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Vaccination strategies for managing brucellosis in Yellowstone bison

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ABSTRACT

Concerns over migratory bison (*Bison bison*) at Yellowstone National Park transmitting brucellosis (*Brucella abortus*) to cattle herds on adjacent lands led to proposals for bison vaccination. We developed an individual-based model to evaluate how brucellosis infection might respond under alternate vaccination strategies, including: (1) vaccination of female calves and yearlings captured at the park boundary when bison move outside the primary conservation area; (2) combining boundary vaccination with the remote delivery of vaccine to female calves and yearlings distributed throughout the park; and (3) vaccinating all female bison (including adults) during boundary capture and throughout the park using remote delivery of vaccine. Simulations suggested Alternative 3 would be most effective, with brucellosis seroprevalence decreasing by 66% (from 0.47 to 0.16) over a 30-year period resulting from 29% of the population receiving protection through vaccination. Under this alternative, bison would receive multiple vaccinations that extend the duration of vaccine protection and defend against recurring infection in latently infected animals. The initial decrease in population seroprevalence will likely be slow due to high initial seroprevalence (40–60%), long-lived antibodies, and the culling of some vaccinated bison that were subsequently exposed to field strain *Brucella* and reacted positively on serologic tests. Vaccination is unlikely to eradicate *B. abortus* from Yellowstone bison, but could be an effective tool for reducing the level of infection. Our approach and findings have applicability world-wide for managers dealing with intractable wildlife diseases that cross wildlife–livestock and wildlife–human interfaces and affect public health or economic well-being.

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1. Introduction

The discovery of new infectious agents and diseases transmissible to humans has raised concerns regarding free-ranging wildlife as a source of emerging human pathogens [1,2]. Humans are often indirectly exposed to wildlife pathogens through infected livestock. The crowding and mixing of wildlife with domestic livestock can increase disease prevalence and transmission potential [3,4] thereby, increasing exposure to humans. Disease transmission risk from wildlife to domestic animals and humans traditionally has resulted in control strategies that negatively impact wildlife populations. Traditional test-and-slaughter programs have been effective for managing diseased livestock but these practices may not be realistic or socially acceptable for wildlife [5,6]. An approach to wildlife disease management is needed that addresses both public health concerns and long-term wildlife conservation. Vaccination is commonly used for disease control in veterinary medicine and wildlife vaccination may offer a promising solution [7]. The success of a vaccination program is influenced by vaccine efficacy and the proportion of the population inoculated. Our ability to deliver efficacious vaccines and monitor their effectiveness is restricted in free-ranging wildlife. Additionally, we will seldom have all the information necessary to predict the effectiveness of a wildlife vaccination program, but management actions will need to move forward despite these uncertainties.

Yellowstone National Park of the western U.S. was created in 1872, and encompasses 9018 km^2 in portions of Idaho, Montana, and Wyoming, but only about 3175 km^2 of this area currently serves as principal bison habitat (Fig. 1). The successful conservation of bison (*Bison bison*) from a low of 23 animals in 1901 to a high near 5000 animals in 2005 has led to an enduring series of societal conflicts and disagreements among various publics and management agencies regarding the potential transmission of *Brucella abortus* to domestic livestock. *B. abortus*, the bacteria causing the disease bovine brucellosis, was introduced to Yellowstone bison by cattle before 1917 and approximately 40–60% of the Yellowstone bison population have been exposed [8]. Since that time, successful conservation increased the abundance of Yellowstone bison from

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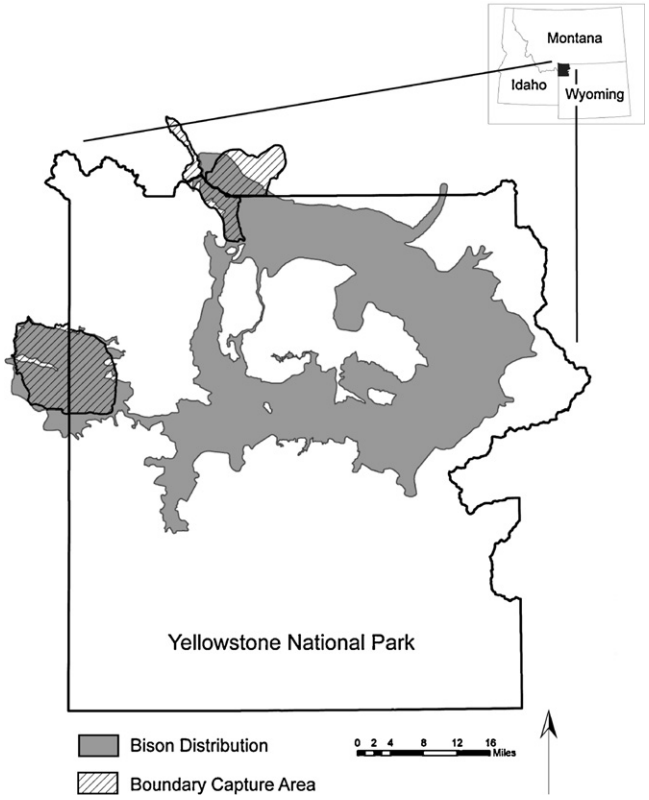

