effective cell-mediated immune responses may clear bacteria and reduce *B. abortus* antibody production.

A limitation of my work may be that I failed to detect low-level chronic infections in adult bison. *Brucella abortus* is known for its intracellular hiding ability which can facilitate long-term persistence (Spera et al. 2006). Consequently, the association of high serologic responses with active infection in older bison may indicate recent exposure or recrudescence of chronic infection. Chronically infected female cattle have been observed to periodically shed *B. abortus* in genital infections (Manthei, DeTray & Goode 1950; Lambert et al. 1960). However, in my study, the relationship between antibody

levels and active infection in older bison suggests that low serologic responses may be indicative of recovery rather than chronic infection. The reactivation of infection is expected to occur from stress and favorable conditions for *B. abortus* proliferation during late gestation (Cheville *et al.* 1998). In 2008, bison were sampled in middle to late gestation during one of the most stressful years in Yellowstone's recorded history (based on high bison population density and winter severity). Thus, I would expect a high rate of recrudescence (i.e., active infection) in older animals if chronic infection was the typical progression of brucellosis in Yellowstone bison.

Management applications

The objective of the present study was not to identify every actively infected bison, but to provide managers with a reliable tool for identifying FPA cutoff values based on specified active infection probabilities and bison age (Table 4.2). This allows managers to take a conservation approach to bison management by targeting infectious animals based on active infection probabilities, which can be adjusted to different phases of a brucellosis reduction program. Currently, brucellosis seroprevalence is high (0.45, White *et al.* 2011), with bison diagnosed as seropositive when net FPA values typically exceed 10-20 mp. This tool allows managers to reduce removals when seroprevalence is high by identifying infectious bison (e.g. probability of active infection > 0.95) with a high level of certainty. Similarly, as immune protection increases against *B. abortus* through vaccination and seroprevalence declines, removal efforts can focus on the majority of *B. abortus* exposed bison (e.g. probability of active infection > 0.55) that are less likely to be actively infected. This targeted approach allows for removing high risk

individuals and increasing herd immunity through vaccination, while promoting bison conservation without large-scale culling.

Brucellosis in the greater Yellowstone area is one of the most challenging issues facing wildlife managers, livestock producers and the concerned public in the western United States. High genetic diversity, unique alleles, and lack of introgression of domestic cattle genes (Halbert 2003) make the Yellowstone bison population critical to the conservation of the species in North America. Because definitive brucellosis tests do not currently exist, the threat of spreading brucellosis has prevented translocation of Yellowstone bison for purposes of genetic augmentation of smaller herds elsewhere in North America. Yellowstone bison management has long relied on serologic tests which overestimate the level of brucellosis infection, the continuation of which may lead to management decisions that reduce bison numbers and reduce long-term viability of this unique population. I have presented a novel method for estimating active brucellosis infection in bison that will allow managers to better assess the relative risk of individual animals and influence culling decisions in a way that should reduce annual losses from this practice. My approach has broad application to balancing disease management with wildlife conservation, such as situations where migratory wildlife pose a risk to livestock and human health or when endangered wildlife may be impacted by infectious disease. The ability to identify infectious individuals, which disproportionately contribute to disease maintenance increases management options beyond traditional culling practices.

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Table 4.1. Age-specific seroprevalence and culture prevalence of *B. abortus* in Yellowstone bison shipped untested to slaughter during the winter of 2008.

Bison Age	Culture Prevalence	Seroprevalence	Seroconversion Rate*
0	0.11 (3/27)	0.10 (3/29)	0.10
1	0.28 (17/61)	0.37 (22/59)	0.21
2	0.43 (6/14)	0.46 (6/13)	0.19
3	0.11(3/27)	0.65 (17/26)	0.23
4	0.16 (4/25)	0.48 (11/23)	0.12
5	0.27 (6/22)	0.81 (17/21)	0.24
6+	0.16 (4/25)	0.54(13/24)	0.10

* Age specific seroconversion rates were estimated using the formula:

$p = 1 - (1 - P_y)^{1/y}$, where p = annual rate of seroconversion, y = age in years,
and P_y = seroprevalence at age y

Table 4.2. Minimum net FPA values for corresponding probabilities of active infection and bison age.

	Probability of Active <i>B. abortus</i> Infection				
	>95%	>85%	>75%	>65%	>55%
0.75	200	175	175	150	125
1.75	175	150	125	100	50
2.75	150	150	125	100	75
3.75	150	150	125	100	75
4.75	175	150	150	125	100
5.75	175	150	150	125	125
6.75	175	150	150	125	125
7.75	175	150	150	125	125
8.75	150	150	150	125	125
9.75	150	150	150	125	125
10.75	150	150	150	125	125
11.75	150	150	150	125	125
12.75	150	150	150	125	125
13.75	150	150	150	150	125
14.75	150	150	150	150	125

Figure 4.1. Age distribution of active *B. abortus* infection in seropositive Yellowstone bison shipped to slaughter during 2008. Values above bars indicate the proportion of culture positive bison for each specified age.

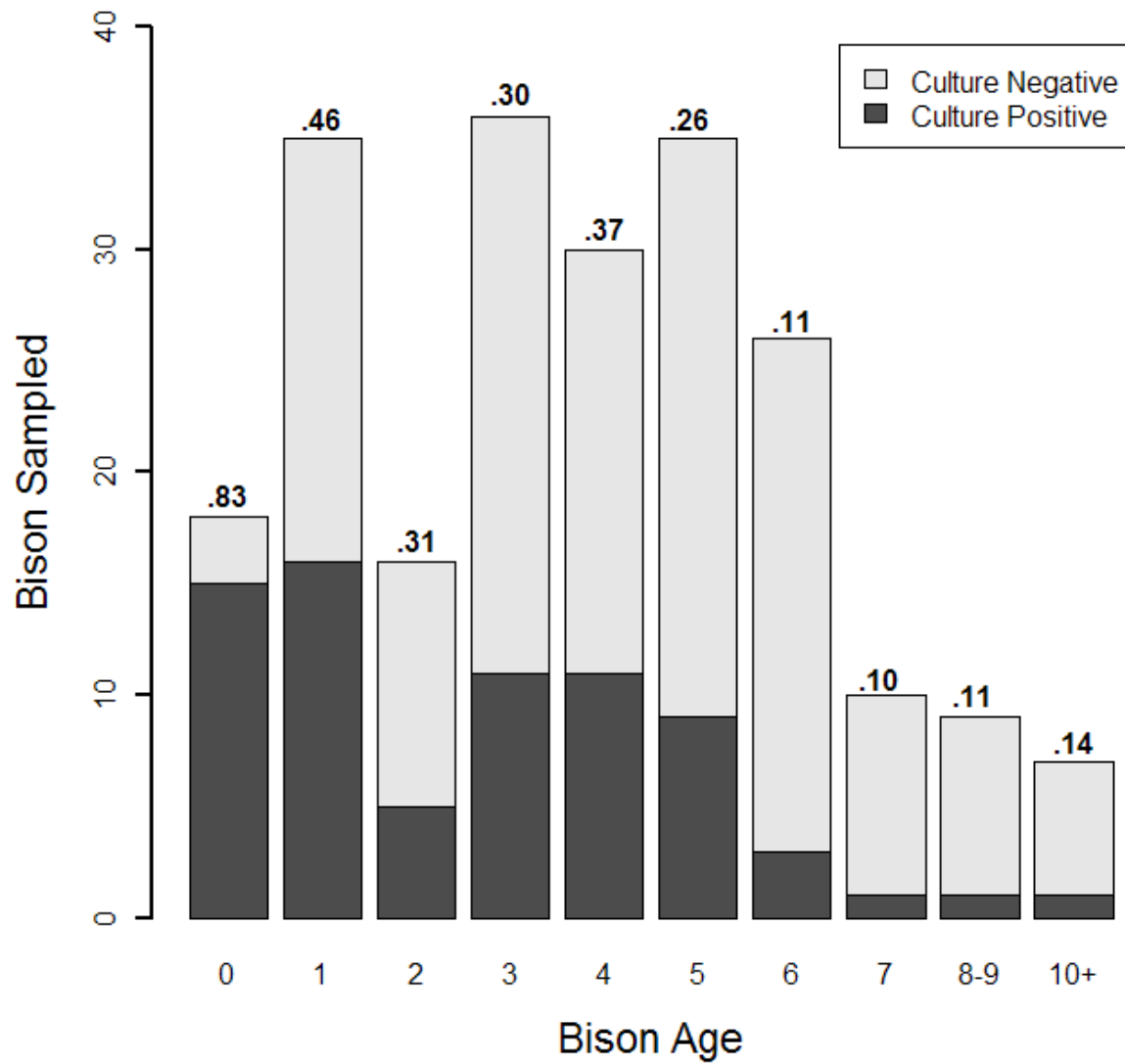


Figure 4.2. Age stratified FPA values (mP) based on *B. abortus* isolation from culture assays of bison.

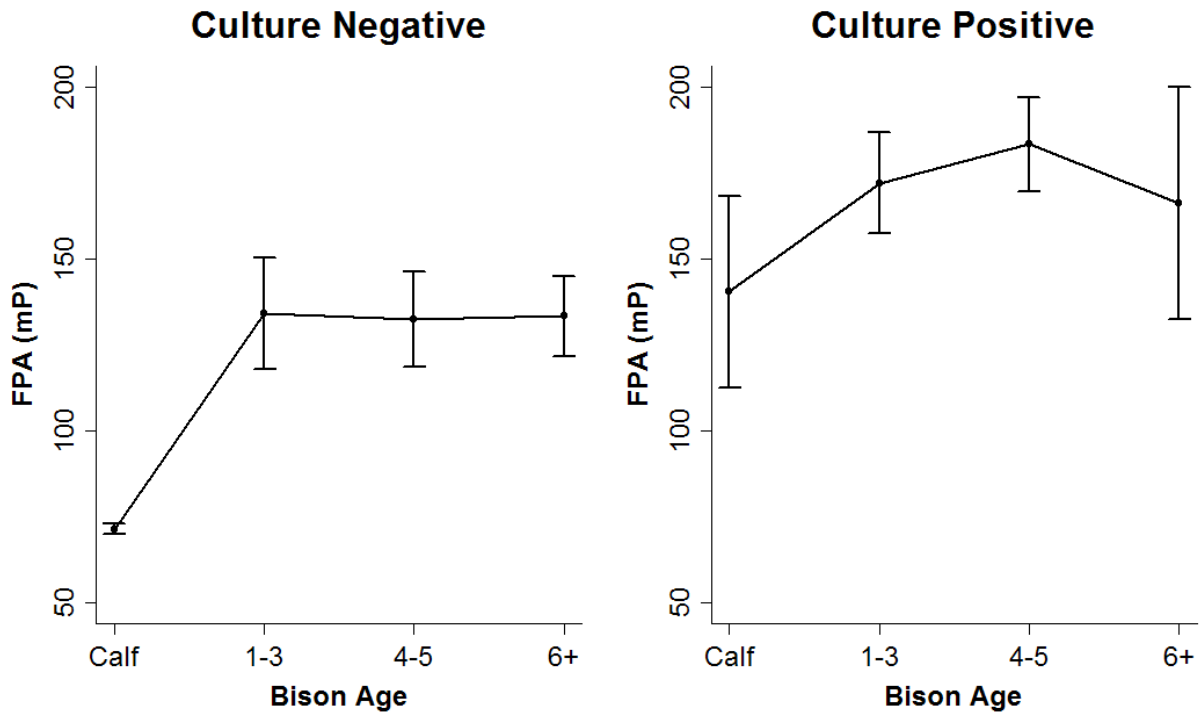


Figure 4.3. Estimated posterior probabilities of active *B. abortus* infection in bison based on shape parameter (p) estimates of a Bernoulli distribution. The shape parameter is the probability of observing active infection with bison age related by, $P = aA_i e^{-bA_i}$. The solid line represents the median and dotted lines represent the 95% credible interval. Points represent observed proportions of culture positive bison for specified ages.

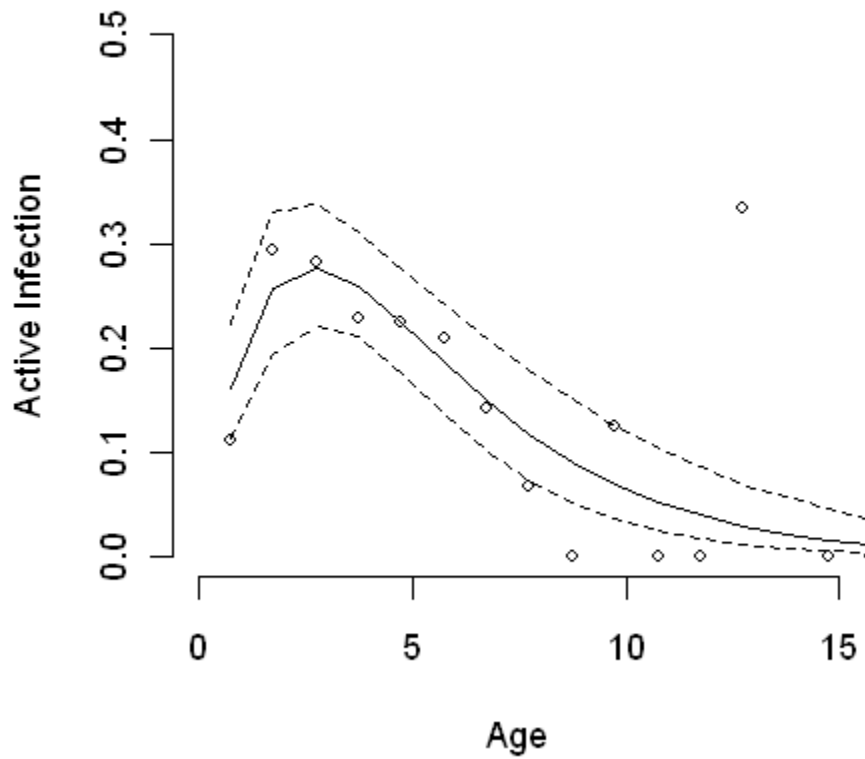
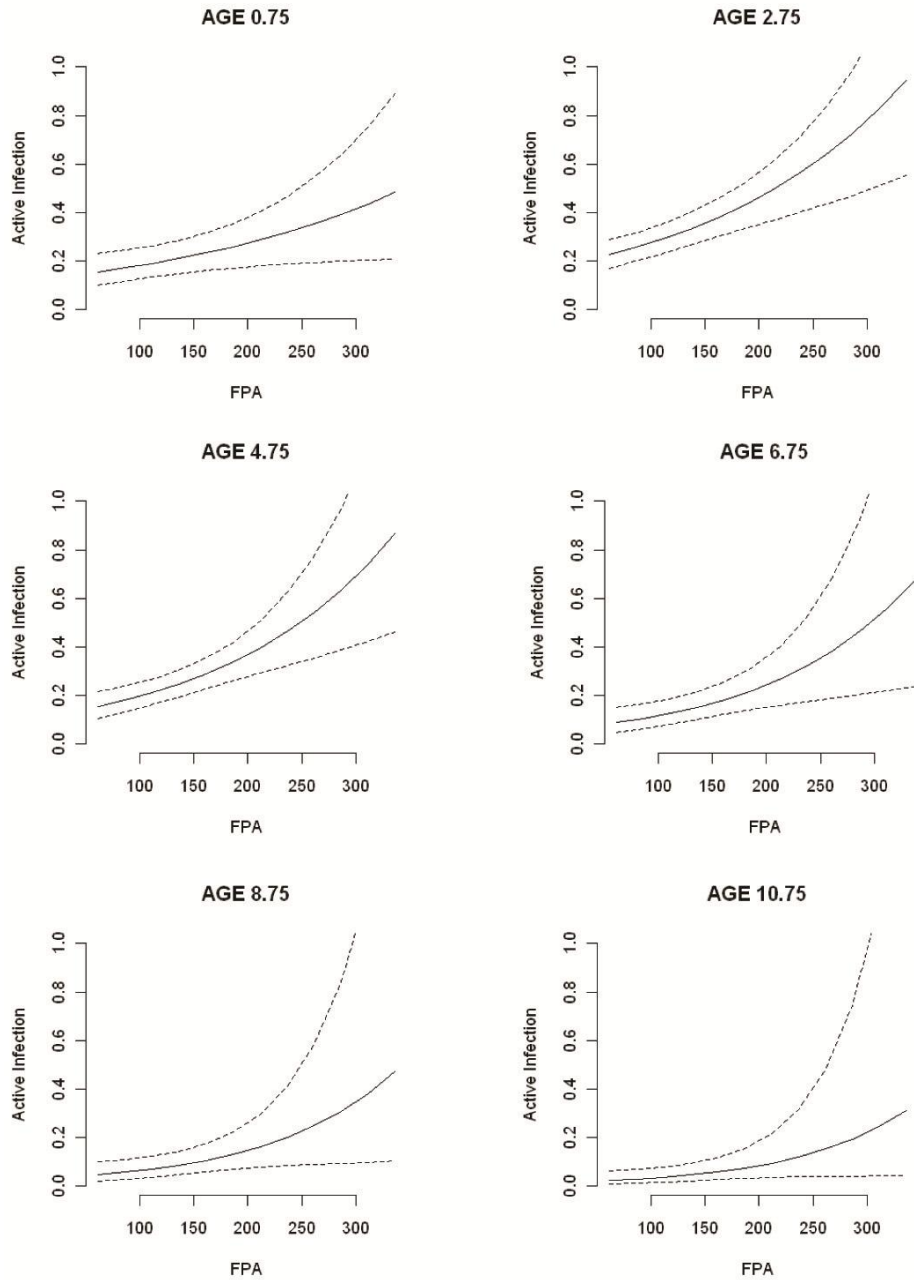


