Detection and Transmission Dynamics of *Brucella abortus* in the Greater Yellowstone Area

By

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IN MEMORY OF

BARBARA R. SCHUMAKER, 1946-2010

WHO SET ME ON THE PATH OF DISCOVERY

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Abstract

Wild, free-ranging, bison and elk in the greater Yellowstone area (GYA) are the last reported reservoir of *Brucella abortus* in the United States. Diagnosis of *B. abortus* infection, potentially leading to brucellosis, is challenging, as there is no perfect reference test. An evaluation of the fluorescence polarization assay (FPA) was performed using serum and tissues from the known *B. abortus*-infected bison herd in Yellowstone National Park (YNP) and serum from privately-owned bison. While the FPA and five other tests had perfect sensitivity in screening of *B. abortus* antibodies in bison, all tests had substantially lower specificity in the YNP herd. However, a Bayesian analysis showed that 59-74% of the culture-negative animals were most likely truly infected. A decision-tree analysis illustrated that the expected cost of FPA testing was comparable to the cost of other serologic tests. The FPA was shown to be highly sensitive but may not be able to differentiate culture-positive and culture-negative animals. For evaluation of tests under field conditions, longitudinal studies should be performed, testing animals throughout the study period and harvesting a subset of subjects at various points to determine their culture status. These studies could further facilitate sound, adaptive management decisions for the GYA.

The ability of bison and elk to concomitantly serve as hosts of *B. abortus*

increases the complexity of the risk of transmission to cattle. This multi-reservoir system poses significant challenges for comprehensive disease management. To address these intricacies, the first spatially-explicit risk assessment of *B. abortus* transmission among elk, bison, and cattle in the northern portion of the GYA was performed. The model used for this assessment was based on spatio-temporal probabilities of bacterial shedding by bison and elk on the northern GYA landscape. Although the model estimated substantial shedding of *Brucella* bacteria from bison in some winters, the most substantial risk of *B. abortus* transmission to cattle was from elk. The estimated percentage of cattle exposure risk from the Yellowstone bison herd was small (0.0-0.3% of total risk) compared with elk, which contributed 99.7-100% of the total risk. Increasing population size resulted in higher herd densities and increased bacterial shedding. Interactive effects between population size and winter severity were major determinants influencing bison movements to lower elevation winter grazing areas, overlapping with federally-regulated domestic cattle grazing allotments. Median total annual risk to cattle from elk and bison was 3.6 cattle-exposure event-days (95% P.I. 0.1-36.6). Natural herd migration and boundary management operations were important in minimizing the contribution of bison to cattle exposure risk, which supports continued boundary management operations for spatio-temporal separation between bison and cattle. Under current management practices, bison risk to cattle grazing in the northern portion of the GYA is expected to be minimal. The comingling of cattle and elk, especially during the late gestation period for elk, should be reduced when spontaneous elk abortions pose a risk for interspecies disease transmission.

The inter- and intra-species contact rates required to maintain brucellosis in the

GYA were previously uncharacterized. Without this knowledge, the likely effects of risk mitigation strategies could not be adequately evaluated. The wildlife risk model described above was used to estimate the spatio-temporal distribution of *B. abortus* shedding events from bison and elk populations in the northern GYA. The percentage of *B. abortus* infectious events in overlapping wildlife populations was calculated, and the risk of *B. abortus* transmission within and between populations was estimated. Bison risk from other bison and from elk showed almost 100% adequacy to transmit the organism once spatio-temporal overlap occurred; however, contact within elk populations was only sufficiently able to produce disease 34% of the time. Transmission risks to elk from elk in other populations or from bison were very small. Minimal opportunity exists for *B. abortus* transmission from bison to elk under current natural conditions in the northern GYA. Under current conditions, management alternatives that reduce bison seroprevalence are unlikely to substantially reduce transmission risk from elk to cattle. Strategies that decrease elk herd densities and group sizes and reduce elk-to-elk transmission could reduce the overall risk to cattle grazing in the northern portion of the GYA.

This research has substantially filled gaps in the understanding of *B. abortus* diagnostics, transmission risk from wildlife to cattle, and transmission dynamics within and between wildlife populations in the GYA. It highlights the need for longitudinal studies of *B. abortus* infection and directs future management actions toward mitigating transmission risks within elk populations as well as from elk to cattle in the northern portion of the GYA. This work should help to justify the most efficient allocation of research and management funding and aid eventual eradication of *B. abortus* from the

United States.

Chapter 1

Detection and Transmission Dynamics of *Brucella abortus* in the Greater Yellowstone Area

Disease management at the wildlife-livestock interface is hampered by the challenge of balancing wildlife conservation with the livelihoods and traditions of livestock producers. The potential for disease transmission between wildlife and livestock exacerbates conflicts between natural resource managers and cattlemen, reduces tolerance for wildlife near livestock operations, and negatively impacts conservation. Therefore, diseases that affect both wildlife and livestock are important in resource management, regardless of their direct impact to the wild animal populations which may serve as their reservoirs. Many important diseases of livestock are shared among multiple species, including foot-and-mouth disease, Rift Valley fever, and Johne's disease (Daszak et al., 2000; Chivian, 2001; Taylor et al., 2001; Woolhouse et al., 2001; Belloy et al., 2004; Cunningham, 2005; Böhm et al., 2009; Tomley and Shirley, 2009). Human population growth and associated landscape changes, as well as competition for grazing lands, have made wildlife-livestock disease transmission more likely by reducing the spatial separation between livestock operations and wildlife habitat (Daszak et al., 2001; Western, 2001).

The US is free of many of the devastating diseases affecting both wildlife and livestock worldwide. However, the government has spent billions of dollars on disease eradication programs for both wildlife and livestock. Pneumonia caused by multiple pathogens from domestic sheep threaten bighorn sheep populations throughout the

western US (Clifford et al., 2009; USDA Forest Service, 2010). Tuberculosis in Michigan deer and cattle populations continues to be a problem (State of Michigan, 2008). Also, recent cases of tuberculosis in captive elk in Nebraska and cattle in California are an indication that the US is far from eradication of these diseases (Olmstead and Rhode, 2004). In addition, multiple recurrences of bovine brucellosis, caused by the bacterium *Brucella abortus* in the states surrounding the greater Yellowstone area have greatly complicated the US eradication effort.

B. abortus is a gram-negative, facultative, intracellular bacterium that causes disease in many domestic and wild animal species including cattle, bison (*Bison bison*), elk (*Cervus elaphus*), and moose (*Alces alces*) (Creech, 1930; Thorne et al., 1978a; Edmonds et al., 1999). Bacteria invade the mucous membranes of ungulates and can cause placentitis with late-gestation abortions in females and orchitis and epididymitis in males (Bercovich, 1998). Increased abortion rates, decreased milk production, loss of condition, infertility, and lameness in cattle have made brucellosis extremely important to beef and milk producers around the world (Manthei and Carter, 1950), restricting international trade in many instances (Wilson and Beers, 2001).

B. abortus was first characterized as the cause of epizootic abortion by Bernard Bang in 1896 (Bang, 1897). The eradication of the disease from the US has been a priority of the federal government since 1934, when a Cooperative State-Federal Brucellosis Eradication Program (BEP) was adopted to reduce the prevalence of brucellosis in cattle, designating it as the most significant livestock disease at that time. Since then, agencies have implemented a variety of livestock, wildlife, and disease risk

management strategies (Cheville et al., 1998). Billions of dollars have been spent eradicating brucellosis from livestock in nearly every state in the US (Wise, 1980).

Zoonotic Implications of Brucellosis

The potential for human infection and large economic losses have made *B. abortus* an important pathogen restricting international trade (Wilson and Beers, 2001). The bacterium has also been classified as an overlap human/livestock select agent by the United States Department of Health and Human Services and the United States Department of Agriculture (USDA) (2002). Brucellosis in humans is characterized by intermittent bacteremia caused by seeding of bacteria from lymph nodes which causes malaise, aching joints, and irregular spikes in body temperature referred to as undulant fever. The recommended treatment of human brucellosis is doxycycline and rifampin (Centers for Disease Control and Prevention, 2007).

In 2006, human brucellosis from exposure to *B. abortus* in cattle, *B. melitensis* in sheep and goats, and *B. suis* in swine was still considered the most common zoonotic infection worldwide (Pappas et al., 2006). In the early years of the BEP, human brucellosis in the US was mainly acquired from contact with infected meat and tissues during slaughter operations. However, *Brucella spp.* also colonize the mammary glands of infected animals and can be transmitted in milk (Young and Suvannoparrat, 1975). In the last two decades, the main cause of human brucellosis in the US was from food-borne infection mainly through importation of soft cheeses from Mexico (Chomel et al., 1994).

Although the US likely has low exposure to *B. abortus* in humans, the increasing number of *B. abortus* infections in Kyrgyzstan is an example of what can happen without appropriate disease control strategies. After the collapse of the Soviet Union, the country was ill-equipped to handle a major livestock disease like brucellosis. These circumstances, combined with the impacts of a depressed economy and poor hygiene, has created the opportunity for a re-emerging zoonotic disease epidemic. In the first six months of 2003, there were 1170 reported cases of human brucellosis in Kyrgyzstan, a 30% increase from the previous year (UN Office for the Coordination of Humanitarian Affairs, 2003). Of those cases, 20% were children or adolescents. Because brucellosis is so difficult and expensive to treat it has been a great detriment to the economy of the country and gives credence to the expenditures on the BEP in the US (Kozukeev et al., 2006).

State-Federal Cooperative Brucellosis Eradication Plan

During the 76-year history of the BEP, it has limited the impacts of brucellosis in cattle throughout the US (Donch and Gertonson, 2008). At the program's inception, 11.5% of adult cattle in the US were infected with the bacterium (Ragan, 2002), and the annual losses to the livestock industry were $400 million with $50 million lost to decreases in milk production alone (Knox, 1947). In 1957, there were an estimated 124,000 herds infected with brucellosis using imperfect surveillance with only 33-50% detection (Ragan, 2002). By 1961, the entire annual loss to the livestock industry was reduced to $25 million (Mingle, 1961), and by 2000 only 6 herds were diagnosed as infected. Studies have shown that, if the program were discontinued, costs would increase

by $80 million annually in less than 10 years (Bittner, 2004). By early 2008, the US and all associated territories were brucellosis-free in livestock. However in June 2008, brucellosis was again detected in cattle herds in Montana and Wyoming (Donch and Gertonson, 2008). Transmission incidents in the last four years in all three states surrounding Yellowstone National Park (YNP) – Idaho, Montana, and Wyoming – have highlighted the importance of wildlife brucellosis on livelihoods and management (Donch and Gertonson, 2008).

Greater Yellowstone Area and Wildlife Populations

YNP was established as America's first national park in 1872, and has become a flagship for wildlife conservation worldwide. Despite its large size of 8,987 square kilometers, YNP is not independent of its surrounding ecosystem, the greater Yellowstone area (GYA). The GYA is one of the largest intact temperate zone ecosystems on earth and includes approximately 28,000 square miles in Montana, Idaho, and Wyoming and encompasses state lands, two national parks, portions of six national forests, three national wildlife refuges, Bureau of Land Management holdings, and private and tribal lands. The GYA is also home to the largest wild and free-ranging elk and bison populations in the US.

Approximately 125,000 elk occupy the GYA across 25 elk management jurisdictions. Agencies manage elk and their habitat resources through complex interagency cooperation. Elk hunting occurs in all involved elk management jurisdictions except YNP. There are also 23 elk feedgrounds in northwest Wyoming (the National Elk Refuge and 22 state operations) that may support approximately 25,000 elk, depending

on winter severity. Approximately 5,000 bison occupy the GYA across trans-boundary bison management jurisdictions in and adjacent to YNP (4,200) and Jackson Hole, Wyoming (800). Bison hunting presently occurs only in select national forest areas in Wyoming, with most bison in Jackson Hole utilizing the feedground on the National Elk Refuge during the winter.

At the turn of the century, only 50,000 elk were reportedly remaining in the entire continental US, mainly inhabiting areas of the GYA (Seton, 1927). Supplemental winter elk feeding began in Jackson Hole, Wyoming in 1910 as an effort to help elk avoid starvation during harsh winters and decrease their impacts on agricultural lands (Smith, 2001). This practice was expanded in 1912, by the creation of the National Elk Refuge, but supplemental feeding has ultimately led to a number of negative consequences. Elk are above management targets in many areas in the GYA (Dickson, 2005). Feeding practices have artificially increased their population density from November to April and allowed for more intraspecies transmission of diseases during the winter months. For example, longer feeding seasons are associated with higher *B. abortus* seroprevalence (Cross et al., 2007).

However, the economics of elk hunting in Wyoming have made the possibility of closing feedgrounds extremely controversial. Unguided hunting on public lands in Wyoming is prohibited for non-residents, and the outfitting industry is a large part of the local economy. In 1980, outfitting businesses in Teton County, Wyoming had direct sales of $2.4 million for big-game hunting (Taylor et al., 1981). Accounting for indirect revenue yielded a total of $4.2 million in annual income from outfitting (Taylor et al.,

1981). Because most hunting revenues are generated in the fall, the outfitting industry helps to bridge the gap between summer and winter tourist seasons.

The continental divide runs from west to east across the southern portion of YNP. The northern GYA includes the Yellowstone bison population and five elk populations (Gallatin-Madison, Gravelly-Snowcrest, Madison-Firehole, northern Yellowstone, and Sand Creek, Idaho), which are distributed across over 1,100 square miles in the northern GYA. Estimates of northern Yellowstone elk were near 25,000 animals in the late 1980s but decreased by approximately 50-60% by 2006 (Eberhardt et al., 2007). The Yellowstone bison population ranges between 2000 and 5000 individuals (Meagher, 1973; Clarke et al., 2005) depending on environmental conditions and management strategies implemented. These bison are important for the conservation of the species because the population is derived from the original wild herd supplemented by an introduced herd containing diverse genetics (Meagher, 1973). In addition, the bison have had no evidence of cattle-hybridization (Halbert et al., 2005). Therefore, disease management activities, including the future potential for movement of individual bison into other herds, are of special interest in this population.

The 2009 summer count for the Yellowstone bison herd was 3,300 animals, divided equally between a central and northern breeding population. The modeled food-limiting carrying capacity for bison within YNP is 6200 individuals (Plumb et al., 2009). However, even at lower population numbers, interactive effects of severe winters and herd density with population numbers greater than 4200 have lead to large-scale dispersal to lower elevations. Plumb et al. (2009), recommended the Yellowstone bison herd be maintained with less than 4500 animals to abate most large-scale movements outside the

park during average winter conditions. Appropriate population management would help avoid contact with cattle, which are grazed (266 in the winter and 1363 in the spring) on public and private lands adjacent to YNP and within habitat occupied by bison and elk during the winter (Kilpatrick et al., 2009).

Brucellosis Pathogenesis in Wildlife

The proximity of cattle-grazing to wildlife populations makes interspecies disease transmission a concern. Wild, free-ranging bison and elk in the GYA persist as the last known reservoir of *B. abortus*-caused brucellosis in the US (Godfroid, 2002). Brucellosis in Yellowstone bison is similar to that of chronically infected cattle (Roffe et al., 1999; Rhyan et al., 2001). In the wildlife host, *B. abortus* is typically transmitted to susceptible individuals after licking a newborn calf of an infected dam or ingesting an aborted fetus or placenta. Once inside the host, *B.abortus* resides in regional lymph nodes and then is transported to other lymph nodes. The sublumbar or supramammary lymph nodes are common targets. *Brucella abortus* uses several strategies to evade detection by the host's immune system (Arenas et al., 2000). The bacterium often takes up residence in host macrophages, where its intracellular signals prevent phagosome-lysosome fusion (Frenchick et al., 1985; Pizarro-Cerda et al., 1998). During the third trimester of pregnancy, the bacterium preferentially invades the placenta and causes fetal death and abortion. Nearly 100% of bison will abort their first calf after infection (Davis et al., 1990; Davis et al., 1991). The typical clinical signs seen in the aborted material are necrotizing placentitis and fetal pneumonia (Rhyan et al., 2001). If the fetus is carried to term, the bacterium may also be vertically transmitted to the calf from the dam's milk.

However, there has been no proven relationship between the serostatus of dam and calf, and most calves are seronegative by six months of age (Fuller et al., 2007).