Fig. 1. Map of the distribution of bison within Yellowstone National Park and location of boundary capture areas for migrating bison. The northern and western boundary capture areas include facilities where bison are tested and vaccinated for brucellosis.

approximately 400 to >4700 in 2007 [9]. A portion of the Yellowstone bison population periodically moves between habitats in the park and adjacent lands in Montana during winter [10], resulting in a risk of brucellosis transmission from bison to cattle on overlapping ranges adjacent to the park [11]. Humans are also susceptible to infection, though brucellosis is no longer a widespread health threat in North America due to the use of sanitary procedures (e.g., pasteurization) in milk processing [12]. When livestock are infected, brucellosis results in economic loss from slaughtering infected cattle herds and imposed trade restrictions [13]. More than $3.5 billion were spent since 1934 to eradicate brucellosis in domestic livestock across the U.S. [8], however the disease remains endemic in bison (*B. bison*) and elk (*Cervus elaphus*) in the greater Yellowstone ecosystem [10]. Many livestock producers and cattle regulatory agencies contend that any risk of brucellosis transmission is unacceptable for economic and public health reasons.

To manage the risk of brucellosis transmission from Yellowstone bison to livestock, the federal government and State of Montana agreed to the Interagency Bison Management Plan [14,15]. This plan established guidelines for implementing hazing, test-and-slaughter, hunting, and other actions affecting bison abundance and distribution near the park boundary, where bison could potentially co-mingle with livestock. The plan also indicates that the National Park Service will conduct a remote delivery vaccination program of vaccination-eligible bison within the park to increase tolerance of untested bison on winter range lands outside the park. The National Park Service is currently considering the implementation of such a program to reduce brucellosis infection in the bison herd. Much remains to be learned about brucellosis epidemiology in bison and how effective vaccination may be, but it will be necessary to make decisions and proceed despite uncertainty. Simulation models can be effective tools for informing this decision-making process by evaluating the effectiveness of different management strategies. Thus, we developed an individual-based model to evaluate alternate vaccination strategies and how brucellosis infection in Yellowstone bison might respond under different approaches.

2. Model context

2.1. *B. abortus* infection

Brucellae are facultative intracellular pathogens, which evade the host's immune system by replicating within the host's white blood cells (e.g., macrophages) [16]. During middle to late gestation, Brucellae that have infected the uterus undergo massive replication in placental cells. The extensive replication causes a rupture compromising placental integrity by allowing the bacteria direct access to the fetus [17]. The resulting abortions and premature calves are highly infectious due to the large number of Brucellae on the fetus, placenta and birth fluids. Following this acute phase of infection, some bison are unable to clear the bacteria and remain infected. The pathogen's ability to establish persistent infections in some animals results in a class of latent carriers. The relapsing of latently infected animals to the infectious state during future pregnancies, is a concern with Yellowstone bison.

Intracellular protection and replication are crucial components of incubation, latency, and chronic infection of Yellowstone bison [18]. Thus, we modeled these aspects of brucellosis infection in bison by including an incubation period in the model. This allowed for deciding whether a pregnant, susceptible bison that was recently exposed would have adequate time to shed *B. abortus*. Also, we addressed latent infection in the model by assuming bison never truly recover from brucellosis and that adult females can potentially shed *B. abortus* throughout their reproductive lives.

2.2. *B. abortus* transmission

Transmission of *B. abortus* in Yellowstone bison is believed to occur primarily through contact with an aborted fetus or infected birth tissues shed during a live birth. The number of exposures that occur during these infectious events depends on the behavior of the bison cow at the time of parturition. Bison tend to give birth in close proximity to other group members, which increases the likelihood of transmission. *B. abortus* is also known to cause mammary gland infections [19] and can be transmitted through infected milk [18,20,21]. Though bacterial numbers in milk are lower than in an infected placenta, they are typically high enough to present a serious risk of transmission [8]. The role vertical transmission (transmission from cow to newborn calf) plays in the maintenance of *B. abortus* in Yellowstone bison is unclear, but may help explain the low frequency of observed abortions, high seroprevalence rates among young animals, and latent infection.