Figure 4.4. Age-specific estimated posterior probabilities of active *B. abortus* infection based on shape parameters (p) estimates of a Bernoulli distribution. The shape parameter is the probability of observing active infection with age and FPA related by, $p = aA_i e^{-bA_i + cF_i + dA_i F_i}$. The solid line represents the median and dotted lines represent the 95% credible interval.



Chapter 5 - Maintenance of brucellosis in Yellowstone bison: Linking seasonal food resources, host-pathogen interaction, and life-history trade-offs

Introduction

The acquisition and allocation of food resources are key factors shaping the life history strategies of organisms (Boggs 1992; Boggs 2009). During periods of food restriction, individuals may face trade-offs in the allocation of limited internal resources toward reproduction, survival, and growth (Stearns 1992; Zera & Harshman 2001). Consequently, investment in immune defense, which has high nutritional costs, may be reduced (Martin, Weil & Nelson 2008). Susceptibility to infectious disease may increase when seasonal food restriction overlaps periods of high nutritional demands because parasite infectivity is influenced by host condition and the timing of physiological tradeoffs (Jolly & Fernandes 2000; Bueler *et al.* 2010). Thus, individuals in poor nutritional condition may be more susceptible to infection, have higher infection intensities, and serve as an important source of infection within the population (Beldomenico & Begon 2009).

In seasonally cold environments, the quality and availability of forage fluctuates seasonally and is often limited for large mammalian herbivores during periods of high reproductive needs (Parker, Barboza & Gillingham 2009). Female ungulates may thus experience their lowest nutritional state near parturition, which can increase their susceptibility to pathogens known to establish chronic infections. Many persistent intracellular disease organisms, such as *Toxoplasma gondii*, *Coxiella burnetii*, and *Brucella abortus*, have life cycles that are timed with periods of high reproductive demands of their hosts (Innes *et al.* 2002; Berri *et al.* 2007; Carvalho Neta *et al.* 2010). The intracellular strategy allows these pathogens to hide

within host cells until the immune system is suppressed (Rhen *et al.* 2003), typically when reproductive costs increase in late gestation. If reproductive demands are prioritized over immune defense, nutrient resources allocated to fetal growth and lactation may lead to a loss of immune protection near parturition (Houdijk, Jessop & Kyriazakis 2001; Nyman *et al.* 2008).

The maintenance of brucellosis in Yellowstone bison may be linked to periods of nutritional stress and reduced immune function, both seasonally and across host life stages. *Brucella abortus* is an intracellular bacterium transmitted primarily through the ingestion of infectious tissues (e.g. fetal membranes and uterine discharges) shed following an abortion or at parturition (Samartino & Entright 1993). Yellowstone bison are seasonal breeders with moderate synchrony in spring calving (Jones *et al.* 2010). This reproductive schedule restricts *B. abortus* transmission to mainly late gestation near parturition when there is an influx of vulnerable hosts (e.g. naïve newborns) into a nutritionally stressed population. Such seasonal conditions favor pathogens that can establish persistent infections enabling transmission during future pregnancies. The endemicity of brucellosis in Yellowstone bison may be a consequence of vulnerability in young animals and the timing of food restriction with seasonal reproductive needs.

Here I provide a conceptual framework describing how brucellosis may be maintained in Yellowstone bison based on life-history strategies of the host and pathogen with seasonal factors influencing infection and transmission (Figure 5.1). For pregnant bison, late gestation is a protein and energy demanding state as increasing demands of fetal development coincide with food restriction. Yellowstone bison are typically in negative energy balance during winter when endogenous reserves (fat and body protein) are used to meet energy requirements until spring green-up (DeGuidice *et al.* 2001). Calving is synchronized with the emergence of highly

nutritious spring forage, which reduces lactational demands and increases calf survival (Rutberg 1987). However, this strategy requires pregnant bison to be in a state of reduced body condition at a time when food is limited and reproductive demands of late gestation are high. The seasonal reduction in protein and energy can create a bottleneck that constrains immune defenses (Buehler, Tieleman, & Piersma 2010) and may open a transmission and infection window for *B. abortus*. The life-history traits of *B. abortus* (persistence, replication rate, and transmissibility) influence bacterial fitness and consequently overlap with the reproductive cycle of Yellowstone bison. Investment of internal resources toward growth and winter survival may increase susceptibility to infection in immature bison, with transmission potential increasing at sexual maturity. In contrast, older bison may have lower infection and transmission rates than younger animals because they are no longer growing and have had time to recover from infection acquired earlier in life.

In this study, I examined how age and nutritional condition affect active brucellosis infection and the intensity of infection in Yellowstone bison. First, I assessed whether seasonal changes in diet quality affect nutritional condition and coincide with reproductive needs in female bison. Next, I tested whether active brucellosis infection and infection intensities varied with host condition and nutrition. Last, I investigated evidence for seasonal changes in immune responses, which may offer protection against *B. abortus*, in relation to nutritional condition. I expected active *B. abortus* infection and the intensity of infection to be greatest in young bison and animals in poor condition across ages. I also expected the magnitude of protective immune responses to be greatest outside the infection and transmission window when bison are in good nutritional condition.

Methods

Study area and seasonality of food

The study was conducted in the Yellowstone National Park (Yellowstone) in the western United States during the winter of 2008, as described in Treanor *et al.* (2011). Yellowstone is over 898,000 ha within the states of Wyoming, Montana, and Idaho, of which >90,000 ha are habitat for bison. The bison population is divided into two separate breeding herds. The central herd occupies the central plateau and the northern herd occupies much of Yellowstone's northern boundary. The park's geologic processes have created a range in elevations that exerts strong control over the distribution of plant species (Marston & Anderson 1991). Plant communities range from grasslands and shrubs at low elevations, coniferous forest at mid elevations, and alpine tundra near peak elevations. Winter ranges for both bison herds consist of large valley bottoms and open meadows comprised of grasses and sage brush (*Artemisia tridentata*) bordered by coniferous forest. Yellowstone winters are often severe, with snow water equivalents (i.e. mean water content of a column of snow) averaging 35 cm and temperatures reaching -42° C. Bison are generalist grazers and their diet is mainly composed of graminoids (grasses and sedges). Winter conditions (e.g. deep snow pack) reduce foraging opportunities within the park, which influences winter movements out of the park where bison are not tolerated because of the risk of brucellosis transmission to privately owned domestic cattle (USDI & USDA 2000a; Geremia *et al.* 2011).

I collected bison fecal samples each month (April 2006 to September 2007) to assess seasonal changes in diet quality. Sampling was determined by the distribution of bison on sampling days. I located bison groups (> 20 individuals), consisting of adult females and associated young, across seasonal ranges. For each group sampled, approximately 5 fecal

samples of equal volume were collected from individual bison and pooled to make a single composite sample (n=143 composite samples) for diet quality analysis. Freshly deposited (nonfrozen) fecal samples were collected into sterile whirl paks (Nasco, Fort Atkinson, Wisconsin) and stored frozen (-20° C). Samples were analyzed for crude protein (CP) and digestible organic matter (DOM) using near infrared reflectance spectroscopy (NIRS). The NIRS technique is an effective method for assessing diet quality in livestock and wildlife (Showers *et al.* 2006; Li *et al.* 2007; Rothman *et al.* 2009).

Bison sampling

During the winter and spring (February-April) of 2008, 1434 Yellowstone bison were consigned to slaughter at facilities in Montana and Idaho. I collected tissue samples immediately post-mortum from 402 slaughtered bison for culture of *B. abortus*. Ages of young bison (< 5 years old) were determined by incisor eruption patterns (Fuller 1959) and by cementum annuli analysis of the first incisor for older bison (≥ 5 years old) with all permanent teeth. Pregnant females were sampled during the third trimester of pregnancy, and pregnancy status was determined by the presence of a fetus in the uterus. Depending on bison sex and age, I collected a section of mammary gland and pairs of the following lymph nodes, which have been identified as the priority tissues for isolating *B. abortus* from Yellowstone bison (Rhyan *et al.* 2001): 1) retropharyngeal, 2) supramammary, 3) superficial inguinal, and 4) internal iliac. Tissues were collected into sterile Whirl-Paks and stored frozen (-20°C) before being sent to the National Veterinary Services Laboratories (Ames, Iowa, USA). *Brucella abortus* isolation from tissue samples was conducted according to established standard operating procedures (USDA-NVSL a). Trained staff minced and then mixed each tissue separately in phosphate buffered saline and plated the macerated tissue suspension onto the agar surface of 5 plates: 1 tryptose agar plate

with 5% bovine serum, 1 tryptose agar plate with 5% bovine serum and antibiotics; 1 tryptose agar plate with 5% bovine serum, antibiotics, and ethyl violet; 1 Farrell plate; and 1 Ewalt plate. Inverted plates were incubated at 37°C with 10% CO₂ for a minimum of 10 days. Plates were observed at 5 days and 10 days for the presence of morphologically suspect *Brucella* colonies. For each plate, suspect colonies were identified as *B. abortus* using traditional biochemical analysis (USDA-NVSL b).

Indicators of nutrition, condition, and immune function

For large herbivores, nutrition refers to the assimilation rate of ingested energy and nutrients, while nutritional condition describes the varying state of an animal's fat reserves and muscle mass that ultimately result in future fitness (Stephenson *et al.* 2002). During periods of food restriction, body reserves are mobilized resulting in metabolic changes that can be measured as adjustments in blood concentrations of hormones and metabolites (Ingvarsen & Friggens 2005). For each bison, whole blood was collected into 10-mL vacutainer tubes containing 15% EDTA (K₃). Blood was centrifuged within 3 hours after collection and separated plasma was stored frozen (-80 °C). Concentrations of plasma non-esterified fatty acids (NEFA, *n* = 352) and urea nitrogen (BUN, *n* = 374) were measured enzymatically in duplicate according to Ballou *et al.* (2008). Plasma concentrations of insulin-like growth factor 1 (IGF-1, *n* = 328) and leptin (*n* = 326) were analyzed by radioimmunoassay in duplicate according to Delavaud *et al.* (2000).

Bison condition was assessed using live weight, backfat measurements, and body condition scoring for each age group. The live weights (kg) of bison (*n* = 262) were recorded at the Stephens Creek capture facility on Yellowstone's northern boundary using an electronic platform scale before being shipped to slaughter houses. All slaughtered bison were skinned and eviscerated, with carcasses split along the midline (midsagittal plane). Subcutaneous backfat

(mm) was measured at the thickest point on carcasses just posterior to the last sacral vertebra. Carcass weights (kg, $n = 214$) were measured after processing on bison without hide, head, blood internal organs, and feet. Since multiple bison were being processed simultaneously, a modified kistner score was used to quickly evaluate the physical condition of carcasses (Kistner Trainer & Hartmann 1980). Body condition scores were determined based on the distribution of subcutaneous fat on the carcass and visceral fat associated with internal organs. Subjective fat indices were rated on a scale from 0 to 5 with 0.5 increments. Scores of three subcutaneous indicator sites (rump, hump, and brisket) and three visceral locations (omentum, cardiac, and perirenal) were used to produce a mean score for each bison. Each of the six sites was scored as 0-1 (little to no fat), 2-3 (moderate amount of fat), 4-5 (abundant fat).

Cell-mediated immune responses are essential for host defense against *B. abortus*, with a bias toward $T_{\text{helper}1}$ responses, characterized by the production of interferon- γ (IFN- γ), being important (Waters *et al.* 2002). Seasonal changes in IFN- γ were assessed from bison captured during 2007 to 2009 as described in Clapp *et al.* (2011). Blood samples were collected from female bison ($n = 69$, ages 1 to 11) held at the Stephens Creek capture facility and from bison captured via chemical immobilization within Yellowstone as part of bison management and monitoring. Blood was collected from the jugular vein into 10-ml vacutainer tubes containing sodium heparin, refrigerated or placed on ice, and processed within 24 hours after collection. Peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation, washed three times with RPMI 1640 and resuspended in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated horse serum (GIBCO BRL), and 100 U/ml penicillin, 100 mg/ml streptomycin. PBMC at 5×10^6 cells/ml in medium were aliquoted into 24-well tissue plates. Duplicate cultures were stimulated with concanavalin A (ConA, 1 μ g/ml) or

unstimulated (medium only). Cultures were incubated at 37°C with 5% CO₂ for 5 days and stored at -80°C until analysis. Interferon- γ in culture supernatants was measured using a bovine capture ELISA. Concentrations of IFN- γ were determined by comparing absorbances of test samples with absorbances of standards within a linear curve fit.

Statistical analysis

Generalized linear models were used to evaluate the effects of age-specific nutrition and condition on active infection and infection intensity. The data for bison age were standardized by subtracting and dividing each value by the mean. Covariates for all analyses were also scaled with respect to bison age. Because condition indicators may be correlated with age, these transformations standardized covariates for each age group as the percentage above or below the mean, which facilitated interpretation of covariate effects.