The incubation period for brucellosis is variable and affected by gestation, exposure dose, age, vaccination, and effects of host-resistance (Nicoletti, 1980). After experimental inoculation of elk, mean length of time between inoculation and a serologic titer was 39 days, and the mean time-to-abortion was 89 days post-infection (Thorne et al., 1979). In cattle, about 20% of calves born to infected dams are seronegative but latently infected (Plommet et al., 1973; Lapraik et al., 1975). Up to 10% of these calves have been known to seroconvert in early adulthood as the stress of pregnancy lowers their immune systems (Wilesmith, 1978). This so-called "heifer syndrome" has been described in bison and elk in addition to cattle (Van Den Born and Vervoorn, 1965; Plommet et al., 1973; Lapraik et al., 1975; Thorne et al., 1978b; Thorne et al., 1979; Catlin and Sheehan, 1986; Olsen et al., 2003). Once an animal is infected, there is little evidence to suggest that it will ever recover from infection, and it is recommended that it be considered a carrier for life, even if no abortions occur (Ragan, 2002).

Brucellosis in the GYA

Brucellosis was first detected among wildlife in the GYA in 1917, when epizootic abortion was described in Yellowstone bison (Mohler, 1917). The now-bison disease was most likely acquired from domestic cattle which were brought into the area for grazing (Meagher and Meyer, 1994). Elk in the southern GYA probably acquired the disease directly from cattle and then transmitted it to the bison presently using Grand Teton National Park. Today, elk populations in the northern GYA have low seroprevalence (i.e.,

exposure; <5%) for *B. abortus*, whereas seroprevalence in Yellowstone bison is high (40-60%) (Cheville et al., 1998). Elk feedgrounds in the southern GYA have increased the prevalence of brucellosis. The average seroprevalence for *B. abortus* among fed elk is around 26% (Aune, 2002; Etter and Drew, 2006). Brucellosis in wildlife does not generally threaten population persistence. Coinfection with bovine tuberculosis reduced the pregnancy rates by 10-15% for bison in Wood Buffalo National Park in the absence of elk competing for grazing land (Joly and Messier, 2005). However in YNP bison, recruitment and population numbers have remained sustainable, aside from boundary removals from the population to reduce transmission risk to cattle.

Although the wildlife populations in the GYA are stable, the ability of bison and elk to concomitantly serve as alternative hosts and sources of *B. abortus* increases the complexity of risk of transmission to cattle. This multi-reservoir system poses significant challenges to comprehensive disease management (Delahay et al., 2009). Understanding the interspecies transmission dynamics of a multi-host system is crucial for disease management (Dobson, 2004; Delahay et al., 2009). Some hosts may be persistent reservoirs of disease, and others may be recurrently infected through pathogen spillover (Power and Mitchell, 2004). Overall, diseases with multiple wildlife hosts are deemed extremely difficult to control and eradicate (Government Accountability Office, 2009).

Brucellosis Diagnostics

In order for a disease management program to be effective, infected animals must be detected. Unfortunately, there is no perfect brucellosis reference test. Although culture of tissues or fluids, such as milk, is frequently used as a standard for *B. abortus* diagnosis,

it is also imperfectly sensitive (Gall and Nielsen, 2004). There are often few detectable bacteria and no obvious signs of infection (i.e., subclinical or latent infection). If an individual clears the infection, it is likely to test positive on serologic tests, yet not shed bacteria. Also, collection, handling, and storage of samples, as well as laboratory techniques can affect the success of culture (Sutherland, 1980; Rhyan et al., 1997; Roffe et al., 1999; Gall and Nielsen, 2004). Laboratory methods require specific media and specialized incubation conditions, and *B. abortus*'s slow growth rate often leads to overgrowth of non-target bacteria on the culture plates. Because of these difficulties, serologic testing is frequently used to determine infection status.

The ideal serologic test should correctly classify an animal's infection status, be able to be performed animal-side, and yield rapid results. However, under field conditions, where an individual has the opportunity for exposure to *B. abortus* organisms, it is impossible to determine whether serologic test-positive but culture-negative individuals are either exposed but not currently infected or truly infected with undetected bacteria due to the lack of sensitivity of culture. Conversely, recently infected individuals may not yet be producing sufficient antibodies for serologic detection. However, these false-negative reactors may shed bacteria when aborting or calving. Also, persistently infected individuals can falsely test negative after the immune response diminishes below the threshold of detection due to lack of repeated exposure. Thus, it is unlikely that all truly infected individuals can be identified by serology alone (Cheville et al., 1998). Roffe et al. (1999) noted that only approximately 46% of sero-positive bison were culture positive from one or more tissues.

As the prevalence of brucellosis decreases in the US, the need for diagnostic tests with high sensitivity and specificity is becoming much more critical for appropriate brucellosis management, with the cost of incorrect test results becoming more substantial. Many serologic tests have been produced to aid diagnosis of *B. abortus* infections; however, all currently-used diagnostic methods were developed and validated for use only in cattle. When applied to wildlife, many cattle tests have been shown to be inaccurate and unpredictable (Morton et al., 1981; Davis et al., 1990). Furthermore, antibodies developed to environmental bacteria, such as *Yersinia enterocolitica O:9*, can cause cross-reactions in commonly-used *B. abortus* screening tests (Kittelberger et al., 1995).

In the last decade, some tests have shown promise in tackling these diagnostic issues. In 1999, Edmonds and colleagues described a western immunoblot designed to differentiate antibody responses to *B. abortus*, *B. melitensis*, and *B. suis*, as well as *Yersinia enterocolitica O:9*. The variation in O-antigens among the different bacterial species results in the host's development of specific antibodies to *B. abortus* that can be differentiated by the western blot (Edmonds et al., 1999). The technique was evaluated for use in detecting *B. abortus* antibodies in elk, and the results were comparable to standard serologic tests (Schumaker et al., 2010). In 2000, Gall et al. validated a fluorescence polarization assay (FPA) for use in detecting serum antibodies for *B. abortus* in bison (Gall et al., 2000). The authors estimated the specificity of FPA and other serologic tests in a population with no epidemiologic evidence of the presence of brucellosis, and a blinded study yielded sensitivity and specificity values of 96.3% and 97.6%, respectively.

Interspecies Disease Transmission

Both elk and bison have been shown to be competent reservoirs for *B. abortus* transmission to cattle (Thorne et al., 1979; Davis et al., 1990). In an elk-cattle pen study by Thorne et al. (1978a), close confinement may have contributed to transmission, but contact was not closer than feedground situations. Also, a report by Flagg (1983) showed evidence of fence-line contact and transmission of *B. abortus* from bison to cattle. While *B. abortus* may be carried in sperm and transmission via artificial insemination is a concern in livestock, males are not considered to be an important source of transmission risk from wildlife to cattle (Thorne, 2001).

The risk period for *B. abortus* transmission is well-defined. In general, data suggest that bison and elk in the northern portion of the GYA exhibit a high degree of birth synchrony, with the majority (80%) of bison calving during late-April to late-May and elk calving between mid-May to mid-June (Cheville et al., 1998; Berger and Cain, 1999). Feed ground data from the southern portion of the GYA in Wyoming have shown birth dates for elk that are later in the year, but parturition events are still unlikely after the third week of June due to the normal pattern of sexual segregation (Cross et al., 2009; Maichak et al., 2009). Including abortions in the last 90 days of pregnancy, late-January to mid-June is the most likely period for *B. abortus* transmission (Roffe et al., 2004).

The probability of *B. abortus* transmission between elk (or from elk to cattle) is likely low during calving (May through June) because elk dams segregate themselves while giving birth and meticulously clean the birth site (Johnson, 1951). Thus, birth sites are dispersed, and the likelihood of other elk or cattle encountering infected birth tissues

and fluids is low. However, transmission risk may be higher during the brucellosis abortion period from February through April when many elk aggregate in larger groups on lower-elevation winter ranges that sometimes include ranch areas with cattle (Hamlin and Cunningham, 2008). Spontaneous abortions by elk that are not segregated from the herd could expose many susceptible elk (or cattle) to infected fetuses and birth tissues (P.J. White, personal communication). Elk that winter at the Madison headwaters showed 53% winter range overlap with Yellowstone bison in December and 76% overlap in May (Ferrari and Garrot, 2002). A meaningful percentage of elk locations (18%) were within 100 meters of bison with comingling correlated with snowpack. However, these elk do not show evidence of an increase in *B. abortus* exposure compared with populations with spatio-temporal separation from bison (Ferrari and Garrot, 2002; Proffitt et al., 2010).

In contrast to elk, bison are gregarious during parturition, and pregnant females have been observed to nuzzle newborn calves (Treanor et al., 2008). Mobbing events of a newborn calf or aborted fetus could contribute to intra-species transmission of *B.abortus* if the dam were infected (Jones et al., 2009). Bison conservation continues to be a priority of the National Park Service; however, for decades, livestock and regulatory personnel have viewed Yellowstone bison as a potential source of pathogens for livestock in the GYA (Meagher and Meyer, 1994). Current management, which maintains spatial and temporal separation between bison and cattle, makes the risk of *B. abortus* transmission from bison to cattle in the northern GYA negligible (Kilpatrick et al., 2009). However, hazing and culling actions by bison managers to maintain this separation have been highly scrutinized and criticized for their economic costs and negative effects to bison. In the last decade, there have been multiple detections of brucellosis in cattle in the GYA

states (Idaho, Montana, Wyoming), with elk identified as the source of infection for nine cases since 2002 (Donch and Gertonson, 2008).

It is unknown how close a susceptible cow would have to be to *B. abortus*-infected tissues before it would be likely to investigate tissues and become exposed to the bacterium. The Starkey Project at the Pacific Northwest Research Station found that forage competition between elk and cattle likely decreases the chance of comingling on winter range (Coe et al., 2005). However, lack of available forage and other environmental pressures during severe winters in the GYA might increase comingling. The number of days a *B. abortus*-contaminated birth site is infective is dependent upon the amount of time that it takes for an infected fetus or tissues to be scavenged or for ultraviolet radiation to degrade the bacteria. Aune et al. (2007) and Cook et al. (2004) found that fetuses would be scavenged prior to ultraviolet degradation of bacteria (mean; range = 18.2; 1-78 days), which was used by Kilpatrick et al. (2009) to estimate the persistence of an infected site.

Brucellosis Status in GYA States

In the last four years, there have been outbreaks of brucellosis in all three states in the GYA (Idaho, Montana, and Wyoming). Idaho lost its brucellosis class-free status in 2006 but regained it in 2007. It has been maintaining a surveillance boundary for the five counties bordering YNP (USAHA, 2009). Idaho requires mandatory official calfhood vaccination for all cattle operations. It has been working to develop herd plans to minimize the risk of *B. abortus* transmission from wildlife to cattle by mainly focusing

on preventing winter feeding of elk, fencing stack yards, securing hay barns, and enclosing winter cattle feedgrounds.

Montana had cattle herds test positive for brucellosis in May, 2007 and June, 2008, causing the state to lose it brucellosis-free status. The state regained its class-free status in July, 2009 and continued its surveillance and Brucellosis Action Plan (BAP) until January 10th, 2010 (6 months following reclassification to class-free status). The high-risk area for the BAP, included the seven counties surrounding YNP (Beaverhead, Madison, Park, Gallatin, Sweet Grass, Stillwater, Carbon). After January, 2010 the more recent risk area included only Beaverhead, Madison, Park, and Gallatin counties. Surveillance of elk provided 880 useable samples, of which 62 (7%) were positive on standard serologic tests. However, only 13 (1.5% of the total) were confirmed positive by the western blot. Seropositive elk were found in five distinct hunting districts.

Wyoming lost its brucellosis-free status in 2004 and regained it in 2006. It had one herd test positive in 2008 but the outbreak was confined to the one herd. That herd was depopulated in October, 2008 and the state has maintained its class-free status. Over 8,000 cattle tested negative as part of the mandatory surveillance required before regaining the state's status. Brucellosis vaccination is required statewide with more stringent testing requirements required within the designated surveillance area (DSA). There is official identification of female cattle over 12 months of age statewide, and serologic testing required within 30 days prior to change of ownership, movement from the DSA, interstate movement, or exit from feeder channels. If a cow is tested during the lower-risk period (July 1 – November 1), it can be moved within 60 days. Cattle tested during the higher-risk period (November 2 – June 30) can be moved within 30 days.

Brucellosis Management

The Interagency Brucellosis Management Plan (IBMP) was established in 2000 to manage the risk of *B. abortus* transmission from bison to cattle by implementing hazing, test-and-slaughter, hunting, and other actions near the boundary of Yellowstone National Park (Plumb and Aune, 2002; Donch et al., 2005). To date, these actions have successfully prevented the transmission of *B. abortus* from bison to cattle (Clarke et al., 2005), and an assessment suggested that the risk of future *B. abortus* transmission is minimal under current management conditions (Kilpatrick et al., 2009). Since 2000, about 3200 bison have been removed from the Yellowstone herd with over 1000 animals, or 20% of the total population, culled during the winter of 2005-2006. These actions have been controversial with animal advocacy groups.

The IMBP was not intended to incorporate potential *B. abortus* transmission between elk and bison, and the resultant risks of transmission between elk and cattle. All recent detections of brucellosis in northern GYA cattle have been qualitatively attributed to elk that may or may not have seasonally occupied YNP (Galey et al., 2005). Due to the intense focus on bison *B. abortus* management during the past decade, elk have received minimal brucellosis management attention until recently and often move freely across the ecosystem and come into close contact with cattle premises.

Due to increased *B. abortus* prevalence in Wyoming elk, more elk *B. abortus* mitigation strategies have been evaluated. A five-year pilot test-and-slaughter program in the Pinedale area by Laura Linn-Meadows lowered *B. abortus* seroprevalence but at a cost of $7000 for each elk removed (USAHA, 2009). The study showed that only 50% of

all test-eligible elk were able to be captured. Of all animals sent to slaughter, half were culture-positive.

Since the early period of the BEP, vaccination has been considered a control method for *B. abortus* transmission. The immune-response necessary for conferring protection to the bacteria is a cell-mediated response, especially from CD8 cytotoxic T cells, which is necessary for attacking intracellular bacteria (Schurig et al., 2002). With *B. abortus*, the lipopolysaccharide (LPS) is the major inducer of antibody responses and involves an incompletely understood intracellular signal involving tumor necrosis factor a, perforin, and gamma-interferon (Zhan et al., 1993; Murphy et al., 2001).

There are two USDA-licensed vaccines that offer a measure of protection against *B. abortus*. The strain 19 vaccine was developed from an isolate of *B. abortus* taken from the milk of a Jersey cow in 1923. The isolate was kept at room temperature for over a year and discovered to have lost some of its virulence (Buck, 1930). Unfortunately, the bacterium kept its O side chain of the LPS, which causes animals vaccinated with strain 19 (S19) to test positive on standard *B. abortus* serologic tests. There are also side effects to S19 vaccination. About 1-2.5% of pregnant cattle vaccinated with S19 abort their calves (Manthei, 1952; Mingle, 1961; Beckett and McDiarmid, 1985). There have also been reports and experimental evidence of a more rare association between lameness in cattle and vaccination with strain 19 (Bracewell and Corbel, 1980; Wyn-Jones et al., 1980; Nicoletti et al., 1986; Rogerson and Morgan, 1986; Corbel et al., 1989; Johnson et al., 1994). The arthropathy is caused by *Brucella* antigen-containing immune complexes which locate in the affected joints. Despite the risks of vaccination, elk are vaccinated with S19 on the Wyoming feedgrounds, except for one site for comparison.

Because the serologic cross-reactions of S19 make it ineffective for test-and-cull methods of *B. abortus* control, other candidate vaccines were explored. A live rifampin-resistant "rough", or devoid of the LPS O-chain, attenuated strain of *B. abortus* labeled "51" by internal laboratory nomenclature was developed by Schurig and colleagues (1991) and was later trademarked by Virginia Tech Intellectual Properties in 1992. Rough *Brucella* 51 (RB51) has proven to be less abortigenic in cattle than S19 while showing similar efficacy. Because RB51 lacks the O-chain on its LPS it does not cross-react on *B. abortus* serologic tests. The vaccine was licensed for use in cattle by USDA-Animal and Plant Health Inspection Service (APHIS) in 1996. However, it has not shown efficacy in elk, which is why S19 is the only vaccine used in this species (Kreeger et al., 2002).