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

A key component of brucellosis transmission is the number of exposures that occur during an infectious event. Thus, the ability of the *Brucella* pathogen to spread could be influenced by group size, composition, cohesion, and the infection status of associates [22]. Yellowstone bison appear to have a dynamic social structure with fluid movements between groups [23–27]. The fundamental social unit is the cow–calf association, which persists for approximately

9 months in male calves and 14 months in female calves [26,28]. There is little evidence that groups of related females form lifelong associations [23], but cows with calves tend to be found more often in groups with other cow–calf pairs [29]. Also, group sizes tend to get larger as habitat becomes more open and generally increase during the spring calving season [25].

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

2.3. B. abortus detection in bison

Identifying the state of brucellosis infection within the Yellowstone bison population relies on diagnostic tests performed on a segment of the bison population captured at the park boundary. Brucellosis infection is diagnosed in bison through serologic tests and bacterial cultures. For serologic tests, the fluorescent polarization assay (FPA) is the diagnostic test of choice for detecting brucellosis in bison because of its high sensitivity (94.5%), specificity (99.5%), and adaptability to field use [30–32]. Serologic tests provide indirect evidence of infection because they detect antibodies (i.e., responses to infection) rather than living bacteria and can result in both false positive and false negative diagnoses. Thus, it is unlikely that the probability of identifying truly infectious individuals can be accomplished by serology alone [8]. Combining serologic testing with tissue culture identified that nearly half (46%) of slaughtered seropositive bison were also culture positive [33]. Based on this work, we estimated that 46% of seropositive bison were culture positive animals and considered to be actively infected. We assumed, based on the use of the FPA as a diagnostic tool, that all actively infected bison and a high proportion of latent infected animals could be diagnosed as positive under boundary capture scenarios.

2.4. Vaccination of Yellowstone bison

The objective of bison vaccination is to stimulate an acquired immune response to *B. abortus* thereby increasing herd immunity and reducing the potential for transmission. The live *B. abortus* strain RB51 (SRB51) is the official brucellosis vaccine for cattle in the U.S., but has the potential to induce abortions in pregnant bison vaccinated in mid-gestation [34]. Though bison calves vaccinated with SRB51 may be safely booster-vaccinated during their first pregnancy, making early gestation a potentially safer period for vaccinating adult pregnant bison [20]. Based on these findings we developed vaccination strategies that would limit the potential for vaccine induced abortions by focusing on reproductively immature bison and adult females during early gestation.

There is uncertainty about the level of protection (i.e., efficacy) SRB51 will provide Yellowstone bison based on experimental studies. Vaccination of bison calves provided protection from abortions and placental infection when challenged with virulent *B. abortus* during their first pregnancy [22]. However, SRB51 was found to have little efficacy in adult and calf bison despite repeated vaccinations [35,36]. Thus, the duration of protection provided by a single dose of SRB51 is unknown and older cows may need to be booster-vaccinated to extend the protection of the vaccine [20]. A key feature of SRB51 is that vaccinated bison remain seronegative when tested with standard serologic tests [37] which prevents the removal of tested vaccinated animals.

Delivery of vaccine poses a problem with free-ranging bison and, currently, the most feasible method of remote vaccine delivery is via biodegradable projectiles (i.e., "bio-bullets"). Ballistic vaccination has been used to inoculate free-ranging elk on feedgrounds in Wyoming [38] and tested experimentally with bison. Ballistic inoculation of bison with photopolymerized SRB51 packaged into bio-bullets induced a significant cell-mediated immune response that was similar to syringe delivery of the vaccine (i.e., parenteral vaccination) [39]. We assume that remote delivery of SRB51 to free-ranging bison would provide protection equal to bison given syringe vaccinations when handled at the boundary. We also addressed waning immune protection in the years following vaccination and included an increase in protection with booster vaccination.

3. The individual-based model

3.1. Model development

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

The IBM tracked information on each female bison born into the population (Fig. 2A). The model used a yearly time step to simulate population level processes and daily time steps to simulate exposure routes during the transmission period (Fig. 2B). The yearly time step components involved mating, natural mortality, exposure to *B. abortus* via elk, and effects of management operations (testing and subsequent removal of seropositive bison at park boundaries). The daily time step detailed the processes (*Brucella* induced abortions and infectious live births) leading to shedding and transmission of *B. abortus* among Yellowstone bison. Male bison were included in yearly outputs, but were not a focal component of the model because their role in maintenance and transmission is expected to be minimal [40]. Age, sex, disease status, reproductive status, and vaccination status were recorded for each female bison modeled.

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

The model included two types of infectious events for simulating horizontal transmission: *Brucella* induced abortions and infectious live births. We assumed that both events had equal transmission potential. We also assumed that infected bison did not fully recover from the disease. These animals had a low probability of shedding the bacteria in future pregnancies while remaining latently infected. In situations where latent cows recrudesced and shed *B. abortus* during an infectious live birth, their calves became infected through vertical transmission (consuming infected milk) at a specified probability.