To evaluate active infection in female bison ($n = 152$), culture status was defined as a binary response variable based on the presence of at least one colony forming unit of *Brucella abortus* on a single culture plate for all tissues assayed. The inverse-logit transform was used to relate the probability of observing a positive culture status to covariates for bison age and condition. Animals ≥ 8 years of age were grouped into a single old-adult age class. A suite of 11 logistic models was developed to represent alternative hypotheses of bison age and the effect of nutritional condition on active infection. Active *B. abortus* infection was expected to correspond with sexual maturation in female bison (Treanor *et al.* 2011), thus I included a quadratic term for age effects in all models. The model suite consisted of additive models, which included all combinations of age, the quadratic effect of age, live weight, and back fat thickness. I expected bison live weight and back fat thickness to be negatively associated with active *B. abortus* infection.

I expected the intensity of *B. abortus* infection to increase during periods of food restriction, as body reserves were metabolized to meet competing nutritional demands. Culture results were reevaluated from actively infected bison (e.g. culture positive, $n = 94$) to identify age-specific effects of nutrition and condition on the intensity of infection using logistic-binomial models. The response was the total number of culture positive plates observed for each individual. Infection intensity was defined as the probability of observing a culture positive result on a single plate, with the probability corresponding to covariates for bison age, condition, and nutrition using the inverse-logit transform. Bison ≥ 7 years of age were grouped into a single old-adult age class. To maximize sample size, the effects of nutrition and condition were analyzed separately. A suite of 22 additive models was developed to assess the effects of condition indicators ($n = 53$ records) on infection intensity. The model suite included all combinations of age, the quadratic effect of age live weight, back fat thickness and body condition score. Because Yellowstone bison become exposed to *B. abortus* as juveniles, I predicted the intensity of infection would be greatest in younger ages (i.e., reproductively immature animals), with infection intensity negatively associated with age and covariates for condition. I also developed a suite of 32 models to assess effects of nutrition (metabolites and hormones; $n = 78$ records) on infection intensity. The model suite included all combinations of age, the quadratic effect of age, and concentrations of plasma indicators of nutrition (BUN, NEFA, IGF-1, and leptin). Again, I predicted the intensity of *B. abortus* infection to be greatest in young bison experiencing undernutrition. I expected infection intensity to be negatively associated with age, BUN, IGF-1 and leptin, but positively associated with NEFA.

Maximum likelihood estimation was used to estimate model parameters using the package `bbmle` in program R (R Development Core Team 2010). Models were ranked using

Akaike's Information Criterion corrected for small sample size (AIC_c); normalized AIC_c weights (w_i) were used to compare candidate models. Models with AIC_c differences (Δ_i) ≤ 2 from the top supported model were considered to offer substantial empirical support, while models with $4 \leq \Delta_i \leq 7$ have considerably less support and models having values $\Delta_i \geq 10$ having essentially no support (Burnham & Anderson 2002). We examined 95% confidence intervals of model parameters to determine the relative magnitude and direction of effects. Normalized AIC_c weights (w_i) were used to compare the fit of candidate models to the data.

Results

Season and age effects

The analysis of bison fecal samples demonstrated a seasonal pattern in diet quality, with peak forage quality occurring immediately following spring green-up and extending into early summer. The period from May to June, which corresponds with the timing of bison calving and high lactation demands, had the largest percentage of crude protein and digestible organic matter (Figure 5.2a). The ratio of digestible organic matter to crude protein (DOM:CP) is a useful metric of rumen efficiency, with a range between 4 and 8 being acceptable and 4 being optimal (Odadi *et al.* 2011). The DOM:CP ratio was within the range expected for weight gain during late spring and summer, while the ratio during winter and early spring (Jan-April) reflected low quality forage (Figure 5.2b).

Reduced body condition and metabolic and hormonal responses of pregnant bison sampled during late gestation (Feb 9 to April 9) implied a prolonged state of undernutrition (Figure 5.3). Mean concentrations of NEFA in pregnant bison increased during the sampling period and were larger than concentrations in reproductively mature nonpregnant bison (Figure 5.3a), indicating a shortage of dietary energy. The rise in BUN and corresponding decline in

IGF-1 (Figure 5.3b) reflect a shortage in dietary protein that coincided with a decline in dietary energy. The mean concentration of plasma leptin and backfat thickness declined during late gestation (Figure 5.3c) further reflecting an increase in fat metabolism and a state of negative energy balance. Annual changes in bison live weight and carcass weight were significantly greater ($P < 0.001$) in young bison below the age of three than in older individuals (Figure 5.4).

The secretion of IFN- γ by stimulated cells was greater ($P < 0.05$) in bison sampled during fall months than winter or spring (Figure 5.5a). Plasma leptin concentrations were lower ($P < 0.05$) in spring than winter (Figure 5.5b) indicating a seasonal decline in energy reserves. Mean IFN- γ responses to unstimulated cells (media only) was near zero for all seasons measured (fall=0, Winter=0.12, and spring = 0.08 ng/ml IFN- γ).

Analysis of infection

The probability of active brucellosis infection was influenced by age and age-specific live weight with the highest probability observed in early reproducing bison (ages 3 to 5). The two top supported models (83% of w_i ; Table 5.1) included covariates for bison age, live weight and back fat. However, the 95% confidence interval for the back fat coefficient spanned zero (Table 5.2) suggesting that it did not improve model performance. The coefficient estimates for bison live weight and age² did not span zero and indicated these covariates were negatively associated with the probability of active infection. Fitting bison age as a quadratic covariate improved model fit by allowing for a curvilinear response such that the probability of active infection could increase early in life, peak during early reproductive years and decline with increasing age. The influence of live weight had less support in models without age² ($\Delta AIC_c = 4.43-4.97$), while models without live weight had essentially no support ($\Delta AIC_c > 10$; $\sum w_i = 0$).

These findings suggest that bison in below average body condition in this early reproducing age group had the highest probability of active brucellosis infection (Fig 5.6a).

In actively infected bison, the intensity of infection was influenced by age and live weight (5.6b). There was strong support for models that included live weight, age, and age² ($\sum w_i = 0.95$; Table 5.1). The coefficient estimates for back fat thickness and body condition score were unstable. For these covariates, the direction of the effect varied between models and the 95% confidence interval for the estimated coefficients spanned zero (Table 5.3). Thus, back fat thickness and body condition scores were found to be unimportant predictors of infection intensity.

For models assessing the influence of bison nutrition on the intensity of infection, the top supported models ($\Delta AIC_c < 3$) included covariates for age, age², BUN, and NEFA ($\sum w_i = 0.86$; Table 5.1). These ranking of these models suggest that the intensity of brucellosis infection is greatest in young bison with lower levels of BUN and higher levels of NEFA (Table 5.1). Leptin was also found to be an important predictor of infection intensity, but the positive direction of the effect was unexpected (Table 5.4). Coefficient estimates for IGF-1 suggest that the plasma concentration of this hormone is not an important predictor of infection intensity, as the estimated confidence interval spanned zero (Table 5.4). These findings suggest that the intensity of *B. abortus* infection in actively infected bison corresponds with a reduction in dietary protein and an increase in fat metabolism and is most pronounced in young animals (fig 5.6c).

Discussion

The dominant mechanism sustaining the endemic level of *B. abortus* infection in Yellowstone bison appears to be the synchrony of food restriction and seasonal reproductive needs in young bison. My results support the hypothesis that seasonal changes in forage quality influence the reproductive cycle in Yellowstone bison. There is a strong relationship between plant phenological stages and the nutritional quality of herbivore diets, with energy and protein content generally being highest in early plant growth stages (Van Soest 1994; Mysterud *et al.* 2001). As I expected, fecal crude protein and digestibility were highest in late spring through early summer and declined to the lowest levels during winter and early spring (Figure 5.1a). The emergence of high quality spring forage may explain the synchrony of calving observed in Yellowstone bison (Rutberg 1984). High quality forage at the onset of the calving period through the breeding season has fitness benefits for bison by increasing calf survival and allowing postpartum females to initiate their next reproductive cycle.

However, Yellowstone bison are also challenged to meet their maintenance and energy needs during winter as dietary protein and energy decline below maintenance levels (DelGuidice *et al.* 2001). In wild adult ruminants, the level of crude protein necessary to maintain minimum protein balance ranges from 5 to 9% (Parker, Barboza & Stephenson 2005). In my study, bison fecal analysis suggested that forage crude protein was below maintenance levels during the winter (Figure 5.2a). Forage digestibility, an indicator of available energy, also declined over the growing season as the amount of indigestible structural tissues increased in growing plants (Larter & Nagy 2001). The ratio of digestible organic matter to crude protein (DOM:CP) indicated that bison had access to high quality forage from May to August (Figure 5.2b), which coincides with lactation demands and replenishing body reserves needed for ovulation in late

summer. However, forage quality was well outside this range during the winter months that coincide with increasing fetal demands in late gestation. The reproductive strategy in Yellowstone bison links calving with the availability of high quality forage, but also ensures that pregnant females are in poor condition when forage quality is low in winter and early spring.

Pregnant bison had elevated plasma NEFA concentrations 2-3 times that of mature, barren females (Figure 5.3a), indicating a state of negative energy balance during late gestation. In ruminants, peak energy requirements coincide with pregnancy demands (Van Soest 1994) which are met by the mobilization of fat reserves. As food restriction and pregnancy advance, non-esterified fatty acid (NEFA) levels rise, indicating a shortage in dietary energy and an increase in fetal energy demands (Yambayamba, Price & Foxcroft 1996). In my study, observed increases in blood urea nitrogen (BUN) and declines in insulin-like growth factor-1 (IGF-1) (Figure 5.3b) indicate that pregnant bison were also experiencing a reduction in protein nutrition during late gestation. Protein restriction decreases BUN; however, during long period of negative energy balance, muscle tissue is catabolized as a gluconeogenic substrate and increases BUN (Harder & Kirkpatrick 1994; Karasov & Martínez del Rio 2007). Additionally, the endocrine hormone IGF-1 is sensitive to dietary protein with reduced levels indicating periods of deficient protein and calorie ingestion (Gomes *et al.* 2003). The decline in plasma leptin and back fat thickness over late gestation (Figure 5.3c) reflect an increase in fat metabolism and an advancing state of negative energy balance. Leptin, a peptide hormone secreted primarily by adipose tissue, tracks available energy stores (fat) and has been linked to fat metabolism and energy balance in many mammals (Spady *et al.* 2009). Further, the ability to communicate the status of fat stores makes leptin an attractive candidate for modulating immune responses in accordance with energetic reserves (Adelman & Martin 2009; French *et al.* 2009; Borghetti *et al.*

2009). Cumulatively, these findings support the hypothesis that food restriction during winter reduces fat and protein stores in Yellowstone bison, which contribute to a decline in nutritional condition.

In my study, active brucellosis infection in female bison was negatively associated with bison age and nutritional condition. Infection probabilities increased during pre-reproductive years, peaked in early reproductive ages (2-4 years old), and declined with increasing age. For each age class, the probability of isolating *B. abortus* was greatest for animals in below-average condition (Figure 5.5 a & b). The high level of active infection in juvenile bison may reflect greater exposure risk resulting from social interactions with infectious pregnant females (Treanor *et al.* 2011). However, the significant effect of reduced body mass suggests nutritional stress increases susceptibility to *B. abortus* infection. Loss of body mass may be the critical factor that mirrors compromised immune function (Nelson, Demas & Klein 2002). For immature bison, prioritizing body reserves for maintenance and growth over immune defense could be the best strategy for survival and reproduction. Survivorship increases with body size in juvenile ungulates (Parker, Barboza & Gillingham 2009), while nutritional restriction increases the age at first reproduction and reduces life-time reproductive success (Gaillard *et al.* 2000). Over-investment in immune defense may result in fitness costs, such as age and size at maturity (Sorci *et al.* 2009). Thus, the increase in *B. abortus* prevalence in juvenile bison may reflect a tradeoff in immune defense for survival and future reproductive success.

Individual bison life stages can represent different habitats for *B. abortus*. Malnourished juveniles may be vulnerable to infection but are a dead-end host for *B. abortus* transmission until females reach sexual maturity and become pregnant. Until then, *B. abortus* may pose little threat to the immune defenses of immature hosts thereby not eliciting costly immune responses. After

establishing infection, *B. abortus* bacteria can persist within their host in low numbers, yet undergo massive replication in pregnant females during late gestation (Cheville, McCullough & Paulson 1998; Carvalho Neta *et al.* 2010). In long-lived ungulates, such as bison, young females reproduce before reaching their adult weight and often bear the energetic costs of growth and lactation simultaneously (Hamel, Côté & Festa-Bianchet 2010). Primiparous bison may have compromised immune function during the *B. abortus* transmission period because they are still growing and body reserves are largely depleted during late gestation. In older bison, the decline in active infection may indicate some level of acquired immunity after experiencing acute infection earlier in life (Treanor *et al.* 2011). My findings support that active *B. abortus* infection is influenced by nutritional condition, with seasonal food restriction facilitating infection of immature bison which become the primary transmission source during early reproductive ages.

When food is limited, mounting an effective immune response to contain and overcome parasitic infections can have considerable nutritional costs (Valderrabano *et al.* 2006; Martin, Weil & Nelson 2008). Consequently, hosts in poor condition might have higher infection intensities because parasites would encounter less opposition to their survival and proliferation (Beldomenico & Begon 2009). My results suggest that infection intensities are exacerbated by seasonal reductions in dietary protein and energy. The association of BUN and NEFA levels with infection intensities indicates that reduced protein intake and elevated fat metabolism (i.e. energy needs) may increase susceptibility to *B. abortus* infection. Deficiencies in dietary protein increased susceptibility to gastrointestinal parasites in wild bovids (Ezenwa 2004), while reductions in total body fat reduced humoral immunity in rodents (Demas *et al.* 2003). Because *B. abortus* is an intracellular parasite, immune defense requires effective T cell-mediated

responses which have high costs in terms of protein and energy (Calder and Jackson 2000; Buehler, Tieleman, & Piersma 2010). Suppression of these defenses may reduce the ability of bison to contain *B. abortus* when infection is concurrent with seasonal food restriction and high reproductive demands.

Leptin plays an important role in the generation and maintenance of T cell responses (e.g. T_{helper} cell 1), which are reduced at low leptin levels (Lord 2002; Bernotiene, Palmer & Gabay 2006). The positive association between leptin and the intensity of *B. abortus* infection in bison was unexpected. Leptin levels and fat reserves indicated bison were in a state of negative energy balance, thus I expected higher infection intensities in animals with lower plasma leptin. However, in this study, leptin levels in bison were comparable to those in food-restricted animals (Delavaud *et al.* 2000; Soppela *et al.* 2008) and may have signaled that energy reserves were depleted. Thus, T cell responses in bison may have been down regulated as leptin levels communicate the amount of energy available for the immune system (French, Dearing & Demas 2011). As a mediator of the inflammatory immune response, leptin increases the production of IFN- γ (Fernández-Riejos *et al.* 2010), which plays a major role in protection against *B. abortus* (Clapp *et al.* 2011). The reduction of IFN- γ and leptin during spring (Figure 5.5) suggests that immune defenses against *B. abortus* may be suppressed in Yellowstone bison during the most critical period.