The efficacy of RB51 in bison remains in dispute (Davis and Elzer, 2002). Olsen et al. (2003) reported that RB51 was efficacious as a calfhood vaccine, whereas data reported by Elzer and Davis (2002) were contradictory. However, there does appear to be consensus that RB51 vaccine is safe as a calfhood vaccine for bison. Also, information about effects on individual bison from vaccination during pregnancy is limited, and there are concerns about abortigenic responses in bison. No abortions occurred when pregnant female bison were vaccinated during their first or second trimesters of gestation (2-5 months after conception; Elzer et al. 1998, Davis and Elzer 2002, Olsen and Holland 2003). However, 2 of 8 pregnant females that were vaccinated during the second half of gestation (i.e., 4.5-6.5 months after conception) aborted their fetuses (Palmer et al. 1996). Also, 50% of seronegative, female bison vaccinated in late pregnancy seroconverted and,

while no abortions occurred, both RB51 and field strain *B. abortus* were shed at parturition (Roffe and Olsen 2002).

Age-specific seroprevalence proportions in Yellowstone bison indicate that approximately 50% of bison are exposed prior to reproductive maturity (Treanor et al. 2007). Thus, early exposure to the vaccine may allow immature bison to develop resistance to infection, which could be maintained by booster vaccinations to reduce the occurrence of *B. abortus*-induced abortions. Seeking to increase tolerance for bison outside YNP and reduce risk of cattle *B. abortus* exposure, the National Park Service has been exploring the option for the remote delivery of the RB51 brucellosis vaccine to various segments of the YNP bison herd (USDI-NPS, 2010). Vaccination of all female bison within YNP is expected to significantly reduce the population seroprevalence of brucellosis (Treanor et al., 2010).

The objective of the vaccination program is to reduce the risk of *B. abortus* transmission to livestock outside YNP by decreasing brucellosis infection in the Yellowstone bison herd. An individual-based model (IBM) was constructed to capture the variability between individuals and estimate responses to both the disease and vaccination for the overall bison population (Treanor et al., 2010). The IBM tracked information on each female bison born into the population. The model used a yearly time step to simulate population level processes and daily time steps to simulate exposure routes during the transmission period. The yearly time step components involved mating, natural mortality, exposure to *B. abortus* via elk, and effects of NPS management operations (testing and subsequent removal of seropositive bison at park boundaries). The daily time step detailed the processes (*B. abortus*-induced abortions and infectious live

births) leading to shedding and transmission of *B. abortus* among Yellowstone bison. Demographic, life history, and management-related information (age, sex, disease status, reproductive status, vaccination status, and management removal) were recorded for each female bison modeled.

The estimated brucellosis seroprevalence has fluctuated between 40-60% in YNP bison during the past 20 years (Cheville et al., 1998). This range of infection prevalence was simulated by the model prior to the analysis of each vaccination alternative. The following three vaccination alternatives were simulated: 1) vaccinating female calves and yearlings captured during boundary management operations, 2) combining remote vaccination using biobullet delivery (Olsen et al., 2006) with boundary vaccination of female calves and yearlings, and 3) vaccinating all female bison during boundary operations as well as by remote delivery. Under each alternative, bison captured at the boundary were tested and test-positive animals removed. The effects of vaccination are likely to play out over a 10-30 year time horizon, during which other ecological factors such as variations in snow pack and predation risk may obscure the effects of vaccination. For example, Cross et al. (2007) suggested winter severity could affect the duration of aggregation by ungulates. If this aggregation coincided with the peak *B. abortus* transmission period, these factors could play an important role in the maintenance of the disease.

Modeling and Risk Assessment

The Yellowstone bison population has been extensively modeled (Peterson et al., 1991a, b; Dobson and Meagher, 1996; Gross et al., 2002; Treanor et al., 2010). However,

none of these models attempt to estimate the contact rates required to maintain *B. abortus* at documented prevalence levels. Early modeling studies of *B. abortus* transmission focused exclusively on bison and used mathematical models to show how individual populations interact with a parasitic bacterial pathogen (Peterson et al., 1991a, b; Dobson and Meagher, 1996). Dobson and Meagher's deterministic state-transition model showed that the proportion of hosts infected with *B. abortus* increases as a function of herd density and that 200 infected individuals were necessary to establish *B. abortus* in the Yellowstone bison herd. Once established the authors speculated that high levels of culling would be required to eradicate the disease.

None of the previous modeling efforts attempted to quantify the *B. abortus* transmission pathways within and between bison and elk in the GYA and the risk to cattle from both wildlife hosts. After decades of high level management and scrutiny, Kilpatrick et al. (2009) provided the first quantitative assessment of the risk of *B. abortus* transmission from Yellowstone bison to cattle grazing in the northern portion of the GYA. Their estimates of bacterial transmission risk were heterogeneous across the spatial landscape and varied with bison population numbers and winter severity. However, Kilpatrick et al. (2009) did not include elk in their analyses or examine explicit spatial information on range overlap between wildlife and cattle.

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Chapter 2

EVALUATION OF THE FLUORESCENCE POLARIZATION ASSAY FOR DETECTION OF *BRUCELLA ABORTUS* ANTIBODIES IN BISON IN A NATURAL SETTING

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Abstract

Bison and elk in the greater Yellowstone area are the last-known reservoir of *Brucella abortus* in the United States. Diagnosis of brucellosis is challenging as there is no perfect reference test. The objectives of this study were to estimate the accuracy of the fluorescence polarization assay (FPA) for the screening of *B. abortus* antibodies in bison in a natural setting. Serum and tissue samples were collected and analyzed from the known brucellosis-infected bison herd in Yellowstone National Park (YNP). Additionally, serum samples from privately-owned bison were serologically tested for brucellosis. While the FPA and five other tests had perfect sensitivity, all tests had substantially lower specificity in the YNP herd. However, a Bayesian analysis showed that as many as 59-74% of the culture-negative animals were most-likely truly infected. A decision-tree analysis showed that the expected cost of FPA testing was comparable to the cost of other serologic tests. The FPA was shown to be highly sensitive but may not be able to differentiate culture-positive and culture-negative animals. There is a need for long-term longitudinal studies to estimate diagnostic accuracy of tests for *B. abortus* in bison.

Key words: *Brucella abortus*, fluorescence polarization assay, bison, *Bison bison*, diagnostic test evaluation, Greater Yellowstone Area

INTRODUCTION

Brucella abortus causes disease in several domestic and wildlife species, including cattle, bison (*Bison bison*), and elk (*Cervus elaphus*) [1-2]. Infection with *B. abortus* causes variable clinical signs in ungulates, including late-gestation abortions, stillbirths, infertility, decreased milk production, loss of condition, and lameness [3]. Such losses in livestock production led the federal and state governments to start a Cooperative State-Federal Brucellosis Eradication Program in 1934. During its 75-year history, the program has limited the impact of brucellosis in cattle throughout the United States [4]. However, recent outbreaks of cattle brucellosis in the three states surrounding Yellowstone National Park – Idaho, Montana, and Wyoming – have highlighted the importance of wildlife brucellosis. Currently, wild, free-ranging bison and elk in the Greater Yellowstone Area (GYA) are the last-known reservoir of *B. abortus* in the United States and wild elk have been implicated in the GYA cattle outbreaks.

Several federal and state agencies are involved in research initiatives designed to identify appropriate strategies to eradicate *B. abortus* from the GYA while maintaining healthy and genetically-diverse populations of bison and elk. The YNP bison are desirable for the conservation of the species because the population is derived from the original wild herd and an introduced herd containing widely diverse genetics [5]. In addition, the GYA bison have had no evidence of cattle-hybridization [6]. Therefore, disease management activities, including the future potential for movement of individual bison into other herds, are of special interest in this population.

Management alternatives for brucellosis – animal translocations, test and cull operations, immunocontraception, vaccination, etc. – are predicated on correct

classification of infection status. Diagnostic accuracy is difficult in the case of brucellosis, where no perfect reference test exists. Although culture of tissues or fluids such as milk is frequently used as a standard for *Brucella abortus* diagnosis, culture is also imperfectly sensitive [7]. There are often few detectable bacteria and no obvious signs of infection (i.e., subclinical or latent infection). If an individual clears the infection, it is likely to test positive on serologic tests yet not shed bacteria. Also, collection and handling of samples and culture techniques can affect the success of culture [8-10]. Because of the difficulties with culture, serologic testing is frequently used to determine infection status. The ideal serologic test should correctly classify an animal's infection status, be performed animal-side and yield rapid results. However, under field conditions, where an individual has the opportunity for exposure to *Brucella* organisms, it is impossible to determine whether serologic test-positive but culture-negative individuals are either exposed but non-infected, or infected with undetectable bacteria due to the lack of sensitivity of culture. Alternatively, those same animals could have antibodies to other bacterial species that cross-react on standard brucellosis serologic tests [11].

In 2000, Gall et al. validated a fluorescence polarization assay (FPA) for use in detecting serum antibodies for *B. abortus* in bison [12]. The authors estimated the specificity of FPA and other serologic tests in a population with no epidemiologic evidence of the presence of brucellosis. Few studies, however, are performed in field conditions where the disease is endemic and animals have an opportunity for exposure to *B. abortus.* The objective of this study was to determine the diagnostic accuracy (sensitivity and specificity) of the FPA in the detection of *B. abortus* antibodies in

unvaccinated bison in a natural setting and to compare the FPA to other tests used to screen for or confirm *B. abortus* serologic responses.

MATERIALS AND METHODS

Subject Selection

Bison samples were acquired from two different sources: the brucellosis-infected bison herd in Yellowstone National Park (YNP) and a collection of privately-owned bison from the Western USA states. Bison are prohibited from having contact with cattle grazing in the GYA by the Interagency Bison Management Plan [13]. In order to prevent this contact, bison that exit park boundaries are hazed back into the park or captured and slaughtered based on their numbers and brucellosis serostatus. For this study, YNP animals were taken from a convenience sample of 190 bison that exited the confines of YNP between February 1996 and March 2001 and were subsequently tested and slaughtered according to state bison management plans. State bison management plans differed during the study, but tended to target animals that tested positive on brucellosis chute-side tests. Therefore, for some time periods, test-positive animals were more likely to be sampled and included in the study than were test-negative animals. As a result, the sample prevalence of brucellosis was higher than the prevalence estimated for the overall population. Animals under one year of age were omitted from the study due to the uncertainty of performance of the serological tests for this age category. None of the YNP animals had been vaccinated against brucellosis. The spectrum of signs seen in this *B.* abortus-infected herd has been described elsewhere [14-17].

The privately-owned bison were part of a collection of animals that existed as a large meta-population. The bison were from locations in 4 states, but belonged to a single owner. There had been periodic mixing of animals from the different locations so the bison were considered, epidemiologically, to be from a single herd. Animals were

handled periodically for disease testing and other studies. For this study, privately-owned bison were enrolled when they were sampled for various reasons unrelated to brucellosis. Serum samples were collected and tested from December 2002 through October 2003. The 189 animals sampled were not selected for any reason that would affect their brucellosis status. Periodic testing for brucellosis over several years since the establishment of the herd had not revealed sero-reactor animals, nor was clinical evidence of brucellosis ever noted. In the absence of any indication of infection in the herd, culture of animals for *B. abortus* had not been attempted. Although some of these bison had been vaccinated against brucellosis, all tests were evaluated using standards for non-vaccinated animals [18]. These standards are more conservative than those for vaccinated animals.

Sample Processing and Testing

For the YNP herd, serum samples were taken from all animals ante-mortem or immediately post-mortem for serologic testing. Animals were humanely euthanized and organ samples were taken on necropsy for bacteriologic culture. Forty-two of the 151 bison were killed by gunshot, an additional 18 animals were euthanized using xylazine (0.5mg/kg by IM injection) and captive bolt, and the remaining 91 animals were processed through corrals and a chute. Blood samples were collected from the jugular or caudal vein of these latter animals, and they were transported for slaughter at a local processing plant. A comprehensive culture technique was used on 23 animals (14 of 18 euthanized; nine of 42 killed by gunshot) [15]. Tissue specimens collected included vaginal, rectal, and uterine swabs, mammary gland (5cm x 5cm x 5cm), uterus (7.6cm length), spleen (10cm x 2.5cm x 2.5cm), blood (30 ml, synovial fluid (1-3 ml from the

stifle), liver and kidney (50-100 grams), bone marrow (5 ml), ileum (7.6cm length), and the following whole lymph nodes: medial and lateral retropharyngeal, tracheobronchial, mediastinal, hepatic, mesenteric, lumbar, medial and lateral iliac, superficial inguinal, superficial cervical, prefemoral, popliteal, mandibular, and parotid. Specimens were homogenized individually in sterile distilled water and the whole volume of the homogenates was then plated onto large *Brucella*-selective culture media. Standard bacteriologic culture was performed on all slaughtered animals as well as the remaining euthanized and gunshot-killed animals (n=128). The standard culture tissues and fluids included blood (10ml), whole retropharyngeal, iliac, and superficial inguinal lymph nodes, and male genitalia (swabbed seminal vesicles, 25-30% of a testicle, and whole epididymis). Blood samples for serology were immediately chilled and serum separated within eight hours of collection. Serum was then frozen at -70C. Tissue specimens, fluids and swabs for culture were immediately chilled and frozen at -70C within eight hours of collection. Some samples may have been stored for as long as 5 years under these conditions. After freezing, tissue and serum specimens were shipped on dry ice to the National Veterinary Services Laboratories (NVSL) for bacteriologic and serologic examination. At NVSL, tissues were homogenized in sterile distilled water and the homogenates were swabbed and then plated on standard *Brucella*-selective culture media.

FPA was performed on most samples at the Montana Veterinary Diagnostic Laboratory (MVDL), although for 5 samples the FPA was done at NVSL. Ten serologic tests were performed on all samples, including fluorescence polarization assay (FPA), standard plate (SPT), standard tube agglutination (STT), card, rivanol, complement fixation (CF), competitive enzyme-linked immunosorbent assay (D-TEC), particle

concentration fluorescence immunoassay (PCFIA), rapid automated presumptive (RAP), and buffered acidified plate antigen (BAPA) tests using methods previously described [19-20]. Serum and tissue samples were handled separately from each other and laboratory personnel were unaware of the results of other tests when performing the analysis.

Serological results were interpreted according to standards specified in the United States Department of Agriculture, Brucellosis Uniform Methods and Rules [18] or standards suggested by the manufacturers (FPA). A spreadsheet was constructed with results from the ten serologic tests and bacteriologic culture [9]. Some animals were missing results from one or more tests, but all had results from at least nine tests, in addition to the FPA. Serum samples from each of the 189 privately-owned bison were evaluated for brucellosis at the MVDL, using a battery of serologic tests including SPT, STT, card, rivanol, CF, FPA, and BAPA. No samples were taken for culture from any of these animals.

Statistical Analysis

Test performance characteristics (sensitivity (Se) and specificity (Sp)) and 95% confidence limits were calculated using culture as the criterion for infection [21]. Suspect test results were included in the calculations but were classified as neither test-positive nor test-negative, thus penalizing both sensitivity and specificity values. Median ages for the culture-positive and -negative animals were compared for statistical significance using the Mann-Whitney test [22]. Test results for all animals were evaluated to determine whether the sampling method (target tissues or full set) had a significant effect

on the culture results and test performance using Pearson's chi-square test. Comprehensive and standard sampling of tissues for bacteriologic culture were compared for differences in performance of all ten serologic tests. Pairwise covariances between FPA and comparison tests were calculated for both sensitivity and specificity as a measure of correlation in results.

A Bayesian analysis of the sensitivity and specificity of the FPA was then performed using a 2-test in 2-population model allowing for conditional correlations as described in section 3.3 of Branscum et al. (2005) [23]. The private bison herd was not used for this analysis because the serologic tests would presumably have had different sensitivities (due to differing vaccination histories) and different specificities (due to potential differences in exposure to *Yersinia* and other cross-reacting environmental pathogens). Also, because the private herd had a presumed zero prevalence it could not be used to evaluate the prevalence of the Yellowstone herd. Instead, the 2 groups for the Bayesian analysis were both derived from the Yellowstone herd and were divided based on culture results into culture-positive and culture-negative populations. Beta distributions for the informative priors of the 2 population prevalences were created from the test results of the culture-negative Yellowstone population with a non-informative prior for the correlation coefficients for positive and negative tests. This analysis was performed four times, comparing FPA with rivanol, STT, card, and PCFIA tests. Posterior inferences were based on 50,000 iterations of each model. A copy of the Winbugs code used for the Bayesian analysis is available from the first author on request.