These findings have application to management efforts attempting to reduce the level of *B. abortus* in Yellowstone bison. During spring, bison seek emerging forage on low elevation ranges outside the park but are frequently pushed back onto high elevation ranges within the park where snow cover delays spring green-up. This management approach maintains separation of Yellowstone bison and cattle outside the park and has been successful at preventing *B. abortus*

transmission from bison to cattle. However, management practices that prevent bison from accessing nutritious food during the *B. abortus* transmission period may contribute to the maintenance of brucellosis in Yellowstone bison. Extending the period of food restriction when reproductive demands are greatest and body reserves are depleted is expected to suppress immune function which may facilitate *B. abortus* transmission and infection. Management practices that concentrate bison in the park during the *B. abortus* transmission window increase transmission potential as bison densities rise near park boundaries. Conversely, management practices that improve the nutritional condition of Yellowstone bison may improve the ability of bison to respond to *B. abortus* infection and also increase the effectiveness of vaccines aimed at reducing transmission.

I have provided a framework that integrates seasonal factors within a life-history context for the purpose of understanding how *B. abortus* is maintained within Yellowstone bison. The reproductive strategy of bison appears to be linked with the seasonal availability of food. Though this strategy increases bison fitness, it may have consequences for *B. abortus* infection. The investment of limited resources toward reproduction over immune defense in young bison may be a major trade-off sustaining infection. These findings further understanding of how physiological tradeoffs favoring survival and reproduction over immune defense can influence infection heterogeneities in wildlife populations.

Table 5.1. Ranking of regression models evaluating active brucellosis infection (logistic) and the intensity of infection (binomial-logistic) in Yellowstone bison. Models are ranked by ascending ΔAIC_c and Akaike weights (w_i).

Model	K	AICc	ΔAIC	w_i
Active Infection				
Age + Age ² + LW + BF	5	155.45	0.00	0.43
Age + Age ² + LW	4	155.61	0.16	0.40
LW	2	159.88	4.43	0.05
LW + BF	3	160.17	4.72	0.04
Age + LW	3	160.38	4.93	0.04
Age + LW + BF	4	160.42	4.97	0.04
Infection Intensity - Condition				
Age + Age ² + LW	4	561.74	0.00	0.32
Age + Age ² + LW + BCS	5	561.76	0.02	0.31
Age + Age ² + LW + BCS + BF	6	562.39	0.65	0.23
Age + Age ² + LW + BF	5	564.18	2.44	0.09
Infection Intensity - Nutrition				
Age + Age ² + BUN + NEFA + IGF-1 + LEP	7	754.23	0.00	0.34
Age + Age ² + BUN + NEFA + LEP	6	754.41	0.18	0.31
Age + Age ² + BUN + NEFA	5	756.16	1.93	0.13
Age + Age ² + BUN + NEFA + IGF-1	6	757.05	2.82	0.08
Age + Age ² + BUN + LEP	5	758.02	3.79	0.05
Age + Age ² + BUN + IGF-1 + LEP	6	758.34	4.11	0.04
Age + Age ² + BUN	4	759.75	5.52	0.02
Age + Age ² + BUN + IGF-1	5	760.97	6.74	0.01

Model notation: LW = bison live body weight; BF= backfat thickness; BUN= blood urea nitrogen; NEFA= non-esterified fatty acid; IGF-1= insulin-like growth factor-1; LEP = leptin.

Table 5.2. Parameter estimates of top supported models (logistic) evaluating active *Brucella abortus* infection in female bison in Yellowstone National Park ($n = 152$). Non-bolded estimates indicate the 95% confidence interval spans zero.

Active Infection	Intercept	Age	Age²	Live WT	Back Fat
Age + Age ² + LW+ BF	-0.68 (-1.22, -0.15)	-0.56 (-1.41, 0.28)	-1.82 (-3.29, -0.35)	-6.84 (-10.85, -2.84)	-0.78 (-1.81, 0.24)
Age + Age ² + LW	-0.69 (-1.22, -0.16)	-0.50 (-1.34, 0.33)	-1.77 (-3.21, -0.32)	-7.65 (-11.52, -3.78)	
LW	-1.19 (-1.59, -0.78)			-7.50 (-11.28, -3.71)	
LW + BF	-1.19 (-1.60, -0.78)			-6.74 (-10.67, -2.81)	-0.67 (-1.67, 0.32)
Age + LW	-1.17 (-1.57, -0.76)	-0.44 (-1.14, 0.25)		-7.16 (-10.93, -3.39)	
Age + LW + BF	-1.17 (-1.58, -0.76)	-0.49 (-1.19, 0.22)		-6.31 (-10.24, -2.39)	-0.73 (-1.74, 0.28)

Model notation: LW = bison live body weight; BF= backfat thickness

Table 5.3. Parameter estimates of top supported models (binomial-logistic) evaluating the influence of age and nutritional condition on *Brucella abortus* infection intensity in Yellowstone bison ($n = 53$). Non-bolded estimates indicate the 95% confidence interval spans zero.

Infection Intensity	Intercept	Age	Age ²	LW	BF	BCS
Age + Age ² + LW	-1.13 (-1.3, -0.96)	-0.38 (-0.61, -0.14)	0.60 (0.24, 0.95)	-1.82 (-2.85, -0.79)		
Age + Age ² + LW + BCS	-1.13 (-1.30, -0.96)	-0.37 (-0.6, -0.13)	0.58 (0.22, 0.93)	-1.65 (-2.70, -0.60)		-0.04 (-0.90, 0.10)
Age + Age ² + LW + BCS + BF	-1.13 (-1.30, -0.96)	-0.38 (-0.61, -0.14)	0.57 (0.21, 0.93)	-1.91 (-3.03, -0.79)	-0.69 (-1.33, -0.04)	0.31 (-0.13, 0.76)
Age + Age ² + LW + BF	-1.13 (-1.30, -0.96)	-0.38 (-0.62, -0.14)	0.6 (0.24, 0.95)	-1.84 (-2.96, -0.72)	0.02 (-0.33, 0.37)	

Model notation: Live Wt = bison live body weight; BF= backfat thickness; BCS = body condition score

Table 5.4. Parameter estimates of top supported models (binomial-logistic) evaluating the influence of age and nutrition on *Brucella abortus* infection intensity in Yellowstone bison ($n = 78$). Non-bolded estimates indicate the 95% confidence interval spans zero.

Infection Intensity	Intercept	Age	Age ²	BUN	NEFA	IGF-1	LEP
Age + Age ² + BUN + NEFA + IGF-1 + LEP	-1.09 (-1.24, -0.94)	-0.55 (-0.70, -0.40)	0.32 (0.15, 0.48)	-0.88 (-1.30, -0.45)	0.17 (0.04, 0.30)	-0.21 (-0.47, 0.05)	0.26 (0.04, 0.48)
Age + Age ² + BUN + NEFA + LEP	-1.08 (-1.23, -0.93)	-0.56 (-0.70, -0.41)	0.31 (0.15, 0.47)	-0.71 (-1.08, -0.34)	0.16 (0.03, 0.29)		0.23 (0.01, 0.44)
Age + Age ² + BUN + NEFA	-1.08 (-1.23, -0.93)	-0.57 (-0.72, -0.43)	0.32 (0.15, 0.48)	-0.71 (-1.08, -0.34)	0.16 (0.03, 0.29)		
Age + Age ² + BUN + NEFA + IGF-1	-1.09 (-1.24, -0.94)	-0.57 (-0.71, -0.42)	0.32 (0.16, 0.49)	-0.83 (-1.25, -0.41)	0.17 (0.04, 0.29)	-0.16 (-0.41, 0.10)	
Age + Age ² + BUN + LEP	-1.07 (-1.22, -0.92)	-0.55 (-0.70, -0.41)	0.30 (0.14, 0.47)	-0.69 (-1.06, -0.32)			0.22 (0.01, 0.44)
Age + Age ² + BUN + IGF-1 + LEP	-1.08 (-1.23, -0.93)	-0.55 (-0.70, -0.40)	0.31 (0.15, 0.47)	-0.83 (-1.26, -0.41)		-0.19 (-0.44, 0.07)	0.25 (0.03, 0.47)
Age + Age ² + BUN	-1.07 (-1.21, -0.92)	-0.57 (-0.71, -0.42)	0.31 (0.15, 0.47)	-0.69 (-1.06, -0.32)			
Age + Age ² + BUN + IGF-1	-1.07 (-1.22, -0.92)	-0.57 (-0.71, -0.42)	0.32 (0.15, 0.48)	-0.79 (-1.21, -0.37)		-0.13 (-0.38, 0.12)	

Model notation: LW = bison live body weight; BF= backfat thickness; BUN= blood urea nitrogen; NEFA= non-esterified fatty acid; IGF-1= insulin-like growth factor-1; LEP = leptin.

Figure 5.1. Conceptual framework describing the endemicity of brucellosis in Yellowstone bison through the integration of seasonal resource availability, host-parasite interaction, and host life history trade-offs. Forage quality declines as reproductive needs increase during late gestation, which in turn decreases nutritional condition and resources available for immune defense. The life cycle of *B. abortus* is linked with the reproductive cycle of bison to facilitate transmission when immune activity is depressed during late gestation. Seasonal food restriction influences physiological tradeoffs favoring growth and reproduction over immune defense in young bison, which increase susceptibility to infection and transmission potential during pregnancy.

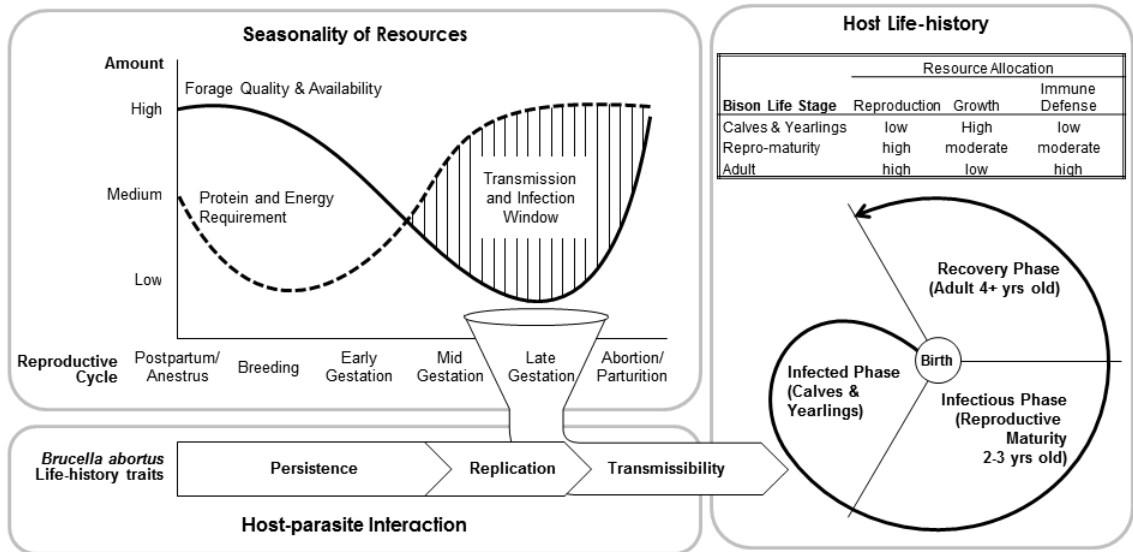
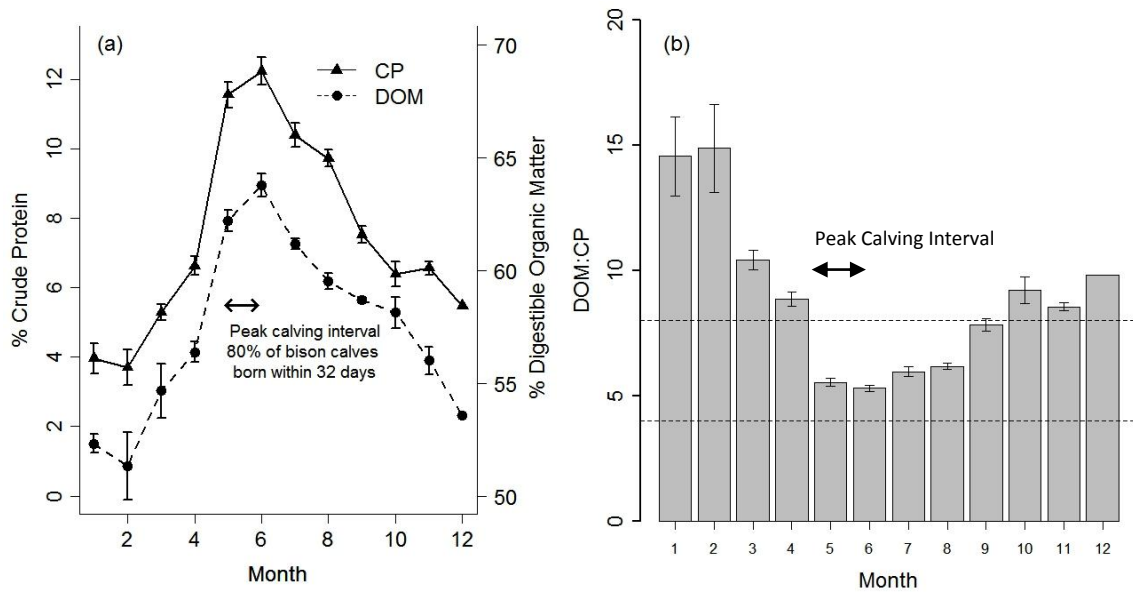


Figure 5.2. Monthly percentage (mean \pm SE) of (a) fecal crude protein (CP) and digestible organic matter (DOM) and (b) DOM:CP ratio for bison in Yellowstone National Park. Bison calving is synchronized with spring emergence of forage high in protein and digestibility. Dietary DOM:CP ratios between 4 to 8 indicate a favorable protein-energy balance to meet reproductive demands.



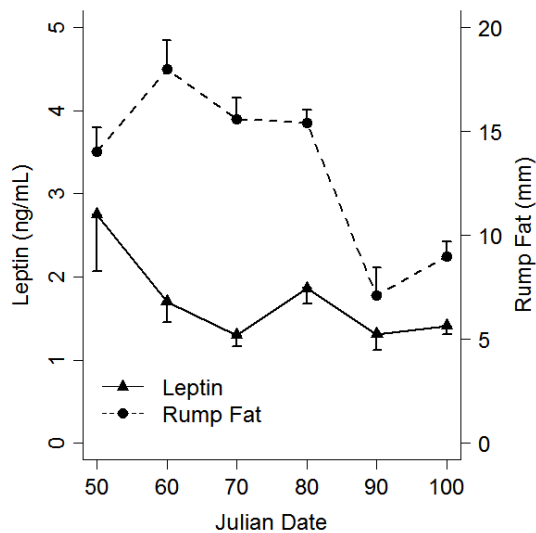
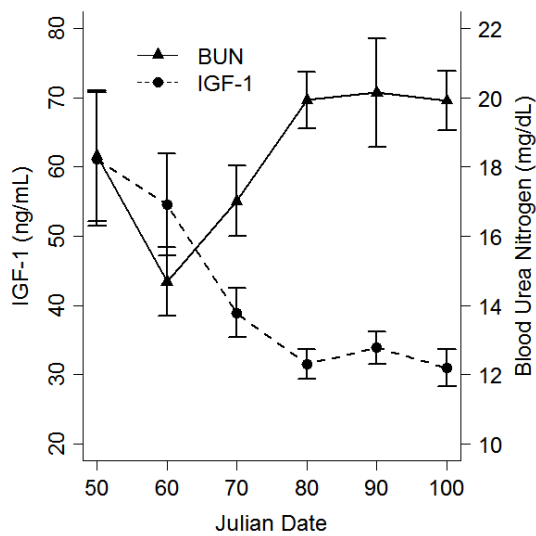
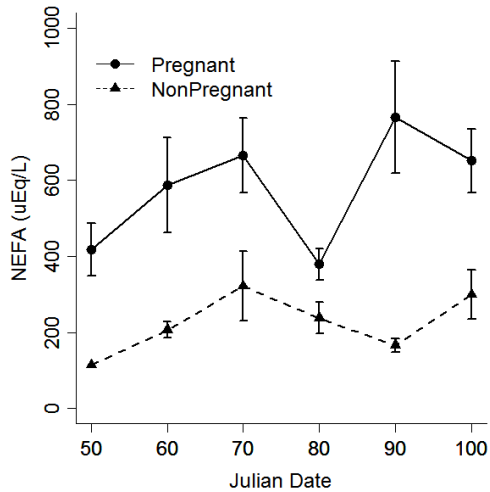


Figure 5.3. Differences in blood metabolites, hormones and body condition in female Yellowstone bison sampled during late gestation. Julian date refers to the numeric date starting on Jan1, 2008 (e.g. 50 = Feb 9 and 100= April 9). Values are presented as mean \pm SE for (a) nonesterified fatty acid (NEFA), (b) concentrations of insulin-like growth factor -1 (IGF-1), and blood urea nitrogen (BUN), and (c) concentrations of leptin and backfat measurements.

Figure 5.4. Differences in body mass (mean \pm SE) with increasing age in female Yellowstone bison. Live weight represents measurements on intact animals including pregnant and nonpregnant adults. Carcass weight represents measurements on processed animals without internal organs and reproductive tissues.

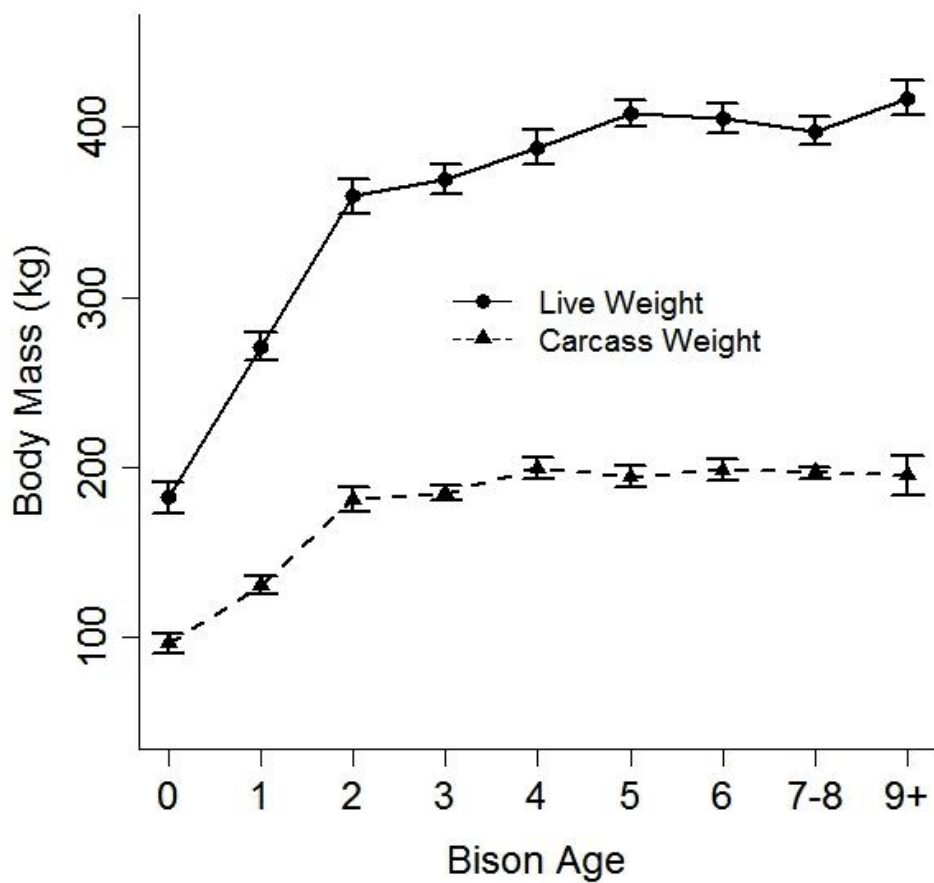


Figure 5.5. Seasonal levels (mean \pm SE) of (a) interferon- γ and (b) leptin in female Yellowstone bison sampled from 2007-2009. The interferon- γ levels were measured by ELISA from the culture supernatants of peripheral blood mononuclear cells stimulated by the mitogen Concanavalin A. Asterisks indicate significant differences in interferon- γ and leptin between seasons relative to spring (Apr-Jun) sampling, $*P < 0.05$.

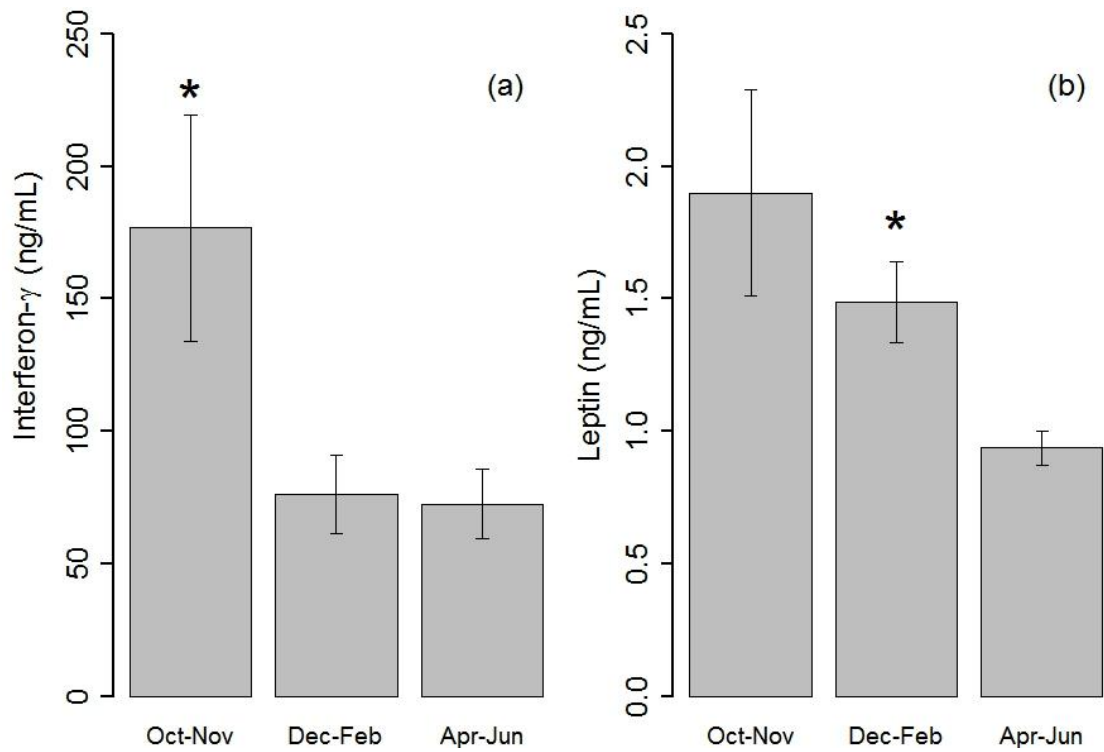
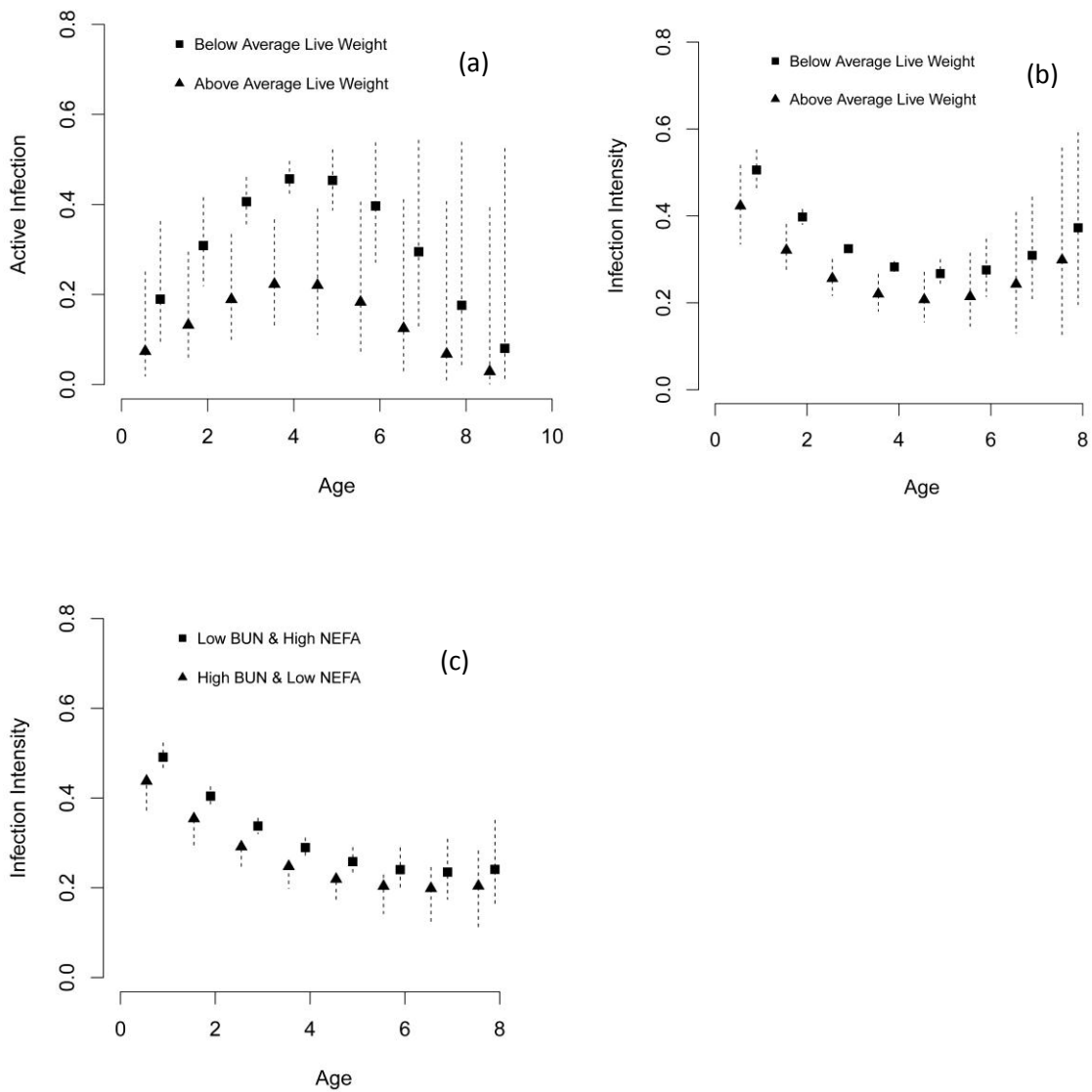


Figure 5.6. Predicted probabilities of active brucellosis infection and intensity of infection across bison age for the dependent variable (a & b) bison live weight and (c) concentrations of blood urea nitrogen (BUN) and non-esterified fatty acid (NEFA). Below and above average live weight and low and high BUN and NEFA represent data from the 20th and 80th percentile, respectively. The dashed lines indicate the 95% confidence limits for the predictions.



Chapter 6 - Comparison of immune responses following vaccination with *Brucella abortus* strain RB51 in captive and free-ranging bison

Introduction

Brucellosis, caused by bacteria in the *Brucella* genus, is a zoonotic disease of concern throughout the world, with at least half a million new cases occurring annually (Vassalos *et al.* 2009). The *Brucellae* are gram negative, facultative intracellular bacteria known to infect a diversity of mammalian hosts. The bacteria were first isolated by David Bruce from human spleens in 1884 (Nicoletti 2002), though the disease has been linked to humans as far back as 79 A.D (Capasso 2002). Currently, the World Health Organization regards brucellosis as the world's most widespread zoonosis (Godfroid 2005). Prevention of human brucellosis depends on control of the disease in animals because humans cannot maintain the disease without an animal host (Corbel 2006). Since no human brucellosis vaccine exists, vaccination of livestock has been the primary method for controlling the spillover of animal infection to humans.

In the United States, vaccination has played a critical role in nearly eradicating bovine brucellosis (*Brucella abortus*) from domestic cattle (Olsen & Tatum 2010). Vaccination of domestic animals reduces the clinical effects of the disease (e.g. abortions and shedding of bacteria in birth tissues and milk), which facilitate transmission. The massive amount of bacteria expelled on fetal membranes and birth tissues drives transmission and maintains the disease (Cheville 1998). Livestock vaccination has significantly reduced brucellosis infection (Corbel 2006), while mass vaccination

programs, which include test-and-slaughter practices, have the potential to reduce brucellosis prevalence toward eradication (Martins *et al.* 2009). Despite the success of livestock vaccination, brucellosis control programs become more complicated when a wildlife reservoir for the disease is present.

In the Greater Yellowstone Area (GYA), the risk of brucellosis transmission from wild bison (*Bison bison*) to cattle (*Bos taurus*) has been a contentious issue between wildlife managers and advocates for bison conservation (Kilpatrick *et al.* 2009). Yellowstone bison have been infected with *B. abortus* since 1917 through the introduction of infected European cattle (Mohler 1917; Meagher & Meyer 1994). Though the disease is not a threat to the long-term survival of the Yellowstone bison herd, concern that bison may transmit brucellosis to cattle on neighboring lands has led to unpopular management practices which prevent bison from migrating outside the park (Bienen & Tabor 2006). Currently, wildlife (bison and elk *Cervus elaphus*) in the GYA are the last reservoirs of *B. abortus* in the United States. In the past decade, brucellosis outbreaks in the three states bordering Yellowstone National Park (Idaho, Montana, and Wyoming) have caused additional economic expenses for the state livestock industries. Though infected elk have been implicated in these outbreaks (Beja-Pereira *et al.* 2009; Higgins *et al.* 2012), the high level of infection and intolerance of bison outside the park have made them a target for brucellosis management.

Traditional brucellosis control methods, such as test and slaughter, have been ineffective at reducing prevalence in Yellowstone bison and are unlikely to be effective without reducing the population to near eradication levels (Dobson & Meagher 1996). Conflicting perspectives on how to manage the risk of brucellosis transmission from

bison to cattle led to the development of an Interagency Bison Management Plan. The plan's objective is to maintain a wild, free-ranging population of bison while addressing the risk of brucellosis transmission to Montana's livestock industry (USDI & USDA 2000). Vaccination of Yellowstone bison was proposed as a method to manage the transmission risk between bison and cattle, but there have been mixed results of bison vaccination using the currently available vaccine.