Decision Analysis

Decision-tree analysis was used to evaluate the costs of various testing scenarios, accounting for the effects of prevalence and the values or costs of test outcomes (true positive, false positive, true negative and false negative) [24]. The tree structure used the sensitivity and specificity results from the Bayesian analysis for the FPA, rivanol, card, STT, and PCFIA tests, which ranked highly on the initial analysis of test accuracy. Also, the PCFIA is considered a highly accurate laboratory test while the card test is the only currently available animal-side test aside from the FPA (Steve Hennager, personal communication). The predictive values of diagnostic test results are related to prevalence since, when prevalence is very high, test-negative animals are more likely to be false-negatives and when prevalence is very low, test-positive animals are more likely to be false-positives. Because of this relationship, the test accuracies were evaluated for hypothetical prevalences of 2%, 50%, and 70%. The 70% prevalence was used to represent the highest reported seroprevalence of brucellosis in the GYA [25]. The 50% prevalence represents the mid-point seroprevalence typically found in the Yellowstone bison population and the 2% prevalence represents a scenario where brucellosis is close to eradication. The costs of the various tests were based on values on the USDA website [26]. The decision tree was linked to an intervention targeted to remove bison either reproductively or physically from a population based on their serologic status.

Costs of false-positive and false-negative test results were calculated for 3 different assumptions of the cost for inaccurate results. For the baseline, the cost of a true-positive or true-negative test would be the personnel and drug/equipment costs for the capture ($600/capture; Rick Wallen, personal communication). The cost of a false-negative result was the unnecessary need to capture another animal to achieve the quota

for the intervention ($1200). The cost of a false-positive result was the removal an animal from the population with an unnecessary intervention. This figure was taken from the approximate yearly budget for bison-related activities in the GYA ($3,000,000) divided by the approximate herd size (3500) to yield a total cost for each bison (~$857). For other assumptions, we doubled the cost of a false-negative or false-positive test result, since some individuals might place a much higher economic value on an individual bison and some might place a much higher economic cost on delaying decreases in the seroprevalence of brucellosis in the herd. Examples of calculations used in the decision analysis are in Figure 1.

RESULTS

The bison sampled from the YNP herd included 58 male and 93 female adult bison ranging from 1-15 years in age (median = 4.0). The privately owned bison consisted of 151 adult female bison, one 9-month-old female, and thirty-seven 1.5-year-old males. None of the 189 privately-owned bison tested positive for brucellosis on any of the tests performed, based on guidelines defined for animals not vaccinated against brucellosis [18]. However, 3 bison were classified as suspect on FPA and a different individual tested suspect on STT. Because there was no history of disease in this closed population and no abortions had been noted, we were confident in classifying all bison from among the privately-owned animals as *B. abortus* negative. However, suspect test results were penalized because they would be non-definitive and would require further testing. Therefore, the specificity of the FPA in the non-infected herd was 98.4% (95%

CI = 95.4 to 99.7) compared to 99.5% for STT (95% CI = 96.2 to 99.9) and 100% for the other tests performed (95% CI = 98.1 to 100).

Brucella abortus was isolated from tissues from 31 adult animals from the YNP herd. Culture-positive animals were statistically significantly younger (median age = 2) than culture-negative animals (median age = 5; $p < 0.0005$). Six tests – FPA, Rivanol, BAPA, RAP, STT, and PCFIA – were 100% sensitive, correctly identifying all the culture-positive animals as infected (Table 1). Six of the 10 tests had one or more suspect results. The tests had substantially lower specificity when using *Brucella* culture results as the criterion for infection. Sixty-three culture-negative animals were positive to at least 6 serological tests. Of all the culture-negative animals, eight of the 15 comprehensively-cultured animals were positive on at least 6 serological tests as were 55 of the 105 animals with a reduced set of tissues cultured. A higher proportion of animals with a full set of tissue samples collected were culture positive (8 of 23; 34.8%) than with target tissues only (23 of 128; 18.0%). The difference was substantial but not statistically significant ($p = 0.154$). There were no statistically significant differences between sensitivities and specificities among tests run on samples that had a full set of tissues cultured versus those that were run on samples with only target tissues cultured.

The pairwise specificity covariances between the FPA and other tests ranged from 0.073 to 0.144 (median 0.106). Estimates of pairwise covariance for sensitivity were zero or negligible. On the basis of the Bayesian analysis (Table 2), the sensitivity and specificity of the FPA ranged from 97.7 to 98.8% and from 97.5 to 98.1%, respectively. The FPA had greater sensitivity than all other tests and also had a higher specificity than

the PCFIA. The potential prevalence of *B. abortus* in the culture-negative animals from the brucellosis-endemic YNP herd was also high, ranging between 59.4 and 74.1%.

Decision-Tree Analysis

The STT had the lowest expected value of test cost for prevalences of 50% and 70% regardless of the assumption relating to false-positive and false-negative test results (Table 3). At 2% prevalence, the STT still had the lowest expected test cost from the regulator perspective (higher false-negative cost), while the card test had the lowest cost for conservationist (higher false-positive cost) and baseline perspectives. Mean cost showed the STT as the lowest ($620) with the FPA ranked second ($647). Most of this difference was attributable to the higher test cost for the FPA ($26/test) compared to the STT ($7/test). Expected mean costs for the other tests were $703 for card, $770 for rivanol, and $943 for PCFIA.

DISCUSSION

The sensitivity of the FPA in this field evaluation of GYA bison was excellent. Only one FPA-negative animal had serologic evidence of brucellosis infection on any other test (a suspect on the CF test), and all culture-positive animals tested positive on the FPA. The Bayesian analysis agreed with this finding (sensitivity 97.7 to 98.8%) and was only different from the 100% values for the traditional calculation because it was a weighted average of the data and a non-informative prior. However, this study highlighted the difficulties of field diagnostic test evaluations. Similar to traditional specificity studies, the serologic tests showed very high specificity when the privately-

owned animals from a population without brucellosis were evaluated. Where *B. abortus* exposure was common, however, the FPA and other serologic tests were not completely accurate in differentiating culture-positive from culture-negative animals.

There are several explanations why bison that tested positive on the FPA or other tests were culture-negative. Their test results could have been false-positives, indicating a problem with the test either through test failure, or cross-reaction with antibodies to a structurally-similar bacterium. The animals could have been exposed but no longer harboring any viable bacteria in their tissues. Finally, sampling and bacterial culture might have missed the infected tissue or section of tissue, or failed to detect *B. abortus* because of contaminant overgrowth. The Bayesian analysis provided evidence in support of this latter explanation by estimating a potential prevalence of *B. abortus* in the culture-negative animals of 59-74%.

Specificity is not usually evaluated in infected herds because of the difficulties in determining whether culture-negative animals are truly infection-free. *B. abortus* culture, although considered the reference test, has a sensitivity of less than 100%, which affects the apparent specificity of any test to which it is compared. It is possible that the FPA detected animals previously exposed to *B. abortus*, incubating infection, or latently infected with the bacterium, but with low antibody concentrations undetectable by less sensitive tests.

In this study, 63 culture-negative animals were positive on at least 6 of the serological tests. This finding is consistent with prior studies in cattle [27] and elk [28] and is likely attributable to the inherent difficulties of culturing brucellae from chronically infected animals [17]. More animals with a full complement of tissues taken

on necropsy had positive cultures for *B. abortus* and hence, it is likely that more of the animals with only target tissues cultured would have been culture-positive if a complete set of tissues had been collected. The difference between the culture-positive percentages for both groups, however, was not statistically significant, nor were there differences in sensitivity and specificity of any of the tests between the two groups. Younger animals sampled from the YNP bison herd were more likely to be culture-positive than older animals. Although the bison sampled were not a representative sample from the whole population, this is consistent with the finding that transmission rates are higher among juveniles than older animals [16].

In the decision-tree analysis, the FPA was comparable with other tests but had a higher expected cost than the STT to which it was compared, mainly due to the higher relative testing costs of the FPA. Field-side testing and future technological development may allow the FPA to better compete with the STT and other tests due to reduced costs and improvements in test performance. The additional advantage of the FPA that was not factored into the decision-tree was its ability to be performed animal-side. Other tests, such as the rivanol and STT are cost- and time-efficient; however, they must be performed at room temperature, which is often not possible in field settings. The card test was also comparable in certain scenarios although it failed to detect one culture-positive animal and has been problematic anecdotally with particulate matter causing false-positive reactions during windy conditions. All tests were run under laboratory conditions which may have improved the calculated performance characteristics of the card test over its field performance.

Findings from the present study indicate the need for further evaluation to assure that current tests are well-suited for their intended purpose. The development of other assays or culture techniques that resulted in a more accurate reference standard would improve diagnostic test evaluations in YNP. Brucellosis testing in the Yellowstone herd requires a test with high sensitivity and reasonable specificity. The emphasis on sensitivity is necessary because the consequences of releasing infected animals are serious, potentially exposing uninfected herd mates as well as domestic animals to *B. abortus*. Animals cannot be held for additional testing, so there is a need to decide immediately, based on an accurate and reliable test. The specificity must be reasonable to prevent identifying uninfected animals as positive, because there is no opportunity to hold those animals and retest later. The FPA is perhaps the most sensitive test for detecting serum antibodies to *B. abortus* in bison but may be unable to differentiate exposed individuals from those with active infections or sequestered bacteria. Further work needs to be performed evaluating the repeatability of the FPA with different operators and under different environmental conditions. A comparison between animal-side testing and bench-top machines is also needed. For evaluation of tests under field conditions, however, longitudinal studies should be performed, testing animals throughout the study period and harvesting a subset of subjects at various points to determine their culture status.

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Figure 1. Example calculations for fluorescence polarization assay (FPA) test accuracy and cost under three different *Brucella abortus* prevalences.

FPA parameters

Test performance characteristics from Bayesian analysis

Sensitivity = 0.9878

Specificity = 0.9804

Total costs

True Positive: $626

True Negative: $626

False Positive: $1483

False Negative: $1226

Equations for test accuracy:

True-Positive Proportion = Prevalence x Sensitivity

True-Negative Proportion = (1 – Prevalence) x Specificity

False-Positive Proportion = (1 – Prevalence) x (1 – Specificity)

False-Negative Proportion = Prevalence x (1 – Sensitivity)

For 70% prevalence:

True-Positive Proportion = 0.7 x 0.9878 = 0.691

True-Negative Proportion = (1 – 0.7) x 0.9804 = 0.294

False-Positive Proportion = (1 – 0.7) x (1 – 0.9804) = 0.006

False-Negative Proportion = 0.7 x (1 – 0.9878) = 0.009

Cost = (0.691 x $626) + (0.294 x $626) + (0.006 x $1483.14) + (0.009 x $1226) = $636

For 50% prevalence:

True-Positive Proportion = 0.5 x 0.9878 = 0.494

True-Negative Proportion = (1 – 0.5) x 0.9804 = 0.490

False-Positive Proportion = (1 – 0.5) x (1 – 0.9804) = 0.010

False-Negative Proportion = 0.5 x (1 – 0.9878) = 0.006

Cost = (0.494 x $626) + (0.490 x $626) + (0.010 x $1483.14) + (0.006 x $1226) = $638

For 2% prevalence:

True-Positive Proportion = 0.02 x 0.9878 = 0.020

True-Negative Proportion = (1 – 0.02) x 0.9804 = 0.961

False-Positive Proportion = (1 – 0.02) x (1 – 0.9804) = 0.019

False-Negative Proportion = 0.02 x (1 – 0.9878) = 0.000

Cost = (0.020 x $626) + (0.961 x $626) + (0.019 x $1483.14) + (0.000 x $1226) = $643

Table 1. Sensitivity and specificity estimates (with 95% exact binomial confidence intervals; CI) and number of suspect test results (Sus.) for 10 tests used to detect *Brucella abortus* infection in serum samples from 31 culture-positive and 120 culture-negative bison (*Bison bison*) from Yellowstone National Park.

Test	Sensitivity % (CI); Sus.	Specificity % (CI); Sus.
Fluorescence Polarization Assay (FPA)	100 (88.8-100)	19.2 (12.6-27.4); [3]
Rivanol	100 (88.8-100)	61.7 (52.4-70.4)
Buffered Acidified Plate Antigen (BAPA)	100 (88.8-100)	36.7 (28.1-46.0)
Rapid Automated Presumptive (RAP)	100 (88.8-100)	36.5 (27.7-46.0)
Standard Tube Agglutination (STT)	100 (88.8-100)	33.3 (25.0-42.5); [15]

Particle Concentration Fluorescence ImmunoAssay (PCFIA)	100 (88.8-100)	32.5 (24.2-41.7); [16]
Card	96.8 (83.3-99.9)	44.2 (35.1-53.5)
Standard Plate (SPT)	83.9 (66.3-94.6); [5]	45.0 (35.9-54.4); [32]
Complement Fixation (CF)	83.9 (66.3-94.6); [2]	34.2 (25.8-43.4); [7]
Competitive Enzyme-linked Immunosorbent Assay (D-TEC)	69.2 (48.2-85.7); [4]	55.1 (45.2-64.8); [15]

Table 2. Summary of a series of Bayesian analyses assuming a two-population model comparing FPA test results for detecting *Brucella abortus* infection in serum samples from culture-negative bison with the corresponding results obtained by four other tests.

	Tests Compared with FPA			
	Rivanol	STT	Card	PCFIA
% iterations FPA higher specificity	21.5	27.4	21.4	100
% iterations FPA higher sensitivity	100	51.6	100	83.9
Test Sensitivity % (95% PI)	61.4 (52.7, 69.7)	97.8 (93.3, 99.8)	77.0 (69.2, 84.0)	95.7 (90.1, 99.2)
Test Specificity % (95% PI)	99.0 (96.2, 100.0)	99.0 (96.2, 100.0)	99.0 (96.1, 100.0)	63.4 (47.2, 78.6)
FPA Sensitivity % (95% PI)	98.8 (95.5, 100.0)	97.7 (91.7, 99.9)	98.8 (95.5, 100.0)	97.8 (92.0, 99.9)
FPA Specificity % (95% PI)	97.6 (93.3, 99.7)	98.1 (94.6, 99.8)	97.6 (93.3, 99.7)	97.5 (93.4, 99.7)
Prevalence in culture-negatives (%)	74.1	59.4	74.1	60.4

Table 3. Costs of five different testing schemes used under three prevalences and three different assumptions of the cost of imperfect test results for *Brucella abortus* exposure testing in bison (*Bison bison*) from Yellowstone National Park.

	Cost of Testing Scheme ($)				
Scenario	FPA	Rivanol	STT	Card	PCFIA
Prevalence = 70%					
Baseline	636	771	**616**	702	747
Assumed Increased False-Positive Costs	645	774	**620**	705	906
Assumed Increased False-Negative Costs	646	1095	**631**	894	780
Prevalence = 50%					
Baseline	638	726	**616**	676	805
Assumed Increased False-Positive Costs	652	731	**621**	681	1070
Assumed Increased False-Negative Costs	645	958	**626**	813	829
Prevalence = 2%					
Baseline	643	618	614	**613**	943
Assumed Increased False-Positive Costs	671	628	624	**623**	1463
Assumed Increased False-Negative Costs	643	627	**614**	618	944
Mean cost	647	770	**620**	703	943

Chapter 3

BISON OR ELK: WHO SHOULD BE THE TARGET OF BRUCELLOSIS CONTROL IN THE NORTHERN GREATER YELLOWSTONE AREA?