Strain RB51 (SRB51), the official live brucellosis vaccine for cattle in the United States, provides significant protection in cattle (Davis and Elzer 2002), but efficacy in bison has been suspect. The efficacy of SRB51 is typically determined by protection from infection and abortion following challenge with a virulent strain of *B. abortus* (e.g. S2308). Early studies concluded that SRB51 did not confer significant protection in vaccinated adult bison despite intensive (three injections) vaccination efforts (Davis & Elzer 1999). More recently, research has shown SRB51 to offer protection against abortion and placental infection when bison calves were vaccinated and later challenged with virulent *B. abortus* during mid-gestation (Olsen *et al.* 2003). The appeal of SRB51 as a vaccine for bison is that it has provided significant protection in cattle, with vaccinated animals remaining negative when tested with standard serologic tests (Olsen *et al.* 1998; Davis & Elzer 2002).

Vaccine protection relies on the stimulation of an immune response to *B. abortus* that can be recalled following field exposure. Protection occurs through the prevention of bacterial excretion in pregnant bison, which disrupts further transmission. The efficacy of SRB51 is expected to be greater under field conditions than under experimental conditions, where known pregnant bison receive an infectious dose of virulent *B. abortus*

during mid-gestation, when they are most susceptible to brucellosis infection (Olsen & Stoffregen 2005). However, vaccine efficacy under field conditions may be influenced by a number of factors, including nutritional stress (Olsen & Johnson 2011). Thus, mounting an effective immune response against *B. abortus* may have condition-related costs influencing the ability of bison to recall protective responses.

Because cellular immune responses play a major role in protection against *B. abortus*, an effective vaccine is expected to generate strong cell-mediated immunity (Clapp *et al.* 2011). As with other intracellular pathogens, immunity to *B. abortus* depends on T-cell-mediated activation of phagocytic white blood cells (macrophages), with interferon- γ (IFN- γ) being the primary cytokine responsible for macrophage activation (Murphy *et al.* 2001; Gorvel & Moreno 2002; Carvalho Neta *et al.* 2010). However, the induction of protective cell-mediated immunity is costly in terms of protein and energy (Calder and Jackson 2000; Buehler *et al.* 2010). For Yellowstone bison, body reserves (e.g. fat) decline as forage quality and availability decrease during late winter and early spring, overlapping the primary *B. abortus* transmission period.

Bison nutrition may be an important factor influencing the effectiveness of a vaccination program to reduce brucellosis prevalence in Yellowstone bison. The purpose of this study was to compare the immunologic responses to vaccination in captive and free-ranging bison and assess the effect of nutritional differences. Additionally, vaccinated free-ranging bison provided the opportunity to evaluate vaccine protection following natural exposure to *B. abortus* within Yellowstone National Park.

Methods

Bison sampling and vaccination

During the winter and spring of 2008, deep snow influenced the movement of bison from high elevation ranges within Yellowstone to low elevation ranges outside the park's boundaries. Over 1647 bison exited the northern boundary between February and April, 2008. Migrating bison were captured and held at the Stephens Creek bison capture facility to prevent them from co-mingling with cattle outside the park. The total population at YNP was estimated at 4694 bison during August, 2007, with 50% of female bison ($n = 881$) testing positive for *B. abortus* exposure.

Bison calves ($n = 112$), which tested negative for *B. abortus* exposure using the fluorescent polarization assay (FPA) and standard card test were transferred from Yellowstone to the Corwin Springs capture facility as part of a bison quarantine feasibility study (MTFWP & USDA-APHIS 2006). All quarantined bison were vaccinated subcutaneously with 1.4×10^{10} colony-forming units (CFU) of SRB51 (Colorado Serum Company, Denver, CO) as yearlings on November 12, 2008. Blood samples were collected by jugular venipuncture from 12 female yearlings (age *c.* 19 months) before vaccination and at 3, 8, 12, 18, and 21 weeks after vaccination (Nov 12, 2008 to April 8, 2009). Bison were sustained on a supplied diet of mixed-grass hay. These 12 pre-pubescent bison constituted the quarantine study group. At each sampling time point, blood was collected into sodium heparin (50mL), EDTA (K₃, 10mL), and serum (10mL) BD Vacutainer Collection Tubes. Serum and plasma were aliquoted and stored at -20 C until analyzed. The heparinized blood used in immunological assays was processed on the day of collection. Because all quarantined bison were required to be

vaccinated, there were no control animals (non-vaccinated bison) in the study. The before-vaccination time point served as a negative control for quantifying immune responses following vaccination.

During winter of 2008, 14 female bison (age *c.* 22 months) that tested negative for *B. abortus* exposure using FPA and standard card test were syringe-vaccinated with SRB51 (1.4×10^{10} CFU) on April 1, 2008. Vaccinated bison were held within the Stephens Creek capture facility for 6 weeks. Blood was collected from 12 of the bison before vaccination (April 1, 2008), 2 weeks after vaccination (April 15, 2008), and 6 weeks after vaccination (May 13, 2008). These 12 bison constituted the Yellowstone study group. All vaccinated bison were fitted with ear tag radio-transmitters (M3620 ATS, Isanti, MN) and released into the park on May 19, 2008. During summer 2008, vaccinated bison matured sexually (fertile estrous) at *c.* 28 months of age and were naturally bred. Nine of the vaccinated bison were located using radio telemetry and re-captured in the field starting 28 weeks after vaccination (Oct 14, 2008, to Jan 20, 2009). Bison were immobilized with carfentanil and xylazine remotely administered using tranquilizing darts. Blood was collected for determining pregnancy status (using serum pregnancy specific protein B, PSPB), diagnosing *B. abortus* exposure (FPA and card test) after release into the park, and evaluating bison immunological status. Bison that converted from seronegative to seropositive status when tested 28 weeks after vaccination were monitored to determine the outcome of their pregnancy (observed abortion or live birth) following natural infection. Bison in the Yellowstone group that reacted positively on the serologic test following release into the park (28 + weeks after

vaccination) were excluded from the study addressing vaccine-induced immune responses because natural exposure may confound vaccine-induced responses.

Blood Analysis

Blood collected for determining immunological responses to vaccination was processed at Montana State University's Biosafety Level 3 Facility-Jutila Research Laboratory. For the quarantined study group blood was processed on the day of collection, while for the Yellowstone study group, blood was refrigerated overnight and processed the next day. The peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood (40 mL) using density centrifugation. The cells were washed three times with media (RPMI 1640) and re-suspended in media supplemented with 2 mM L-glutamine, 10% heat-inactivated horse serum (GIBCO BRL), 100 U/ml penicillin, and 100 mg/ml streptomycin. The PBMCs (5×10^6 /ml in medium) were aliquoted into 24-well plates. Duplicate cultures were stimulated with the mitogen concanavalin A (ConA, 1 μ g/ml), heat-killed *B. abortus* (S2308, 1 μ g/ml), or unstimulated (medium only). ConA is a strong mitogen used to induce T-cell proliferation. Cell cultures were incubated at 37°C with 5% CO₂, harvested after 5 days, and stored at -80°C until analyzed. Immune responses of bison to vaccination were monitored by gamma interferon (IFN- γ) using a bovine capture ELISA from the supernatants of the cell cultures. Concentrations of IFN- γ were determined by comparing the absorbance of test samples with the absorbance of standards within a linear curve fit. For the quarantine and Yellowstone study groups, mean IFN- γ concentrations at time points following vaccination were compared with concentrations before vaccination using a paired *t* test. Individual bison in both study groups were given a vaccine responder classification

(strong, weak, and none) based on IFN- γ production from isolated cells following stimulation with *B. abortus* antigen, with the assumption that strong responses may indicate some level of protection induced through vaccination. Bison were classified as strong responders (>20 ng/mL IFN- γ), weak responders ($2 > \text{ng/mL IFN-}\gamma < 20$), or non-responders (None) (< 2 ng/mL IFN- γ) based on the largest concentration of IFN- γ measured at any sampling time point following vaccination.

For analyzing indicators of nutrition in bison blood, plasma was separated from whole blood within 3 hours of collection and stored frozen -80 until assayed for metabolites and hormones. The hormones leptin and Insulin-like growth factor-1 (IGF-1) regulate immune responses in relation to protein and energy metabolism (Borghetti *et al.* 2009). Plasma leptin and IGF-1 concentrations were determined by radioimmunoassay (RIA) at the University of Missouri, division of animal sciences. The plasma metabolites urea nitrogen (BUN), an indicator protein nutrition, and non-esterified fatty acid (NEFA), an indicator of fat metabolism, were estimated using capture ELISA at Texas Tech University's department of animal and food sciences. Serum collected for pregnancy diagnosis was analyzed by Biotracking (Moscow, ID) using PSPB assay.

Statistical analysis

To assess the influence of nutrition on the induction of cellular immune responses in sampled bison, a suite of 15 regression models were developed. The level of IFN- γ produced by isolated PBMCs following treatment with the mitogen ConA was used as a continuous response variable. A log-transformation of the response variable (\log_{10} IFN- γ : ConA) was used to improve normality. Plasma hormones and metabolites, leptin, IGF-1,

BUN, and NEFA, were model predictor variables. The data set included complete records for each variable ($n = 81$). Models were ranked using Akaike's Information Criterion corrected for small sample size (AIC_c), and normalized AIC_c weights (w_i) were used to compare candidate models. Models with AIC_c differences (Δ_i) ≤ 2 from the top supported model were considered to offer substantial empirical support, while models with $4 \leq \Delta_i \leq 7$ have considerably less support and models having values $\Delta_i \geq 10$ having essentially no support (Burnham & Anderson 2002). All statistical analyses were conducted in R (R Development Core Team 2010).

Results

Immune response to vaccination

Bison inoculated with SRB51 showed elevated IFN- γ responses to *B. abortus* antigen across sampling time points following vaccination (Figure 6.1). For both study groups, the mean IFN- γ response increased during the initial period (8 weeks) following vaccination, though sampling time points for the 2 study groups did not overlap. IFN- γ levels were significantly greater ($p < 0.05$) for quarantined bison at time points after vaccination in comparison to before vaccination, while IFN- γ responses for the Yellowstone group were statistically different ($P < 0.05$) only at 6 weeks after vaccination in comparison to before vaccination. For both study groups, the proportion of bison (0.42) that showed strong responses (IFN- $\gamma > 20$ ng/mL) following SRB51 vaccination was similar (Table 6.1). However, this was less than 50% of bison in each study group. Strong responders from both study groups showed similar initial responses to vaccination, with recall responses at the long-term time point (> 20 weeks post vaccination) being statistically indistinguishable ($P > 0.05$) for both groups (Table 6.1).

Protection from natural infection

Three incident cases of natural *B. abortus* exposure were observed in the vaccinated Yellowstone study group following release into the park in spring of 2008. Though vaccination with SRB51 does not provide protection from the establishment of *B. abortus* infection, it has demonstrated protection against the induction of abortions in vaccinated bison under experimental conditions (Olsen & Tatum 2010). All three of the bison, identified as positive for *B. abortus* exposure using serologic tests (FPA and card) at 28+ weeks after vaccination, were diagnosed as pregnant via serum PSPB. Of the three bison, one had a *B. abortus* induced abortion, one gave birth to a live calf, and one was never observed with a calf (Table 6.2). During spring, 2009, the aborting female was observed with a retained placenta and confirmed as actively infected with *B. abortus* based on isolation of the bacteria from vaginal exudate. Isolated PBMCs from the aborting cow produced low levels of IFN- γ when stimulated with *B. abortus* antigen at 6-weeks post vaccination and at 28+ weeks post vaccination following the field exposure to *B. abortus*. Though not protective, the high antibody levels in the aborting cow indicate a strong humoral response to natural infection. The cytokine interleukin-4 (IL-4), which suppresses the production of IFN- γ was produced by the aborting cow.

Effects of nutrition on immune response

In comparison, IFN- γ responses to heat killed *B. abortus* and ConA treatment were not significantly different between the Yellowstone and quarantine study groups when measured 20 weeks after vaccination (Figure 6.2 a & b). The mean values of plasma leptin, IGF-1, and BUN were different between the study groups at the long-term post-vaccination time point (Figure 6.2 c-e), while there was no difference in plasma

NEFA levels (Figure 6.2f). Plasma, IGF-1, leptin, and BUN levels had a significant positive effect on the induction of IFN- γ following treatment with the mitogen Con A (Figure 6.3 a-c), while plasma NEFA had a significant negative effect (Figure 6.3d).

The induction of IFN- γ following treatment of PBMCs with ConA (\log_{10} IFN- γ :ConA) was influenced by indicators of bison nutrition. The top supported model based on results of model selection (38% of w_i ; Table 6.4) included covariates for plasma leptin, IGF-1, and NEFA. There was strong support for the top 4 models ($\Delta AIC_c = 0 - 2.60$; $\sum w_i = 0.93$), all of which contained IGF-1 and NEFA. Models without both of these covariates had less support ($\Delta AIC_c = 4.75 - 20.95$; $\sum w_i = 0.07$). The second most supported model (30% of w_i , Table 6.4) indicated that the slope coefficients for IGF-1 ($\beta_{IGF-1} = 0.016 \pm 0.004$ [mean \pm SE], $P = 0.001$) and NEFA ($\beta_{NEFA} = 0.002 \pm 0.000$ [mean \pm SE], $P < 0.001$) had a significant effect on the level of IFN- γ by cells stimulated with ConA. These results suggest that bison experiencing under nutrition, indicated by decreasing plasma IGF-1 and increasing plasma NEFA, may have reduced cellular immune responses measured by the production of IFN- γ from isolated cells stimulated with the mitogen ConA.

Discussion

Vaccination has been one of the most successful methods for preventing disease in domestic animals, but vaccination of wildlife presents numerous challenges (Plumb *et al.* 2007). In particular, pathogens that are able to maintain persistent infections within wildlife populations are especially difficult to manage through vaccination. Currently, there are few effective vaccines available for protecting wildlife from persistent bacterial diseases, such as bovine tuberculosis, salmonellosis, and brucellosis. Vaccines that

generate long-lived cellular responses against intracellular pathogens have not provided consistent protection (Seder and Hill 2000). Since much of our understanding of wildlife diseases comes from treating similar diseases in humans and domestic animals, a greater understanding of how to apply disease management approaches to wildlife is needed. In contrast to domestic animals, the effectiveness of wildlife vaccination may be complicated by a variety of ecological and climatological factors. Additionally, because wildlife species are valued by society, livestock methods, which are not aligned with wildlife conservation, are unlikely to be socially acceptable (Nishi, Shury & Elkin 2006).

In Yellowstone, vaccination of bison is recognized as a potential method for reducing the risk of brucellosis transmission from bison to cattle, while also conserving this wildlife resource. My results suggest that a single vaccination of SRB51 provided to female, yearling bison may offer some protection from clinical disease (*B. abortus* induced abortions) in *c.* 50% of vaccinated animals. Here I am assuming that the proportion of bison in both study groups that were classified as strong responders based on *in vitro* responses would have acquired some level of protection. However, the abortion and subsequent isolation of *B. abortus* in one of the three naturally infected bison underscores that SRB51 does not offer complete protection from clinical disease. Additionally, these findings further indicate that vaccination with SRB51 will not prevent positive reactions on standard serologic tests if vaccinated bison are exposed to an infectious dose of *B. abortus* field strain (Olsen *et al.* 2009).