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Abstract

Wild, free-ranging, bison and elk in the greater Yellowstone area (GYA) are the last reported alternate hosts of *Brucella abortus*-caused brucellosis in the United States. The ability of bison and elk to concomitantly serve as reservoirs of *B. abortus* increases the complexity of risk of transmission to cattle, and presents serious challenges for comprehensive disease management. We present the first spatially-explicit risk assessment of brucellosis transmission among elk, bison, and cattle in the northern portion of the GYA. We used a modeling approach based on spatio-temporal probabilities of bacterial shedding by bison and elk on the northern GYA landscape. Interactive effects between population size and winter severity were major determinants influencing bison movements to lower elevation winter grazing areas, overlapping with federally-regulated domestic cattle grazing allotments. Increasing population size resulted in higher herd densities and increased bacterial shedding. Median total risk to cattle from elk and bison was 3.6 cattle-exposure event-days (95% P.I. 0.1-36.6). The estimated percentage of cattle exposure risk from the Yellowstone bison herd was small (0.0-0.3% of total risk) compared with elk which contributed 99.7-100% of the total risk. Natural herd migration and boundary management operations were important in minimizing the contribution of bison to cattle exposure risk, which supports continued boundary management operations for spatio-temporal separation between bison and cattle. Under current management practices, bison risk to cattle grazing in the northern portion of the GYA is expected to be minimal. The comingling of cattle and elk, especially during the late gestation period for elk, should be reduced, as spontaneous elk abortions pose a risk for interspecies disease transmission.

Keywords: bison, brucellosis, disease management, disease modeling, elk, GIS, population dynamics, risk model, spatially explicit model, wildlife health

INTRODUCTION

Wild, free-ranging, bison and elk in the greater Yellowstone area (GYA) are the last-known reservoirs of bovine brucellosis (*Brucella abortus*) in the United States (Godfroid 2002). Because both bison and elk are competent reservoirs of *B. abortus*, comprehensive disease management is a challenge and the aggregated risks of pathogen transmission to cattle is increasingly complicated (Delahay et al. 2009). In addition to *B. abortus*, many multiple-host pathogens cause the most important livestock diseases listed by the World Organisation for Animal Health, including rinderpest, foot-and-mouth disease, Johne's disease, and Rift Valley fever and comprise 80% of the pathogens of domestic animals (Woolhouse et al. 2001).

Understanding the interspecies transmission dynamics of a multi-host system is crucial for disease management (Dobson 2004, Delahay et al. 2009). Some hosts may be persistent reservoirs of pathogens and others may be recurrently infected through spillover (Power and Mitchell 2004). In addition, multi-host systems present differing and complex surveillance and control challenges. Effective surveillance plans provide early detection of emerging infectious diseases and "spillover" disease events, provided the diagnostic tests used are accurate. However, when using diagnostic tests on species for which the test was not developed, there are many issues with test performance. In addition, there can be problems with cross-reactivity on diagnostic tests by commensal organisms of no disease significance (Kittelberger et al. 1995). Overall, having multiple wildlife hosts and reservoirs greatly complicates disease management (Government Accountability Office 2009).

Brucellosis was first detected in Yellowstone bison in 1917 (Mohler 1917) and was most likely introduced from domestic cattle (Meagher and Meyer 1994). The control and eradication of the disease from the United States has been a priority since 1934, when the federal government sought to reduce the prevalence of the most significant livestock disease at that time. At different times and under different jurisdictions, brucellosis management strategies have included combinations of capture, test, and slaughter of test-positive animals; vaccination; surveillance; and forced spatial-temporal separation from livestock through hazing or slaughter (Donch et al. 2005). Since the inception of the Interagency Bison Management Plan in 2000, bison have been actively managed to prevent spatio-temporal overlap with cattle (Clarke et al. 2005). This active management of bison has prevented bison-cattle transmission of *Brucella*; however, until recently, elk have received minimal risk management attention. In the last decade, there have been multiple detections of brucellosis in cattle in the GYA states (Idaho, Montana, Wyoming), with elk identified as the source of infection for nine cases since 2002 (Donch and Gertonson 2008). Bison conservation continues to be a priority of the National Park Service; however, for decades, livestock and regulatory personnel have viewed Yellowstone bison as the highest priority wildlife source of transmission of pathogens for livestock in the GYA (Meagher and Meyer 1994).

Kilpatrick et al. (2009) provided the first quantitative assessment of the risk of *B. abortus* transmission from Yellowstone bison to cattle grazing in the northern portion of the GYA. Their estimates of bacterial transmission risk were heterogeneous across the spatial landscape and varied with bison population numbers and winter severity. We extended this work by (1) including elk as a source of *B. abortus* transmission, (2)

evaluating explicit spatial information on range overlap between wildlife and cattle, (3) providing risk estimates of *Brucella* transmission from both bison and elk to cattle grazing in southwestern Montana, and (4) evaluating the role of winter severity and population size on the spatial distributions of bison and elk and, hence, the overall potential for brucellosis transmission to cattle.

MATERIALS AND METHODS

Study area and wildlife host populations

The GYA is one of the largest intact temperate zone ecosystems on earth and includes portions of Wyoming, Idaho, and Montana. It is also home to the largest wild and free-ranging elk and bison populations in the United States. Elk and bison populations in the GYA are variably infected with *B. abortus*, the cause of cattle brucellosis. Elk populations in northern GYA have a low seroprevalence (i.e., exposure; <5%) of *B. abortus*, whereas seroprevalence in Yellowstone bison is high (40-70%) (Cheville et al. 1998).

One bison population with between 2000 and 5000 individuals (Meagher 1973, Clarke et al. 2005) and five elk populations (Gallatin-Madison, Gravelly-Snowcrest, Madison-Firehole, northern Yellowstone, and Sand Creek, Idaho) are distributed across 3,000 km^2 in the northern GYA. Estimates of northern Yellowstone elk were near 25,000 animals in the late 1980s, but decreased by approximately 50-60% by 2006 (Eberhardt et al. 2007). Domestic cattle (266 in the winter and 1363 in the spring in 2006) are grazed on public and private lands adjacent to Yellowstone National Park (YNP) and within habitat occupied by bison and elk during the winter (Kilpatrick et al. 2009). Federal and

state management agencies have attempted to decrease the risk of *B. abortus* transmission from bison to cattle using hazing and bison culling to maintain spatio-temporal separation from cattle (U.S. Department of Interior [USDI] and U.S. Department of Agriculture [USDA] 2000).

Brucellosis infection and transmission

For *B. abortus* transmission to occur from wildlife to cattle, the following requirements must be met: (1) the wildlife must be infected; (2) infected wildlife must be on allotments or private land where cattle are grazed outside of the National Park; (3) pregnant wildlife must shed *Brucella* into environment (through abortion, birth fluids, or post-partum via placenta); and (4) *B. abortus* must persist on the landscape long enough for grazing cattle to come into contact with bacteria. The probability of *B. abortus* transmission between elk (or from elk to cattle) is likely low during calving (May through June) because pregnant dams isolate themselves while giving birth and meticulously clean the birth site (Johnson 1951). Thus, birth sites are dispersed, and the likelihood of other elk encountering infected birth tissues and fluids is low. However, transmission risk is likely higher during the brucellosis abortion period from February through April when many elk aggregate in larger groups on lower-elevation winter ranges that sometimes include ranch areas with cattle (Hamlin and Cunningham 2008).

Risk model development

We assessed the risk of bacterial shedding from third-trimester abortions and infectious live parturition events for bison and elk populations in the northern GYA. We

created a stochastic *Brucella* shedding risk model, which was parameterized using a combination of published peer-reviewed data, unpublished data, and expert opinion on winter severity, animal locations, serologic test results, and population demography (Table 1, also see Supporting Information). We fit statistical distributions to data using @RISK v5.0 (Palisade Corporation, Ithaca, New York) to address the variability and uncertainty of parameters.

Exposure area and wildlife tolerance

It is unknown how close a susceptible cow would have to be to *B. abortus*-infected tissues before it would be likely to investigate them and become exposed. The Starkey Project at the Pacific Northwest Research Station found that forage competition between elk and cattle likely decreases the chance of comingling on winter range (Coe et al. 2005). However, lack of available forage and other environmental pressures during severe winters in the GYA likely increase comingling which is observed annually by National Park Service staff. Because there was a high degree of uncertainty associated with this comingling parameter, we chose to model it as a discrete distribution with equal probabilities from 50 to 250 meters, by 50-meter increments. We then evaluated the effect of this assumption in a sensitivity analysis.

To account for active management of bison in contrast to elk, we included a wildlife tolerance factor. The tolerance factor was defined as how much access bison are given to cattle grazing allotments as a percentage of the access given to elk. Because there are no specific data on this parameter, we modeled it as a uniform distribution

between 0-100% and evaluated it in the sensitivity analysis with 10% increments from 0-100%.

Birth synchrony

In general, data suggest bison and elk in the northern portion of the GYA exhibit a high degree of birth synchrony, with the majority (80%) of bison calving during late-April to late-May and elk calving between mid-May to mid-June (Cheville et al. 1998, Berger and Cain 1999). Feed ground data from the southern portion of the GYA in Wyoming have shown birth dates for elk later in the year, but parturition events are still unlikely after the third week of June due to the normal pattern of sexual segregation (Cross et al. 2009, Maichak et al. 2009). We assumed a 285-day gestation period for bison and a 250-day gestation period for elk, with the initiation of an abortion window for bison in January and for elk in the second week of February (Fig. 1). The model parameterization is consistent with the timing of culture-positive results from aborted elk fetuses submitted by personnel from the Wyoming Game and Fish Department (Cross et al. 2009, Maichak et al. 2009). We fit statistical distributions to parturition data obtained from published and unpublished sources and used our risk model to estimate the percentage of pregnancies that would fail or result in a live parturition with the potential for bacterial shedding using @RISK v5.0 (Palisade Corporation, Ithaca, New York).

Bacterial versus fetal tissue persistence

The number of days a *Brucella*-contaminated birth site is infective is dependent upon the amount of time that it takes for an infected fetus or tissues to be scavenged or

for ultraviolet radiation to degrade the bacteria. Aune et al. (2007); unpublished data) and Cook et al. (2004) found that fetuses would be scavenged prior to ultraviolet degradation of bacteria (mean ± SD = 18.2 ± 20.1 days). We used a distribution with similar characteristics, BetaGeneral(2, 6.93, 1, 78), for consistency and comparability with other models (Kilpatrick et al. 2009).

Winter severity and kernel density estimation

We estimated winter severity by summing daily snow pack estimates (measured in snow water equivalents [SWE], or the amount of water in a column of snow) from October 1 to April 30, based on the snow pack model described by Watson et al. (2009). We categorized winters during 1988-2008 as mild, average, or severe, with an average winter falling within the range of the median snowpack ± 0.5 SD of the 30 years of data.

We performed spatial data manipulations and analyses using ArcGIS v9.3 (Environmental Systems Research Institute, Redlands, California). We obtained bison spatial information from aerial surveys conducted during 2000-2008 by the National Park Service. We grouped the data from these years by the previously-defined winter severity classifications to separate population spatial distributions for the three different types of winters. We then focused on the spatial locations during June when cattle were grazing on allotments (2000-2002, 2007-2008) and weighted spatial data points by the observed group size at that location.

We used Animal Space Use v1.3 (Horne and Garton 2009) to determine the appropriate bandwidth for our home range kernels. Next, we calculated home range distributions using a 95% fixed kernel estimator with Hawth's tools v3.27 (Rodgers and

Carr 1998, Beyer 2004, Fieberg 2007). Because the primary season for cattle exposure began in June, we used the May-June spatial data.

We bootstrapped elk home-range kernels from minimum convex polygons representing the distributions of various elk populations (Hamlin and Cunningham 2008). We randomly assigned hypothetical individual animal locations within the bounds of the distribution. Then, subsequent points for each individual were approximated using spatial spread statistics on individual animal movements for elk from the northern Yellowstone herd (YNP, unpublished data). The bootstrapping was performed using R statistical language v2.11.0 (R Development Core Team 2010) and several R packages (Stabler 2006, Rowlingson et al. 2009, Lewin-Koh et al. 2010). Locations and usage of cattle grazing allotments were provided by the National Forest Service and the Animal and Plant Health Inspection Service. Our information on cattle grazing practices was limited to operations using public grazing allotments. We masked the home range kernels to show specific overlap regions with cattle grazing allotments and calculated the percentage of volume overlap using R.

Statistical Analyses

We ran the risk model for 50,000 iterations to assess the variability of the exposure risk outputs. We determined the median number of abortion-days and infectious birth-days for both bison and elk. The numbers of abortion or infectious birth-days were defined as the number of infectious events (abortions or births) multiplied by the amount of days that each of those events will persist on the landscape. We determined the cattle exposure risk from both the Yellowstone bison herd as well as the five elk populations in

the northern portion of the GYA. Probability intervals (95%) were estimated based on the 2.5^{th} and 97.5^{th} percentiles of the iterated values. Maps of the probability of infectious events across the northern portion of the GYA were made using ArcGIS. We also evaluated data from the Montana Department of Livestock on the number of bison migrating from YNP into Montana during 2000-2008 compared to the number of bison in YNP.

Model parameter sensitivity analysis

We performed a general sensitivity analysis of all input parameters in @RISK to determine which model parameters were most influential on *Brucella* exposure risk. In addition, the radius of exposure for an infectious event and wildlife tolerance factors were varied and the resultant change in the percentage of risk from bison exposure was evaluated. The model was updated for 50,000 iterations for each parameter value. Also, we evaluated the creation of bison home-range kernels by comparing the spatial distribution and associated risk of cattle *Brucella* exposure between the kernel derived solely from June bison locations and a kernel derived from May and June locations.

RESULTS

Neither wildlife population had any projected infectious parturitions in January and February, so the only shedding in that time period was from abortions (Table 2). Bison began showing infectious parturitions in April, while all parturitions for elk were in May and June. Infectious event maps showed variable shedding across the northern GYA landscape (Fig. 2). Of the total number of infectious events in the northern Yellowstone

elk population, 13.5% (95% P.I. = 2.5 to 45.0) were abortions as opposed to infectious parturitions. In bison, 16.5% (95% P.I. = 10.6 to 21.4) of the infectious events were abortions. The estimated annual cattle risk of exposure to a bison brucellosis infectious event was small (≤0.01 cattle-exposure event-days/year; Table 3). More risk was estimated in average and severe winters than for mild winters (Table 3). Two populations of elk in the northern portion of the GYA (Madison-Firehole and Sand Creek) had no detectable spatio-temporal overlap with cattle grazing allotments (see Supporting Information). Cattle risk estimates for exposure to an elk brucellosis infectious event were two orders of magnitude higher than for bison when elk range overlapped with cattle grazing allotments. Risk estimates for the Gallatin-Madison, Gravelly-Snowcrest, and Northern Yellowstone elk populations were 2.7, 1.6, and 1.9 cattle-exposure event-days, respectively. Bison migration data showed that the largest scale out-migration (2007-2008) occurred during a severe winter, with fewer animals in the YNP bison herd than the next largest migration (2005-2006) during an average winter (Table 4). Both of these migrations occurred during years when the Yellowstone bison herd was larger than 4400 animals.

Sensitivity analysis

The cattle exposure risk to a brucellosis event was most sensitive to the: (1) radius of exposure from each infectious event; (2) number of days infectious tissue would persist on the landscape prior to scavenging; (3) proportion of seropositive elk in the northern portion of the GYA; (4) proportion of elk shedding *Brucella* organisms; and (5) the adult female proportion of elk. Altering the radius of exposure by 50-meter

increments from 50 to 250 meters yielded median exposure risks of 0.5 to 4.4 cattle-exposure event-days. Changing the wildlife tolerance factor from 0.1 to 0.9 caused the percentage of total risk attributable to bison to change from 0.1% to 0.6%. Using bison spatial locations from both May and June when deriving their home-range kernel increased greatly increased the amount of cattle exposure risk and increased the percentage of risk attributable to bison from ≤1% using June alone to ≥60%.

DISCUSSION

Although our results support substantial shedding of *Brucella* bacteria from bison in some winters, the most substantial risk of bacterial transmission to cattle was from elk. Future risk estimates for bison depend on adaptive management of the population. Interactive effects between population size and winter severity were major determinants influencing bison movements to lower elevation winter grazing areas and overlap with federally-regulated domestic cattle grazing allotments. However, during the critical period of potential *B. abortus* exposure to cattle, the risk from Yellowstone bison was minimal. Natural movements of animals back to higher elevation summer ranges and boundary management operations were important in minimizing the contribution of bison to cattle exposure risk, which supports continued boundary management operations for spatio-temporal separation between bison and cattle. Under current management practices, bison risk to cattle grazing in the northern portion of the GYA is expected to be small.

Maintaining spatial and temporal separation between bison and cattle, is believed to make the risk of *B. abortus* transmission from bison to cattle in the northern GYA

negligible (Kilpatrick et al., 2009), but risk of transmission among bison remains high, accounting for the documented high prevalence (Cheville et al. 1998). Behavioral differences between species may also contribute to differences in pathogen prevalence. Spontaneous abortions by elk that are not segregated from their herd could expose many susceptible elk and cattle to infected fetuses and birth tissues (P.J. White, personal communication). In contrast, bison are gregarious during parturition, and pregnant females have been observed to nuzzle newborn calves (Treanor et al. 2008). Mobbing events of a newborn calf or aborted fetus could contribute to intra-species transmission of bacteria if the dam was infected (Jones et al. 2009).

Our results are consistent with the conclusion of Kilpatrick et al. (2009) that bison under current management practices are not likely to transmit *B. abortus* to cattle grazing in the northern portion of the GYA. However, we disagree with assuming that, under a "no plan" strategy (i.e., without management), the risk of bacterial transmission from bison to cattle would be low due to animal migration back to higher elevation grazing lands in Yellowstone National Park. This conclusion does not take into account the seasonal or environmental conditions, which may delay natural migration and does not consider that, without intensive management intervention, there is little doubt that bison would continue to expand their range and disperse to suitable habitat areas outside the northern and western boundaries of the park where cattle could come into contact with *Brucella* bacteria shed on birth tissues (Plumb et al. 2009). Lack of consideration of boundary management operations makes accurate predictions of future spatial movements and locations of bison unlikely. The predictions and conclusions here are reasonable because bison are currently restricted to only a small fraction of their original

range by active hazing into the Park as needed during the winter and spring to reduce contact with cattle. Spatial risk estimates are inextricably tied to current policy conditions and must be revisited as wildlife populations are adaptively managed.

The strength of our conclusions is based on the spatial and temporal resolution of the data used to parameterize the model. The cross-sectional nature of the bison aerial survey data limited our investigation to herd movement patterns. Also, the limited availability of appropriate elk location data, prohibited us from exploring how seasonal changes in elk distributions altered local risk of shedding.

This model provides the first spatially-explicit framework for assessing the risk of bacterial shedding of *B*. *abortus* by bison and elk across the northern portion of the GYA. It may be expanded to include the entire GYA, or serve as a template for models of other diseases. The next steps in exploring the risk of *B. abortus* transmission in the northern GYA are to continue to refine our model with new data, especially on spatial locations of cattle and wildlife, as well as animal movements. The underlying disease dynamics between elk and bison also need to be examined to estimate what frequency or rate of interspecies pathogen transmission is necessary to be maintaining the current prevalence in elk and bison populations in the northern GYA and relative impact that alternative management strategies can have on overall transmission.

In addition to overlap, the major contributors to risk were wildlife population size and the number of elk that were shedding *Brucella* bacteria. While elk currently have a lower density of shedding events throughout their range, they have a larger spatio-temporal overlap with cattle and are more tolerated by managers and livestock keepers on public grazing allotments. Thus, the predominant source of risk to cattle in the northern

portion of the greater Yellowstone area is from elk. With increased disease prevalence due to increased winter densities or other factors, elk are likely to contribute greatly to the overall level of bacterial shedding on the northern GYA landscape (Fig. 2) and will continue to represent the vast majority of risk of *B. abortus* exposure to cattle grazing in the northern portion of the GYA. Therefore, brucellosis management efforts should focus more on the comingling of cattle and elk during the critical abortion period to more effectively decrease risk of transmission.

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Table 1. Input parameters for a *Brucella abortus* transmission model used to assess the risk of an infectious event occurring in elk and bison populations in the northern greater Yellowstone area.

Description of variables	Statistical distribution (parameters) [Mean, SD]	Source
Shedding proportion	Beta (12,14) [0.46, 0.10]	(Roffe et al. 1999)[a]
Fetal persistence	BetaGeneral (2, 6.93, 1, 78) [18.25, 10.19]	(Aune et al. 2007)
Bison		
Number of animals Fit from 2000-2008 data	Logistic (3788.53, 450.13) [3788.53, 816.45]	(National Park Service, unpublished data)
Age proportion (of total population): Fit from 2004-2008 data		(National Park Service, unpublished data)
2-3 year-old females	BetaSubjective (0.043, 0.047, 0.04736, 0.053) [0.047, 0.002]	
4+ year-old females	Pareto (46.43, 0.35123) [0.36, 0.01]	
Proportion pregnant:		
2-3 year-old	Uniform (0.71, 0.79) [0.75, 0.02]	(Yellowstone Center for Resources 2008)
4+ year-old	Uniform (0.76, 0.89) [0.83, 0.04]	
Proportion seropositive 2+ year-old (sampled at boundary capture facility)	Beta (331.0, 211.6) [0.61, 0.02]	(National Park Service, unpublished data)

Percentage shedding by abortion:

First pregnancy females	BetaSubjective (0.65, 0.78, 0.78, 0.9) [0.78, 0.07]	(Davis et al. 1990)
Mature females	BetaSubjective (0.01, 0.1, 0.09, 0.15) [0.09, 0.03]	(Peterson et al. 1991)
Birth synchrony	Normal (40.57, 13.33) [40.57, 13.33] Day 1 = April 1	(Berger and Cain 1999)

Elk

Adult female proportion Fit from 2000-2008 data	BetaSubjective (0.52, 0.73, 0.7, 0.8) [0.7, 0.06]	(National Park Service, unpublished data)
Adult female: yearling	10:1	(National Park Service, unpublished data)
Proportion pregnant: Fit from 2000-2006 data		(National Park Service, unpublished data)
Yearling	BetaSubjective (0.1, 0.33, 0.32, 0.4) [0.32, 0.03]	
Adult	BetaSubjective (0.78, 0.82, 0.815, 0.84) [0.82, 0.01]	

Percentage of shedding by abortion:

First pregnancy females	Beta (13.3, 14.4) [0.48, 0.09]	(Thorne et al. 1978)
Mature females	Beta (1.2, 6.8) [0.15, 0.12]	
Birth synchrony	Poisson (32.526) [32.526, 5.703] Day 1 = May 1	(Maichak et al. 2009)

Gallatin-Madison

Number of animals	Normal (7807, 793)	
Fit from 2000-2008 estimates (sightability corrected using 1.322 correction factor)	[7807, 793]	(Hamlin and Cunningham 2008)

B. abortus seropos. proportion	Beta (3.1, 101.5) [0.03, 0.02]	

Gravelly-Snowcrest (Hamlin 2006)

Number of animals	Uniform (10,900, 11,570)	
Fit from 2004&2006 data	[11,235, 193]	
B. abortus seropos. proportion	Beta (3.1, 101.5) [0.03, 0.02]	

Madison-Firehole

Number of animals	Loglogistic (236.8, 196.2, 1.4)	
Fit from 2000-2008 estimates (sightability corrected using 1.322 correction factor)	[757.2, N/A]	(Hamlin and Cunningham 2008)
B. abortus seropos. proportion	Beta (3.1, 101.5) [0.03, 0.02]	

Northern Yellowstone

Number of animals	Lognormal (9742, 3801, Shift (3396))	
Fit from 2000-2008 estimates (sightability corrected using 1.322 correction factor)	[13,137, 3800]	(Cross et al. 2009)
B. abortus seropos. proportion	Uniform (0.01, 0.05 [0.03, 0.01]	(Barber-Meyer et al. 2007)

Sand Creek, Idaho

Number of adult females, 2006 (sightability corrected using 1.322 correction factor)	1,413	(Mark Drew, Idaho Department of Fish and Game, unpublished data)
B. abortus seropos. proportion	Beta (0.9, 100) [0.01, 0.01]	

a – study generalizes statistic for seropositive female bison

Table 2. Median number of abortion-days and infectious parturition-days and 95% probability intervals (P.I.) for bison and elk in the northern portion of the greater Yellowstone area.

	Bison		**Elk**	
Season	Abortion-days (95% P.I.)	Infectious Paturition-days (95% P.I.)	Abortion-days (95% P.I.)	Infectious Parturition-days (95% P.I.)
Jan.-Feb.	3.3 (0.6, 10.9)	0 (0, 0)	0.01 (0.00, 0.06)	0 (0, 0)
Mar.-Apr.	9.4 (1.7, 31.2)	16.9 (3.2, 52.7)	4.7 (0.4, 37.7)	0 (0, 0)
May-Jun.	2.2 (0.3, 6.1)	58.7 (11.0, 182.7)	2.9 (0.2, 23.2)	50.1 (6.5, 239.8)
Total	14.9; (2.6, 48.2)	75.6 (14.2, 235.4)	7.6 (0.6, 61.0)	50.1 (6.5, 239.8)
% of total shedding	16.5% (10.6, 21.4)	83.5% (78.6, 89.4)	13.5% (2.5, 45.0)	86.5% (55.0, 97.5)

Table 3. Median cattle risk of exposure to a *Brucella abortus* infectious shedding event from the Yellowstone bison population for the month of June using home range estimates for mild, average, and severe winters with 95% probability intervals (P.I.). The units for risk are cattle-exposure event-days.

	Cattle Risk of *Brucella* Transmission (95% P.I.)	**% of Total Exposure Risk From Bison (95% P.I.)**
Mild	<0.01 (0, 0.01)	<0.1 (0, 0.2)
Average	0.01 (0, 0.12)	0.3 (0, 1.8)
Severe	0.01 (0, 0.13)	0.3 (0, 2.1)

Table 4. Yellowstone bison population estimates and the corresponding number of out-migrating bison in the Western Management Area (WMA) during the month of February for mild (M), average (A), and severe (S) winters from 1999 to 2008.

Year	Bison	Bison in WMA (February)
1999-2000 (A)	2500	1
2000-2001 (M)	3000	10
2001-2002 (A)	3400	6
2002-2003 (A)	4100	10
2003-2004 (A)	4250	1
2004-2005 (M)	4400	2
2005-2006 (A)	**5000**	**157**
2006-2007 (M)	4000	No Data
2007-2008 (S)	**4700**	**182**

Fig. 1. Probability distributions for infectious parturitions and abortions by bison and elk in the northern portion of the greater Yellowstone area.

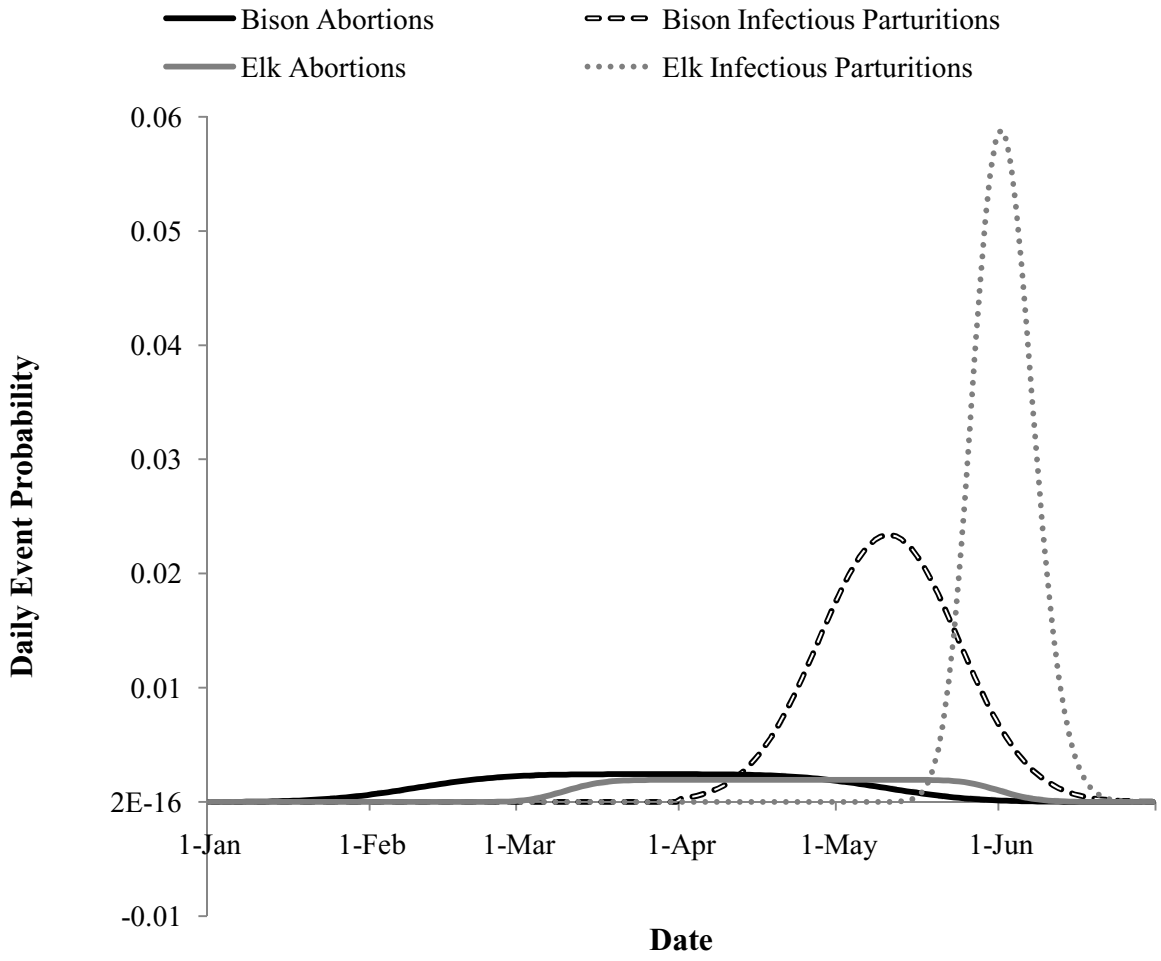
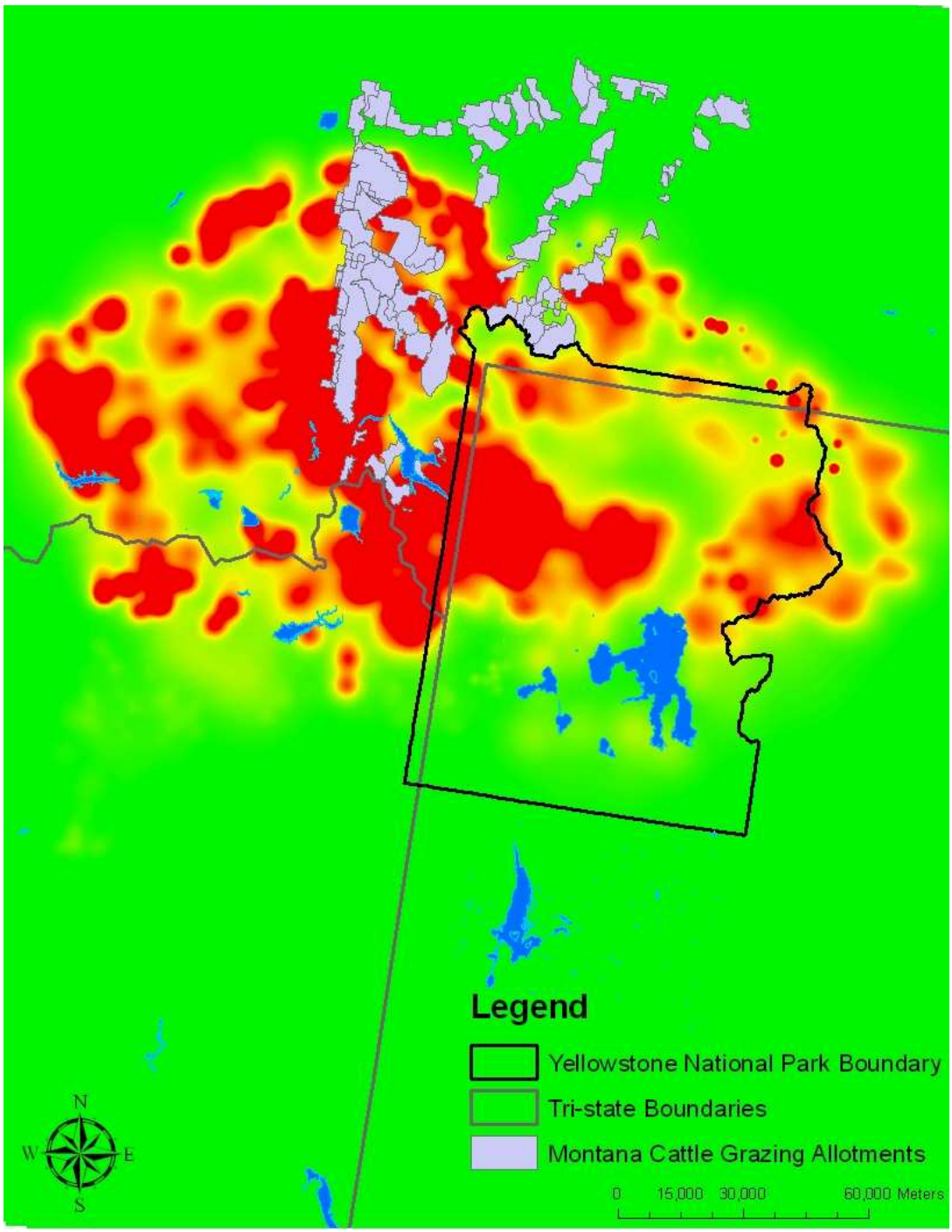


Fig. 2. Map of total *Brucella abortus* shedding events during June in the northern portion of the greater Yellowstone area based on an average winter. Red areas indicate higher levels of shedding while yellow areas indicate lower levels of shedding.