Though vaccinated bison experienced different environmental conditions during the study period, the immune response to vaccination was similar between the two groups. The antigen-induced production of IFN- γ increased in both study groups

following vaccination in comparison to pre-vaccination levels, with peak IFN- γ production at about 6 weeks after vaccination.

The production of IFN- γ in response to antigen seemed to be deficient in a similar proportion (0.58) of bison (weak and non-responders) within both study groups. The lack of IFN- γ production in all bison prior to vaccination and in un-stimulated cells (media only) confirmed that the specificity of the low measurable responses in most bison were in response to *B. abortus* antigen. Also, there was no statistical difference in IFN- γ production in cells stimulated with mitogen ConA across responder classes, which indicated cell cultures in all responder classes were viable. Thus, these findings support that variation in individual immune responses are likely to play a role in the efficacy of SRB51.

In experimental efficacy studies, bison challenged with virulent *B. abortus* will abort their pregnancy in a shorter interval and at a higher percentage compared to cattle. The mean exposure dose for Yellowstone bison that results in abortion is unknown. In cattle, 10^3 CFU of particular challenge strains have not induced clinical effects, while exposure to 700,000 bacteria resembles the epidemiologic features of infected cattle herds (Olsen & Johnson 2011). In the present study, I have no information on the infectious dose received by the bison that had a *B. abortus*-induced abortion. However, in contrast to experimental studies that challenge animals in mid-gestation, the three seropositive bison in the Yellowstone group were exposed before they reached sexual maturity, with the aborted pregnancy occurring the following spring. The high antibody levels detected in the aborting individual is consistent with active *B. abortus* infection identified in young bison with elevated antibody titers (Roffe *et al.* 1999; Treanor *et al.*

2011). These findings suggest that SRB51 will not prevent persistent infection and the onset of clinical disease in a proportion of juvenile bison vaccinated before reaching sexual maturity.

Vaccine protection against *B. abortus* infection requires vaccinated bison to have strong recall responses, which induce the proliferation of T-cells and the production of IFN- γ when exposed to field strain bacteria (Wyckoff 2002). Mean IFN- γ production, in response to *B. abortus* and mitogen stimulation, was not significantly greater in the Yellowstone group than in the quarantined group when sampled 20 weeks after vaccination (Figure 6.2 a-b). I expected responses to be greater in the quarantine group, which was assumed to be under less nutritional stress. However, based on the time of year when sampling occurred for the long-term time point (20 weeks after vaccination), neither group may have been undernourished.

Because bison were not available for both study groups concurrently, animals in each study group were vaccinated at different times of the year. For the Yellowstone group, sampling animals 20 weeks after vaccination occurred in fall and early winter, while for the quarantine group sampling 20 weeks after vaccination occurred during spring. In comparison, plasma levels of IGF-1 and leptin were higher for the Yellowstone group, while BUN levels were higher for the quarantine group. Plasma concentrations of leptin and IGF-1 are regulated by dietary energy and protein levels (Gomes *et al.* 2003; Chelikani *et al.* 2009), which may have been greater for the Yellowstone group as there was no restriction on their ability to graze. For the quarantine group, higher levels of BUN reflect that they were supplied a mixed grass hay diet over the winter (Table 6.3, Figure 6.2e), though overall intake may have been lower than in

the free-ranging Yellowstone group. The protein content in the diet available to the quarantine bison was probably higher than the natural forage available to the Yellowstone group because dietary protein in bison forage declines in late summer through early spring (Chapter 5). More importantly, plasma NEFA levels indicated that fat metabolism was similar in both groups (Figure 6.2f), suggesting little difference in the mobilization of energy reserves at the time of sampling. In my analysis, Plasma NEFA was identified as an important factor influencing IFN- γ production in cells stimulated with mitogen (Table 6.4). Thus, the production of IFN- γ may be further reduced during periods of nutritional restriction, such as late winter through early spring.

The transmission window for *B. abortus* overlaps periods of food restriction and increasing pregnancy demands for Yellowstone bison (Chapter 5). In the present study, production of IFN- γ in response to mitogen stimulation was influenced by nutritional factors, which suggests that recall responses to *B. abortus* following vaccination may be depressed during the critical period. Pregnant bison are typically in negative energy balance during winter with nutritional deficit increasing as parturition approaches (DeGuidice *et al.* 2001). Protective cellular immune responses are costly in terms of protein and energy and may be limited during periods of nutritional restriction (Cunningham-Rundles, McNeely, & Moon 2005; Buehler *et al.* 2010). During late gestation, pregnant bison have elevated levels of NEFA accompanied by declines in IGF-1, leptin, BUN and fat reserves, which, in combination, influence greater tissue colonization by *B. abortus* in infected animals (Chapter 5). The hormones leptin and IGF-1, play a prominent role in the modulation of cellular immune responses, though their function is compromised during periods of protein and energy restriction (Lord *et al.*

1998, Borghetti *et al.* 2009, Xu & Wang 2010). For pregnant Yellowstone bison, nutritional condition and the availability of dietary nutrients decline as nutritional needs increase during late gestation. Thus, protective immune responses acquired through vaccination may be compromised during late gestation when *B. abortus* transmission typically occurs.

Though vaccination of free-ranging bison is challenging, it has potential to be a useful method for reducing brucellosis infection (Treanor *et al.* 2010, Ebinger *et al.* 2011). Under confined and free-ranging conditions, vaccination with SRB51 induced similar immunological responses in bison. However, the magnitude of the *in vitro* immune responses in the present study were considerably lower than responses observed in experimental studies (Olsen *et al.* 2009). These findings suggest that a single vaccination of SRB51 does not offer strong protection from clinical disease. However, the immune responses may be improved if vaccinated animals receive booster vaccinations.

Vaccination alone is unlikely to result in a large-scale reduction of brucellosis prevalence in Yellowstone bison. Methods that combine large-scale vaccination coverage with test and slaughter practices have been effective at reducing brucellosis in cattle (Poester *et al.* 2006; Martins *et al.* 2009). However, Yellowstone bison are a valued wildlife species, making intensive culling socially unacceptable to many stakeholders (Bienen & Tabor 2006) and making vaccination a more suitable approach for managing brucellosis. Because brucellosis has little effect on the Yellowstone bison population, the relevant disease management question is whether vaccination of bison can reduce the risk of *B. abortus* transmission to livestock while conserving Yellowstone

bison. Findings of this study suggest that protective immune responses may be reduced in bison during periods of nutritional restriction. Therefore, seasonal nutrition may play a role in the effectiveness of bison vaccination, with protective immune responses reduced during the primary *B. abortus* transmission period. Further research identifying how seasonal nutrition influences vaccine induced responses may help improve the effectiveness of wildlife vaccination programs.

Table 6.1. IFN- γ (ng/mL; mean \pm SE) produced by peripheral blood cultures stimulated with heat killed *B. abortus* following vaccination with SRB51 for Quarantine and Yellowstone bison study groups.

Quarantine Bison		Weeks After Vaccination				
Responder Class ^a	0	3	8	12	18	21
Strong (<i>n</i> = 5)	0.16 \pm 0.10	61.68 \pm 33.74	17.57 \pm 6.86	9.88 \pm 4.83	13.35 \pm 10.24	13.76 \pm 5.72
Weak (<i>n</i> = 5)	0.16 \pm 0.12	1.63 \pm 0.62	1.12 \pm 0.47	1.23 \pm 0.80	2.60 \pm 1.46	1.46 \pm 0.90
None (<i>n</i> = 2)	0.08 \pm 0.08	0.14 \pm 0.03	0.29 \pm 0.04	0.05 \pm 0.05	0.05 \pm 0.05	0.18 \pm 0.18

Yellowstone Bison		Weeks After Vaccination			
Responder Class	0	2	6	28+	
Strong (<i>n</i> = 5)	1.91 \pm 1.28	38.35 \pm 18.0	98.85 \pm 59.47	14.77 \pm 4.85	
Weak (<i>n</i> = 4)	1.49 \pm 1.49	1.43 \pm 1.43	5.97 \pm 2.46	0.79 \pm 0.79	
None (<i>n</i> = 3)	0	1.50 \pm 0.31	0.46 \pm 0.46	NA	

^a Individual bison were assigned to responder classes based on the largest concentration of IFN- γ measured for any time point following vaccination (strong : >20 ng/mL IFN- γ , weak: 2 > ng/mL IFN- γ < 20, none: < 2 ng/mL IFN- γ).

Table 6.2. Immune responses and observed pregnancy outcomes in free-ranging Yellowstone bison that seroconverted from negative to positive status following SRB51 vaccination.

Bison ID	IFN- γ (ng/mL)				IL-4 (ng/ml)	Antibody Response	Pregnancy Outcome ^a
	0 wks	2 Wks	6 Wks	28+Wks	28+ Wks	FPA (mP) at 28+ Weeks ^a	
357	0	0	0	17.5	0	165.6	Calf observed
361	6.0	2.9	9.3	134.5	0	168.6	No calf observed
370	NA	NA	2.0	4.4	2.7	228.0	Abortion ^c

^a Antibody levels identifying seroconversion at 28+ weeks post vaccination using fluorescent polarization assay (FPA)

^b All three bison were diagnosed pregnant using serum pregnancy specific protein B (PSPB)

^c Bison Yell-370 was observed with retained placenta and *B. abortus* was cultured from vaginal exudates

Table 6.3. Nutritional indicators (mean \pm SE) for quarantine and Yellowstone study groups based on responder classification.

Indicator	Quarantine Responder Class ^a			Yellowstone Responder Class	
	Strong	Weak	None	Strong	Weak
Leptin	1.56 \pm 0.54	1.11 \pm 0.21	1.0 \pm 0.41	2.49 \pm 0.53	3.02 \pm 0.58
IGF-1	60.87 \pm 8.98	57.9 \pm 13.78	43.35 \pm 1.47	77.29 \pm 4.32	74.76 \pm 3.56
UN	30.72 \pm 1.51	32.88 \pm 1.37	32.95 \pm 1.05	19.3 \pm 4.08	8.4 \pm NA
NEFA	321.94 \pm 90.33	481.74 \pm 81.46	552.35 \pm 57.25	448.57 \pm 116.45	449.1 \pm NA

^a Individual bison were assigned to responder classes based on the largest concentration of IFN- γ measured for any time point following vaccination (strong : >20 ng/mL IFN- γ , weak: $2 >$ ng/mL IFN- γ $<$ 20, none: $<$ 2 ng/mL IFN- γ).

Table 6.4. Ranking of regression models evaluating the influence of nutrition on IFN- γ production (\log_{10} IFN- γ) from peripheral blood cultures stimulated with mitogen (ConA) in quarantine and Yellowstone bison study groups ($n = 81$). Models are ranked by ascending ΔAIC_c and Akaike weights (w_i).

Model	K	AIC _c	ΔAIC_c	w_i
Leptin + IGF-1 + NEFA	5	209.01	0.00	0.38
IGF-1 + NEFA	4	209.46	0.45	0.30
Leptin + IGF-1 + BUN + NEFA	6	210.92	1.91	0.15
IGF-1 + BUN + NEFA	5	211.61	2.60	0.10
Leptin + NEFA	4	213.49	4.48	0.04
Leptin + BUN + NEFA	5	214.45	5.44	0.02
NEFA	3	217.86	8.85	0.00
BUN + NEFA	4	219.43	10.42	0.00
Leptin + IGF-1 + BUN	5	222.12	13.11	0.00
Leptin + BUN	4	222.84	13.83	0.00
IGF1 +BUN	4	225.04	16.03	0.00
Leptin + IGF-1	4	225.45	16.44	0.00
IGF-1	3	227.12	18.11	0.00
Leptin	3	227.42	18.41	0.00
BUN	3	229.47	20.46	0.00

Model notation: BUN= blood urea nitrogen; NEFA= non-esterified fatty acid; IGF-1= insulin-like growth factor-1

Table 6.5. Parameter estimates for the model with the lowest AIC_c value in Table 6.4.

Parameter	Estimate	SE	t-value	P
Intercept	4.031	0.334	12.057	<0.0001
Leptin	0.221	0.136	1.621	0.109
IGF-1	0.013	0.005	2.587	0.012
NEFA	-0.002	0.000	-4.473	<0.0001

Model notation: NEFA= non-esterified fatty acid; IGF-1= insulin-like growth factor-1.

Figure 6.1. IFN- γ production (mean \pm SE) by bison peripheral blood cultures following vaccination with SRB51 for quarantine and Yellowstone study groups.

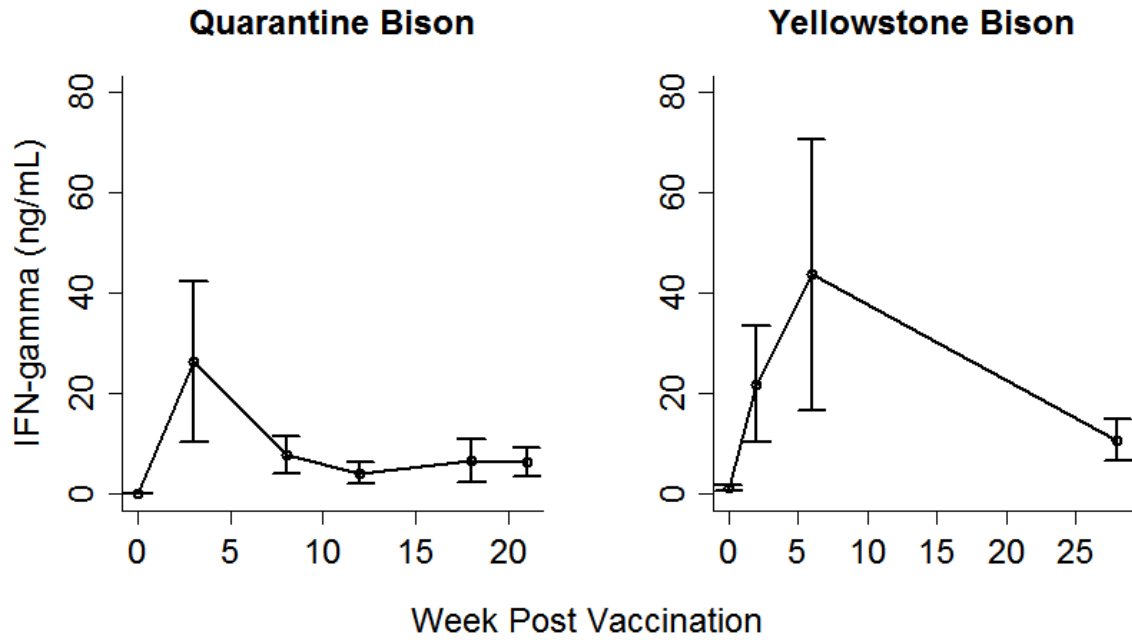


Figure 6.2. Levels of IFN- γ , hormones, and metabolites in bison from the quarantine and Yellowstone study groups sampled 20 weeks after vaccination with SRB51. Values are presented as mean \pm SE for (a) IFN- γ production by bison PBMC stimulated with heat-killed *B. abortus*, (b) IFN- γ production by bison PBMC stimulated with mitogen (ConA), (c) plasma leptin, (d) plasma insulin-like growth factor-1 (IGF-1), (e) blood urea nitrogen (BUN), and (f) plasma non-esterified fatty acid (NEFA). Asterisks indicate significant differences between study groups, * $P < 0.05$.