Online Supporting Information

Table S1. Median cattle risk of exposure to a *Brucella abortus* infectious shedding event from five elk populations in the northern portion of the greater Yellowstone area and 95% probability intervals (P.I.). The units for this risk are given as cattle-exposure event-days/year.

Elk Population	Cattle Risk of *Brucella* Exposure (95% P.I.)
Gallatin-Madison	2.7 (0.1, 27.0)
Gravelly-Snowcrest	1.6 (0.1, 15.8)
Madison-Firehole	0.0 (0.0, 0.0)
Northern Yellowstone	1.9 (0.1, 20.3)
Sand Creek	0.0 (0.0, 0.0)

Fig. S1. Risk model equations for: (a) risk of cattle exposure to a wildlife brucellosis infectious event; (b) total infectious event-days, (b) abortion-days, and (c) infectious live parturition-days from elk and bison in the northern portion of the greater Yellowstone area.

(a)

Risk ∝

(Number of cattle on allotment) × (Number of days cattle are at-risk) × (Number of wildlife infectious event-days) × [(Area of Event Exposure) / (Area of Allotment)] × (Wildlife Tolerance Factor[†])

(**b)**

Number of infectious event-days =

(Number of abortion-days) + (Number of infectious live parturition-days)

(c)

Number of abortion-days =

(Number of animals) x

[(Proportion first pregnancy) x (Age-specific pregnancy proportion) x (Age-specific shedding proportion) x (proportion of first pregnancy females aborting) +
(Proportion mature females) x (Age-specific pregnancy proportion) x (Age-specific shedding proportion) x (proportion of mature females aborting)]

x (Proportion of total abortions expected to occur in the time window)

x (Bacterial persistence proportion)

(d)

Number of infectious live parturition-days =

(Number of animals) x

([(Proportion first pregnancy) x (Age-specific pregnancy proportion)

x (Age-specific shedding proportion) x (proportion of first pregnancy females not aborting)] +

[(Proportion mature females) x (Age-specific pregnancy proportion)

x (Age-specific shedding proportion) x (proportion of mature females not aborting)])

x (Proportion of total infectious live-parturitions expected to occur in the time window)

x (Bacterial persistence proportion)

† - Defined as the access that bison have on grazing allotments as a percentage of the access that elk are given to allotments.

Chapter 4

Who infects whom? Interspecies transmission dynamics of brucellosis in the northern greater Yellowstone area

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Abstract

Bison (*Bison bison*) and elk (*Cervus elaphus*) in the northern portion of the greater Yellowstone area (GYA) remain reservoirs capable of transmitting *Brucella abortus* bacteria to livestock. However, the inter- and intra-species contact rates required to maintain brucellosis in the GYA have not previously been characterized. Without this knowledge, the likely effects of risk mitigation strategies cannot be adequately evaluated. We used a risk model to estimate the spatio-temporal distribution of *B. abortus* shedding events from bison and elk populations in the northern GYA. The percentage of *B. abortus* infectious events in overlapping wildlife populations was calculated, and the risk of *B. abortus* transmission within and between populations was estimated. Bison risk from other bison and from elk showed almost 100% adequacy to transmit the organism once spatio-temporal overlap occurred; however, contact within elk populations was only approximately 34% adequate. Transmission risks to elk from elk in other populations or from from bison were very small. Minimal opportunity exists for *B. abortus* transmission from bison to elk under current natural conditions in the northern GYA. Under current conditions, management alternatives that reduce bison seroprevalence are unlikely to substantially reduce transmission risk from elk to cattle. Strategies that decrease elk herd densities and group sizes and reduce elk-to-elk transmission could reduce the overall risk to cattle grazing in the northern portion of the GYA.

Keywords: bison, Brucella abortus, *infectious disease, model, elk, population management, risk, transmission, wildlife*

INTRODUCTION

Bison (*Bison bison*) and elk (*Cervus elaphus*) populations in the northern greater Yellowstone area (GYA) are variably infected with *Brucella abortus*. Elk populations in the northern GYA have a relatively low seroprevalence (i.e., exposure; <5%) of *B. abortus*, whereas seroprevalence in Yellowstone bison is high (40-60%) (Hobbs et al. 2009). While bison most likely acquired brucellosis from cattle grazing in the GYA (Meagher and Meyer 1994), *B.abortus* has been eradicated from livestock in the US, and wildlife in the northern greater Yellowstone area (GYA) remain a source for potentially transmitting *B. abortus* bacteria to livestock.

The Interagency Brucellosis Management Plan (IBMP) was established in 2000 to manage the risk of *B. abortus* transmission from bison to cattle by implementing hazing, test-and-slaughter, hunting, and other actions near the boundary of Yellowstone National Park (YNP) (Plumb and Aune 2002). To date, these actions have successfully prevented the transmission of *B. abortus* from bison to cattle (Clarke et al. 2005), and assessments suggest the risk of future *B. abortus* transmission is minimal under current management conditions (Kilpatrick et al. 2009, Schumaker et al. 2010). Conversely, elk in the northern GYA have received relatively little brucellosis management attention until recently and often move freely across the ecosystem and come into close contact with cattle. All detections of *B. abortus* infection in northern GYA cattle in the last decade have been attributed to elk (Donch and Gertonson 2008).

Having multiple hosts increases the complexity of *B. abortus* transmission dynamics (Dobson 2004, Delahay et al. 2009). There is still an insufficient understanding of much of these dynamics, and this information is crucial for disease management. Elk

with significant home-range overlap with Yellowstone bison do not show evidence of an increase in *B. abortus* exposure compared with populations with spatio-temporal separation from bison (Ferrari and Garrot 2002, Proffitt et al. 2010). Apparently lower *B. abortus* exposure in elk may be due to differences in the immunological responses or reproductive behavior of the wildlife hosts. Without better knowledge of the inter- and intra-species contact rates that maintain *B. abortus* prevalence in the GYA, the likely effects of risk mitigation strategies cannot be evaluated thoroughly.

The Yellowstone bison population has been extensively modeled (Peterson et al. 1991, Dobson and Meagher 1996, Gross et al. 2002, Treanor et al. 2010). However, none of these models attempt to estimate the contact rates required to maintain *B. abortus* at documented prevalence levels. We extended past modeling efforts by quantifying the transmission dynamics within and between elk and bison populations in the northern GYA. We also determined the bison and elk intra- and inter-species contact rates required to maintain documented prevalence levels in elk in the northern GYA.

MATERIALS AND METHODS

Study area and wildlife host populations

The GYA is one of the largest intact temperate zone ecosystems on earth and also home to the largest wild and free-ranging elk and bison populations in the United States. One bison population with between 2,000 and 5,000 individuals (Meagher 1973, Clarke et al. 2005) and five elk populations – Gallatin-Madison (GM), Gravelly-Snowcrest (GS), Madison-Firehole (MF), northern Yellowstone (NY), and Sand Creek, Idaho (SC) are distributed across 3,000 km^2 in the northern GYA. Estimates of northern Yellowstone elk

were near 25,000 animals in the late 1980s, but decreased by approximately 50-60% by 2006 (Eberhardt et al. 2007). Median estimates fit from multiple data sets for the other four elk populations were: 7,807 for GM; 11,253 for GS; 757 for MF; and 1,413 for SC, respectively (Table 1).

Risk model

The estimation of the *B. abortus* transmission potential within and between elk and bison populations in the northern GYA employed a previously developed risk model (Schumaker et al. 2010). The model estimated the number and spatiotemporal distribution of *B. abortus* shedding events from third-trimester abortions and infectious live parturition events from one bison and five elk populations in the northern GYA (Figure 1). The stochastic model was parameterized with statistical distributions fit to winter severity, animal location, serologic testing, demographic and epidemiologic data using @RISK v5.5 (Palisade Corporation, Ithaca, New York, USA; Table 1). The assumptions for the model were: 1) adult females are the primary source of infection; 2) the critical season of transmission is between January 1 and June 30; 3) no fully immune state exists; and 4) random mixing of animals occurs within a population.

Risk calculation

Risk of *B. abortus* transmission is a combination of spatiotemporal overlap of at-risk individuals, the number and location of infectious events within their own or a neighboring population of bison or elk, and behavioral and disease factors that allow transmission within and between wildlife populations (Equation 1). These factors could

include the relative dominance of one species over another, which might include driving a group of animals off grazing land where infectious material could reside. They also could include transmission rates for *B. abortus* within and between elk and bison, respectively, once exposed to the pathogen.

Numbers of infectious events in each wildlife population were taken from the results of 50,000 iterations of the stochastic risk model and distributed to fixed kernel density estimations of wildlife home ranges as described elsewhere (Schumaker et al. 2010). Overlap among elk and bison populations was calculated using the Spatial Analyst extension in ArcGIS v9.3 (Environmental Systems Research Institute, Redlands, California). Rasters were converted to ASCII files and the percentage of volume overlap was calculated using R statistical language v2.11.1 (R Development Core Team 2010) and the raster R package (Hijmans and Van Etten 2010).

Statistical analyses

Equations were created for *B. abortus* transmission risk using medians of the risk model parameters (Table 2). The probabilities of adequate contact – contact, which would result in transmission if the exposed animal were susceptible to infection – from spatiotemporal overlaps of wildlife with *B. abortus* infectious events were listed as unknowns. These probabilities were estimated from the data as uniform distributions, using the observed minimum and maximum values. The Solver Add-in for Excel v2007 (Microsoft Inc., Redlands, WA, USA) was used to optimize a solution using 0.000001 precision, 5% tolerance, 0.0001 convergence, tangent estimates, forward derivatives, and Newton search algorithm. Then the risk model was run for 50,000 iterations to assess the

distribution of transmission risk within and between GYA wildlife populations. The median was determined and 95% probability intervals were estimated based on the 2.5th and 97.5th percentiles of the iterated values. *B. abortus* incidence in the wildlife populations was also estimated (Equation 2).

RESULTS

Bison overlaps within their own population and with elk were almost 100% adequate for *B. abortus* transmission, while elk overlaps with bison shedding were less than 0.1% adequate. Elk overlaps within their own population ranged from 33.8-34.0% adequate for *B. abortus* transmission. Elk overlaps with elk from other populations were only 1.4-1.6% adequate for *B. abortus* transmission, but 24 to 60 times more adequate for transmission than potential contacts from bison. As a percentage of total risk, bison transmission risk from within their own population was three times higher than from elk (Table 3). Conversely, elk risk from bison ranged from <0.1 – 0.5% of total risk. In the GM, GS, and NY populations, risk from other elk populations ranged from 0.3-7.7% of total risk. In those populations, risk from within their own population ranged from 92.1-99.4% of total risk. However, in the MF and SC populations risk from within their own population was only 27.1 and 39.9%, respectively, compared to 72.3 and 59.9% from other elk populations.

DISCUSSION

This study found that minimal opportunity exists for *B. abortus* transmission from bison to elk under natural conditions in the northern GYA. The reasons for this lower

probability of adequate contact for *B. abortus* transmission, even when spatiotemporal overlap occurred, are likely immunological or behavioral. Differences in the immune systems of elk compared with bison may make them less susceptible to infection. These immunological differences may also account for the different responses of elk and bison to vaccination, leading to the failure of elk to be protected by RB51 vaccination while bison acquire some protection from the vaccine (Kreeger et al. 2002, Olsen et al. 2003). Also, anecdotally, bison are more dominant than elk and may drive elk off grazing areas, increasing their opportunity for exposure to elk infectious material but decreasing the opportunity for elk to be exposed to bison infectious material (Rick Wallen, personal communication).

In addition, reproductive behavioral differences likely account for decreased transmission risk for elk compared with bison. The probability of *B. abortus* transmission between elk (or from elk to cattle) is likely low during calving (May through June) because pregnant dams isolate themselves while giving birth and meticulously clean the birth site (Johnson 1951). Thus, birth sites are dispersed, and the likelihood of other elk encountering infected birth tissues and fluids is low. However, transmission risk may be higher during the potential abortion period from February through April when many elk aggregate in larger groups on lower-elevation winter ranges that sometimes include ranch areas with cattle (Hamlin and Cunningham 2008). Spontaneous abortions by elk that are not segregated from their herd could expose many elk to infected fetuses and birth tissues (P.J. White, personal communication). In contrast, bison are gregarious during parturition, and pregnant females have been observed to nuzzle newborn calves (Yellowstone Center for Resources 2008). Mobbing events of a newborn calf or aborted

fetus could contribute to intra-species transmission of bacteria if the dam were infected (Yellowstone Center for Resources 2009).

The MF and SC elk populations had the lowest estimated transmission risk. The SC population was spatio-temporally distant from the YNP bison herd, while the MF elk had increased overlap with Yellowstone bison (Ferrari and Garrot 2002, Proffitt et al. 2010). A lower median seroprevalence in the SC population (0.01 compared with 0.03 in all other elk populations) and the small population size in both herds resulted in decreased estimated shedding in these populations. Therefore, a higher percentage of total risk to these populations came from outside elk sources rather than in the other three elk populations.

Probabilities for elk having adequate contact with other elk for *B. abortus* transmission were 24 times higher within their own population than from other elk populations. Because, behaviorally, most risk comes from spontaneous abortions, it is understandable that these abortions occur more frequently within a single elk population than during periods of comingling of multiple populations. For bison, transmission risk could potentially come from within their own population or from GYA elk. However, because there was a single population of bison, it decreased the ability to differentiate the relative probabilities for adequate contact from bison or elk shedding.

Estimates for transmission risk and transmission incidence were of the same order of magnitude. However, the equation for incidence used the lifespan of the wildlife species as the duration of infection. The assumption that all infected animals were infected at birth created an overestimate of duration, which resulted in an underestimate

of incidence. This helps to account for a transmission incidence lower than the total transmission risk in bison as well as two populations of elk.

The National Park Service is exploring the remote delivery of the RB51 brucellosis vaccine to female Yellowstone bison to reduce abortions from this non-native disease and increase tolerance for bison outside YNP (USDI-NPS 2010). Vaccination is expected to significantly reduce the population seroprevalence of *B. abortus* infection (Yellowstone Center for Resources 2008). However, because bison rarely transmit *B. abortus* to elk, management alternatives such as vaccination that reduce bison seroprevalence are unlikely to reduce transmission from elk to cattle. However, these practices would increase the tolerance for bison outside YNP boundaries, as they would decrease the potential for transmission from bison to cattle. The reduction in practices that increase elk herd densities and group sizes or the implementation of strategies to reduce elk-to-elk transmission should be promoted to reduce the overall risk to cattle grazing in the northern GYA.

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Table 1. Input parameters for a *Brucella abortus* transmission model used to assess the risk of an infectious event occurring in elk and bison populations in the northern greater Yellowstone area.