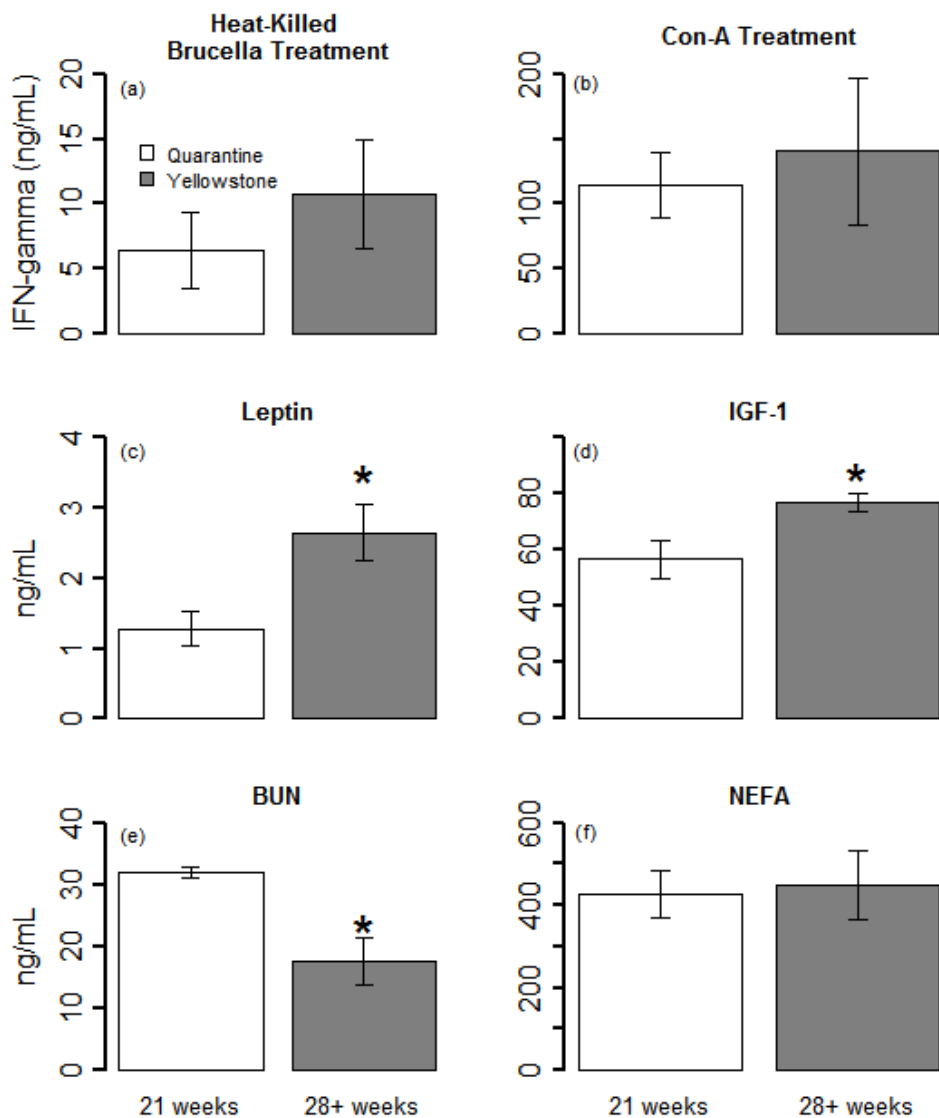
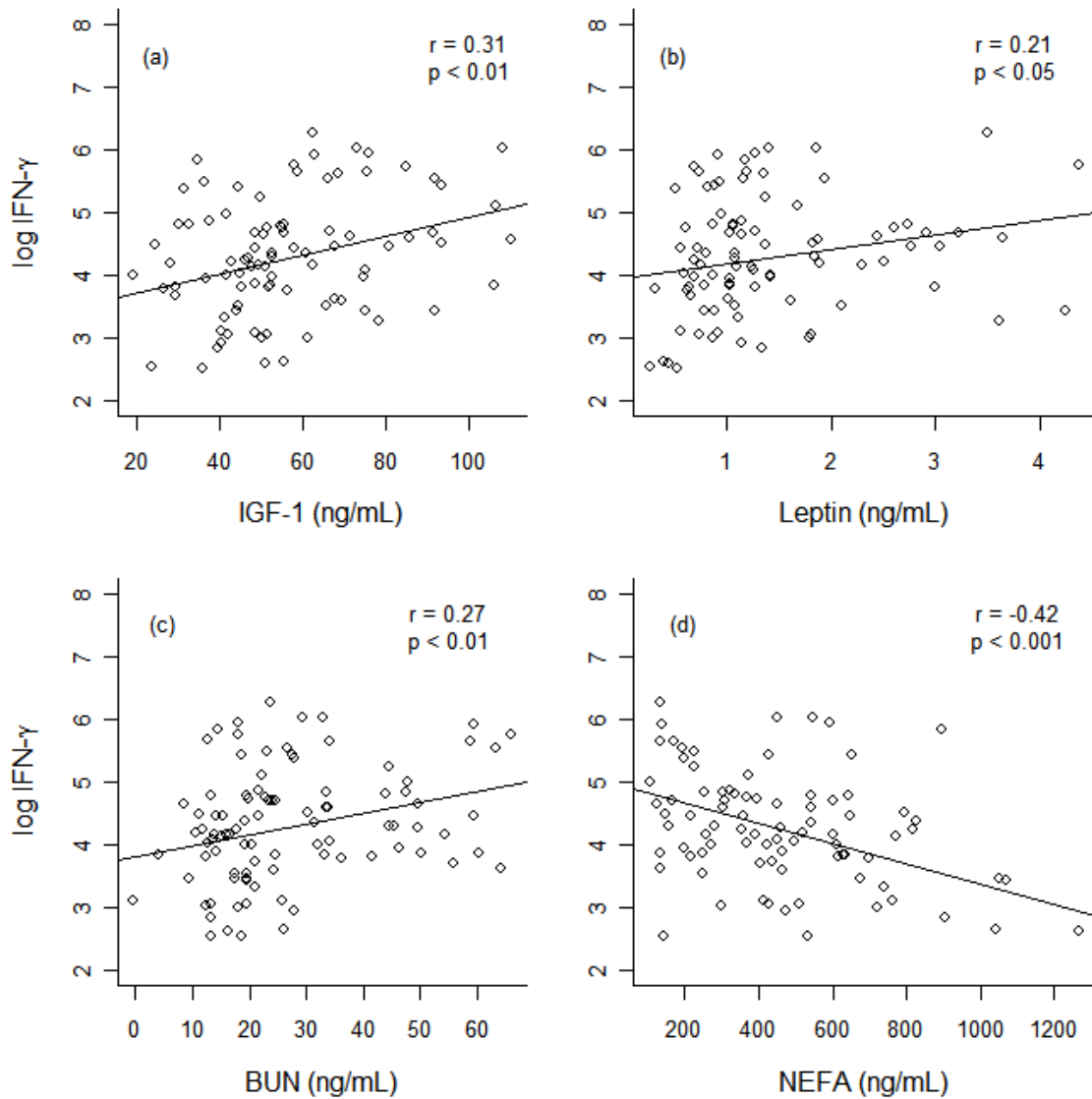


Figure 6.3. Bivariate analyses of the relationship between \log_{10} -transformed IFN- γ production (ng/mL) of bison peripheral blood cultures stimulated with mitogen (ConA) and (a) plasma insulin-like growth factor-1 (IGF-1), (b) plasma leptin, (c) blood urea nitrogen (BUN), and (d) plasma non-esterified fatty acid (NEFA).



Chapter 7 – Conclusions and Future Directions

The focus of this dissertation research has been to advance our understanding of how brucellosis is maintained within Yellowstone bison and to use this information to improve disease management practices. Though there has been much research conducted on brucellosis in domestic animals, there has been far less involving free-ranging wildlife. In chapter 2, I provided a review of intracellular pathogens known to establish long-term infections and cause abortions in their mammalian hosts. For wild ungulates, the reduction of immune defenses near parturition may be better understood in relation to life history tradeoffs involving the allocation of internal resource. Seasonal food restriction during pregnancy has the potential to limit resources available for immune defense and ultimately leads to endemically infected wildlife populations. The work presented in chapter 1 might be expanded to address whether the prevalence of abortive pathogens increases in wild ruminants at northern latitudes. If nutritional restriction increases susceptibility to these disease agents, wild ungulates may have higher infection rates in geographical regions where food availability is seasonally restricted.

For Yellowstone bison, the endemicity of brucellosis infection might be influenced by the timing of food restriction with increasing reproductive demands (chapter 5). Seasonal food restriction was found to reduce nutritional condition during late gestation, with the probability of active brucellosis infection being highest for bison in below-average condition (chapter 5). Variation in winter severity across years may influence brucellosis transmission, with more bison unable to contain *B. abortus* during

severe or prolonged winters. Such heterogeneity in *B. abortus* transmission may help explain the observed fluctuations in bison seroprevalence over past decades. Future brucellosis surveillance efforts could be conducted to assess whether incidence rates, an estimate of new *B. abortus* exposures within a given year, increase in marked bison in years following severe winters.

Primiparous bison may be the primary transmission source sustaining brucellosis in the Yellowstone population (chapter 4 & 5). The greater intensity of infection observed in reproductively immature bison suggests an inability of young animals to contain infection. Further research is needed on how nutritional condition, the level of endogenous protein and energy reserves, influences susceptibility to infection in young bison. Does *B. abortus* behave differently in immunocompromised young animals, which may lead to increased transmissibility, compared to chronically infected older animals? Additionally, bison exposed to multiple persistent disease agents may decrease immune responsiveness, exacerbating clinical disease. Joly and Messier (2005) found that bison which were positive for both tuberculosis and brucellosis were less likely to be pregnant or to survive the winter than bison positive for one or neither disease. Monitoring Yellowstone bison for co-infection of additional abortive disease agents will help better assess disease impacts on the population and improve disease management efforts.

Persistent diseases can decrease long-term conservation of wild ungulates, especially when management practices, such as culling are implemented to control disease transmission from wild ungulates to domestic animals and humans. Although wildlife vaccination has potential to reduce disease prevalence, vaccines that induce long-

lived cellular responses are lacking. In chapter 6, I compared the immunologic responses to vaccination in captive and free-ranging bison. In yearling female bison, the immune response to a single vaccination of SRB51 may offer some protection in approximately 50% of vaccinated animals. Overall, immune responses following vaccination were similar between both study groups including the proportion of individuals within each study group that showed either strong, weak or essentially no response following vaccination. This individual variation is expected to reduce vaccine efficacy when vaccination is applied at the population level. Factors, such as seasonal food restriction and loss of body reserves may play an important role in the effectiveness of wildlife vaccination programs. Research is needed to link within host processes (e.g. nutrition) with the induction of protective immune responses against persistent pathogens. Protective immune responses induced through vaccination may be limited if vaccines are delivered to undernourished animals.

The abortion in one of the three vaccinated bison that were naturally exposed to *B. abortus* (chapter 6) confirms incomplete protection from clinical disease. This study suggests that a single vaccination of SRB51 does not provide strong protective responses. Wild ungulates in temperate and northern environments may need to receive multiple vaccinations to build up immunological memory needed to defend against persistent infectious diseases. To monitor the effectiveness of wildlife vaccination programs, further research is needed to establish measurable correlates of immune protection. In chapter 6, I measured an important indicator of cell-mediated immune response, IFN- γ , following vaccination. Future vaccination studies would benefit from measuring a broader range of indicators, such as multiple cytokines and the proliferation of T-

lymphocyte subpopulations to better assess protective responses. A comprehensive immunological profile could also improve vaccination models by explicitly modeling individual variation in host immune responses to vaccination.

The individual-based model (chapter 3) helped evaluate how different vaccination strategies might reduce the level of brucellosis infection in Yellowstone bison. Model simulations suggested that eradication of brucellosis is unlikely with the currently available vaccine and delivery options. However, reducing the level of infection may be achievable but would require a long-term investment. The main factors limiting success of a bison vaccination program are consistent delivery methods of an efficacious vaccine. Currently, there is much work being conducted on vaccine development, which could be applied to brucellosis reduction in Yellowstone bison. However, effective delivery methods providing large scale coverage to the target population are under-developed. The variability in individual immune responses to vaccination underscores the need for delivery methods that ensure vaccinated animals receive the intended dose of the vaccine. For this reason, remote delivery of vaccine to free-ranging bison may not be a credible option. Alternatively, establishing temporary capture facilities within Yellowstone where bison could be vaccinated, marked, and released would maximize coverage of the vaccine with a reliable delivery method (syringe delivery). Additionally, this approach would allow bison to receive booster vaccinations, with marked individuals available to monitor the program's effectiveness.

Long-term monitoring will be an essential component of any brucellosis reduction program for Yellowstone bison. Until diagnostic tests are improved, seroprevalence is expected to be the primary metric by which the level of brucellosis infection is

determined. Thus, monitoring the effectiveness of vaccination will require a surveillance program which recognizes the limitations of serologic tests in making determinations on the effectiveness of vaccination. Bison in early reproductive ages represent a large demographic of the population and may be the primary source maintaining brucellosis infection (chapter 4). However, antibodies produced against *B. abortus* decline slowly and probably overestimate the level of infection in older bison. Thus, serologic tests can be misleading without an understanding of how they relate to active infection. Further research is needed to better link antibody levels with active *B. abortus* infection and indicators of protective immunity, such as IFN- γ . Since *B. abortus* is a persistent pathogen known to establish long-term infection, we can never be certain that bison have completely recovered from infection by clearing all bacteria. Therefore, improving our ability to identify potentially infectious individuals will advance management practices that are more aligned with bison conservation.

Until effective methods to reduce brucellosis are developed, the best approach to control the risk of brucellosis transmission from bison to cattle is to maintain spatial and temporal separation. Management agencies should continue to allow bison migration to essential winter range areas in and adjacent to Yellowstone National Park, but actively prevent dispersal and range expansion to outlying private lands until there is tolerance for bison in these areas (Plumb et al. 2009). Additionally, Kilpatrick *et al.* (2009) recommended the cessation of cattle grazing in areas where bison leave the park in winter and compensating ranchers for lost earnings and wages. Conservation groups and government agencies have successfully used, and are still pursuing, this strategy with willing landowners (U.S. Department of the Interior et al. 2008). However, further

efforts are needed from wildlife managers, livestock producers, and the public to balance long-term bison conservation with brucellosis risk management.

Brucellosis risk management in the Greater Yellowstone Ecosystem is one of the great challenges facing large mammal conservation in North America. Effective management practices will require a diverse range of integrated methods which include maintaining separation of livestock and wildlife, managing habitat to reduce brucellosis transmission, and reducing disease prevalence in wildlife. The long-term success of these management practices will depend on sound science and support from the stakeholders involved. Otherwise, efforts to balance brucellosis management with wildlife conservation are unlikely to be successful.

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PEER REVIEWED PUBLICATIONS

Treanor, J.J., Geremia, G., Crowley, P.H., Cox, J.J., White, P.J., Wallen, R.L., Blanton, D.W. (2011) Estimating probabilities of active brucellosis infection in Yellowstone bison through quantitative serology and tissue culture. *Journal of applied Ecology* **48**, 1324-1332.

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