Description of variables	Statistical distribution (parameters) [Mean, SD]	Source
Shedding proportion	Beta (12,14) [0.46, 0.10]	(Roffe et al. 1999)[a]
Fetal persistence	BetaGeneral (2, 6.93, 1, 78) [18.25, 10.19]	(Aune et al. 2007)
Bison		
Number of animals Fit from 2000-2008 data	Logistic (3788.53, 450.13) [3788.53, 816.45]	(National Park Service, unpublished data)
Age proportion (of total population): Fit from 2004-2008 data		(National Park Service, unpublished data)
2-3 year-old females	BetaSubjective (0.043, 0.047, 0.04736, 0.053) [0.047, 0.002]	
4+ year-old females	Pareto (46.43, 0.35123) [0.36, 0.01]	
Proportion pregnant:		
2-3 year-old	Uniform (0.71, 0.79) [0.75, 0.02]	(Yellowstone Center for Resources 2008)
4+ year-old	Uniform (0.76, 0.89) [0.83, 0.04]	
Proportion seropositive 2+ year-old (sampled at boundary capture facility)	Beta (331.0, 211.6) [0.61, 0.02]	(National Park Service, unpublished data)

Percentage shedding by abortion:

First pregnancy females	BetaSubjective (0.65, 0.78, 0.78, 0.9) [0.78, 0.07]	(Davis et al. 1990)
Mature females	BetaSubjective (0.01, 0.1, 0.09, 0.15) [0.09, 0.03]	(Peterson et al. 1991)
Birth synchrony	Normal (40.57, 13.33) [40.57, 13.33] Day 1 = April 1	(Berger and Cain 1999)

Elk

Adult female proportion Fit from 2000-2008 data	BetaSubjective (0.52, 0.73, 0.7, 0.8) [0.7, 0.06]	(National Park Service, unpublished data)
Adult female: yearling	10:1	(National Park Service, unpublished data)
Proportion pregnant: Fit from 2000-2006 data		(National Park Service, unpublished data)
Yearling	BetaSubjective (0.1, 0.33, 0.32, 0.4) [0.32, 0.03]	
Adult	BetaSubjective (0.78, 0.82, 0.815, 0.84) [0.82, 0.01]	

Percentage of shedding by abortion:

First pregnancy females	Beta (13.3, 14.4) [0.48, 0.09]	(Thorne et al. 1978)
Mature females	Beta (1.2, 6.8) [0.15, 0.12]	
Birth synchrony	Poisson (32.526) [32.526, 5.703] Day 1 = May 1	(Maichak et al. 2009)

Gallatin-Madison

Number of animals	Normal (7807, 793)	
Fit from 2000-2008 estimates (sightability corrected using 1.322 correction factor)	[7807, 793]	(Hamlin and Cunningham 2008)

B. abortus seropos. proportion	Beta (3.1, 101.5) [0.03, 0.02]	

Gravelly-Snowcrest (Hamlin 2006)

Number of animals	Uniform (10,900, 11,570)	
Fit from 2004&2006 data	[11,235, 193]	
B. abortus seropos. proportion	Beta (3.1, 101.5) [0.03, 0.02]	

Madison-Firehole

Number of animals	Loglogistic (236.8, 196.2, 1.4)	
Fit from 2000-2008 estimates (sightability corrected using 1.322 correction factor)	[757.2, N/A]	(Hamlin and Cunningham 2008)
B. abortus seropos. proportion	Beta (3.1, 101.5) [0.03, 0.02]	

Northern Yellowstone

Number of animals	Lognormal (9742, 3801, Shift (3396))	
Fit from 2000-2008 estimates (sightability corrected using 1.322 correction factor)	[13,137, 3800]	(Cross et al. 2009)
B. abortus seropos. proportion	Uniform (0.01, 0.05 [0.03, 0.01]	(Barber-Meyer et al. 2007)

Sand Creek, Idaho

Number of adult females, 2006 (sightability corrected using 1.322 correction factor)	1,413	(Mark Drew, Idaho Department of Fish and Game, unpublished data)
B. abortus seropos. proportion	Beta (0.9, 100) [0.01, 0.01]	

a – study generalizes statistic for seropositive female bison

Table 2. Risk equation matrix for inter- and intra-species wildlife *Brucella abortus* transmission risk within and between bison and elk populations in the northern greater Yellowstone area.

	***B. abortus* transmission risk equations**		
Population at-risk	**From Bison Population**	**From other Elk Population**	**From own Elk Population**
Bison	(1)	(2)	N/A
<u>Elk Populations</u>			
Gallatin-Madison (GM)	(3)	(4)	(5)
Gravelly-Snowcrest (GS)	(6)	(7)	(8)
Madison-Firehole (MF)	(9)	(10)	(11)
Northern Yellowstone (NY)	(12)	(13)	(14)
Sand Creek, Idaho (SC)	(15)	(16)	(17)

(1) – (Bison shedding) * λ

(2) – (Elk shedding overlap) * γ

(3) – (Bison shedding overlap with GM) * δ

(4) – (GS,MF,NY,SC shedding overlap with GM) * ε

(5) – (GM shedding) * θ

(6) – (Bison shedding overlap with GS) * δ

(7) – (GM,MF,NY,SC shedding overlap with GS) * ε

(8) – (GS shedding) * θ

(9) – (Bison shedding overlap with MF) * δ

(10) – (GM,GS,NY,SC shedding overlap with MF) * ε

(11) – (MF shedding) * θ

(12) – (Bison shedding overlap with NY) * δ

(13) – (GM,GS,MF,SC shedding overlap with NY) * ε

(14) – (NY shedding) * θ

(15) – (Bison shedding overlap with SC) * δ

(16) – (GM,GS,MF,NY shedding overlap with SC) * ε

(17) – (SC shedding) * θ

Probabilities of adequate contact, given spatiotemporal overlap

λ – Bison from bison

γ – Bison from elk

δ – Elk from bison

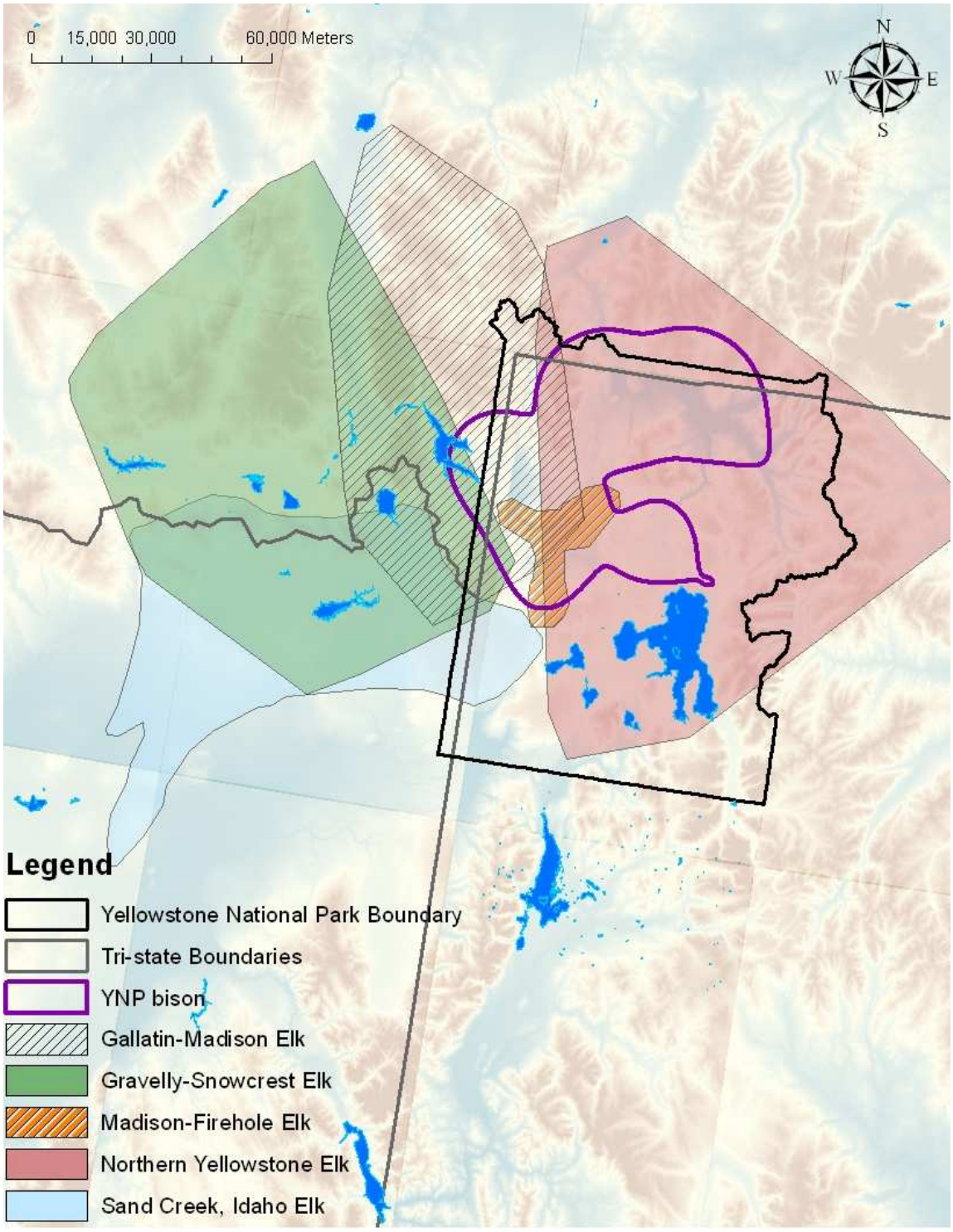
ε – Elk from other elk population

θ – Elk from own population

Table 3. Median risk, percentage of total, and 95% probability interval (P.I.) of *Brucella abortus* risk of transmission within and between bison and elk populations in the GYA using home range estimates for average winters. Units of risk are female exposure event-days.

	***B. abortus* transmission risk; % of total risk (95% P.I.)**		
Population at-risk	**From Bison Population**	**From other Elk Population**	**From own Elk Population**
Bison	91.1; 78.3 (22.7, 242.4)	25.2; 21.7 (4.5, 98.5)	N/A
Elk Populations			
Gallatin-Madison	0.01; 0.2 (0.003, 0.03)	0.4; 7.7 (0.1, 1.3)	4.8; 92.1 (0.9, 18.0)
Gravelly-Snowcrest	0.002; <0.1 (0.001, 0.006)	0.06; 0.9 (0.01, 0.22)	6.9; 99.1 (1.3, 25.8)
Madison-Firehole	0.006; 0.5 (0.001, 0.016)	0.8; 72.3 (0.2, 3.1)	0.3; 27.1 (0.05, 1.8)
Northern Yellowstone	0.03; 0.4 (0.007, 0.09)	0.02; 0.3 (0.003, 0.06)	7.8; 99.4 (1.4, 31.5)
Sand Creek, Idaho	0.001; 0.2 (0, 0.001)	0.3; 59.9 (0.05, 1.0)	0.2; 39.9 (0.01, 1.6)

Figure 1. Map of bison and elk population distributions in the northern portion of the greater Yellowstone area based on an average winter.



Equation 1. Risk equation for inter- and intra-species wildlife *Brucella abortus* transmission risk model

Risk $\propto$

(Number of number of animals in the at-risk population) × (Seronegative female proportion in the at-risk population) × (Number of infectious event-days from the source of risk) × (Proportion of shedding events overlapped by at-risk population) × (Proportion of at-risk population exposed to risk source) x Pr (Adequate Contact | Overlap)†

† - Probabality of contact which would result in transmission if the exposed animal was susceptible, given overlap occurs

Equation 2. Incident Cases = (Prevalent Animals) / (Median Duration of Infection)$^{\alpha}$

Chapter 5

Conclusions

This dissertation research was initiated concurrently with the establishment of the Yellowstone Wildlife Health Program (YWHP). The YWHP, a cooperative partnership between Montana State University, the University of California, Davis, and YNP, was created to help answer meaningful scientific research questions and establish professional networks to funnel the answers to these questions back to YNP. An organizational workshop listed brucellosis among the highest priority research needs of YNP and identified risk assessments, transmission dynamics, and diagnostic test evaluations as specific scientific needs (Schumaker et al., 2007).

The dissertation evaluated the fluorescence polarization assay (FPA) in a natural setting, and found that the sensitivity of the FPA in detecting *B. abortus* antibodies was excellent. A Bayesian analysis agreed with this finding; however, the study highlighted the difficulties of field diagnostic test evaluations. Similar to traditional diagnostic test evaluations, the serologic tests showed very high specificity when the privately-owned animals from a population without brucellosis were evaluated. Where *B. abortus* exposure was common, however, the FPA and other serologic tests were not accurate in differentiating culture-positive from culture-negative animals. In the study, 63 culture-negative animals were positive on at least 6 of the serological tests. Younger animals sampled from the YNP bison herd were more likely to be culture-positive than older animals. Although the bison sampled were not a representative sample from the whole population, this is consistent with the finding that transmission rates are higher among juveniles than older animals (Rhyan et al., 2009). In a decision-tree analysis, the FPA was

comparable to other tests but had higher expected costs than the standard tube tests, mainly due to increased costs of testing equipment and supplies.

Findings from the study indicate the need to assure that tests being used to make management decisions are well-suited for their intended purpose. The development of additional assays or culture techniques that result in a more accurate reference standard would improve disease management programs in YNP. The FPA is perhaps the most sensitive test for detecting serum antibodies to *B. abortus* in bison but may be unable to differentiate exposed individuals from those with active infections or sequestered bacteria. Nevertheless, because the FPA showed perfect specificity in the unexposed privately-owned bison it is likely to have high specificity. This is supported by the results of the Bayesian analysis. Further work needs to be performed evaluating the repeatability of the FPA with different operators and under different environmental conditions. A comparison between animal-side testing and bench-top machines is also needed; however, the FPA is an ideal candidate as a screening test due to its ability to detect small levels of *B. abortus* antibodies. For evaluation of tests under field conditions, longitudinal studies should be performed, testing animals throughout the study period and harvesting a subset of subjects at various points to determine their culture status.

This dissertation also presented the first spatially-explicit framework for assessing the risk of bacterial shedding of *B. abortus* by bison and elk across the northern portion of the GYA. Although our results support substantial shedding of *B. abortus* from bison in some winters, the most substantial risk of *B. abortus* transmission to cattle was from elk. Future risk estimates for bison depend on adaptive management of the population. Interactive effects between population size and winter severity were major determinants

influencing bison movements to lower elevation winter grazing areas and overlap with federally-regulated domestic cattle grazing allotments. However, during the critical period of potential *B. abortus* exposure to cattle, the risk from Yellowstone bison was minimal. Natural movements of animals back to higher elevation summer ranges and boundary management operations were important in minimizing the contribution of bison to cattle exposure risk, which supports continued boundary management operations for spatio-temporal separation between bison and cattle. Under current management practices, bison risk to cattle grazing in the northern portion of the GYA is expected to remain small.

In addition to spatio-temporal overlap of wildlife home ranges and cattle grazing allotments, the major contributors to risk were wildlife population size and the number of elk that were shedding *B. abortus*. While elk currently have a lower density of shedding events throughout their range, they have a larger overlap with cattle and are more tolerated by managers and livestock keepers on public grazing allotments. With increased disease prevalence due to increased winter densities or other factors, elk will likely contribute greatly to the overall level of bacterial shedding on the northern GYA landscape and represent the vast majority of risk of *B. abortus* exposure to cattle grazing in the northern portion of the GYA. Therefore, brucellosis management efforts should increasingly focus on the comingling of cattle and elk during the critical abortion period to more effectively decrease risk of transmission.

Continued exploration of the brucellosis risk model found that minimal opportunity exists for *B. abortus* transmission from bison to elk under natural conditions in the northern GYA. The reasons for this lower probability of adequate contact for *B.*

abortus transmission, even when spatio-temporal overlap occurred, are likely immunological or behavioral. The risk model may be expanded to include the entire GYA or serve as a template for models of other diseases. As additional data become available, especially additional spatial locations of cattle and wildlife and animal movement information, the model can be refined for even more targeted management decisions. Current work is using the model to evaluate the relative impacts that alternative management strategies can have on overall *B. abortus* transmission.

The National Park Service is exploring the remote delivery of the RB51 brucellosis vaccine to female Yellowstone bison to reduce abortions from this non-native disease and increase tolerance for bison outside YNP (USDI-NPS, 2010). Vaccination is expected to significantly reduce the prevalence of *B. abortus* in bison (Yellowstone Center for Resources, 2008). However, management alternatives, such as vaccination, that reduce *B. abortus* prevalence in bison are unlikely to reduce transmission from elk to cattle. These practices would still increase the tolerance for bison outside YNP boundaries, however, as they would decrease either the actual potential for transmission from bison to cattle or the perceived potential for transmission. The reduction in practices that increase elk herd densities and group sizes or the implementation of strategies to reduce elk-to-elk transmission should be promoted to reduce the overall risk to cattle grazing in the northern GYA.

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