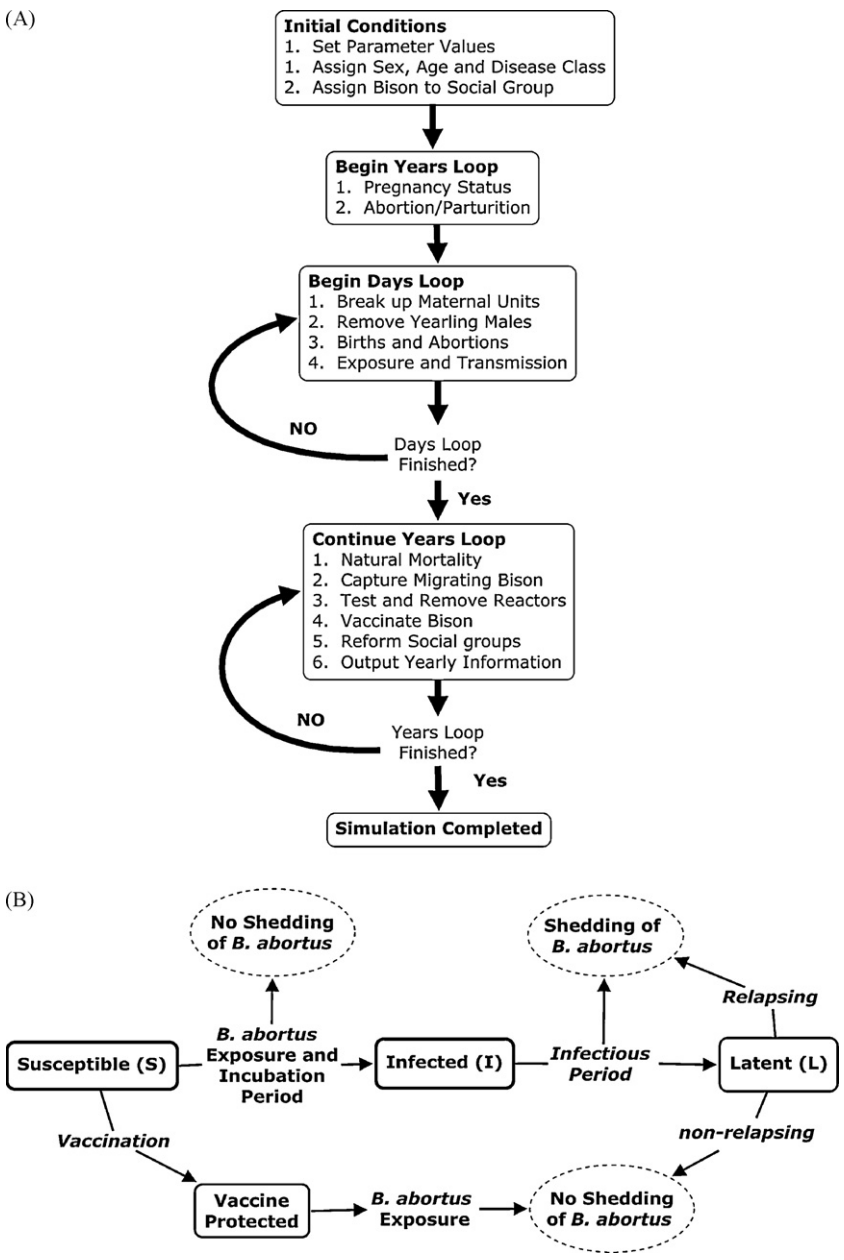

Fig. 2. Flow diagram of individual-based model processes influencing the state of *B. abortus* infection in Yellowstone bison. The sequences simulated (panel A) for the three vaccination alternatives were run for a 30-year period with yearly processes controlling population demographics and vaccination status and daily processes detailing *B. abortus* exposure and transmission. Changes in disease state and vaccination status (panel B) were based on rules of exposure and vaccine efficacy.

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

3.2. Model processes

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

The annual time step began with bison becoming pregnant based on estimates of age-specific pregnancy rates. Pregnant bison were given either a pregnancy date or an abortion date depending on the individual's disease class. The abortion period included the last trimester (90 days) of gestation (287 days) before the live birth period (61 days). Depending on their disease status, pregnant bison had a non-infectious live birth (i.e., *Brucella* not shed;

Table 1
Default parameter values for an individual-based model predicting how brucellosis infection in Yellowstone bison might respond under alternate vaccination methods.

Parameter/variable	Value	Source
Pregnancy rate (Pr)		National Park Service
2-year olds	0.71	
3-year olds	0.79	
4-year olds	0.76	
Adults (5 years+)	0.89	
Calving rate (Cr)	0.71	National Park Service
Birth period (Bdays)	61 days	[43]
Abortion period (Adays)	90 days	[8]
Death rate (Dr)		[44]
0–2 years	0.2	
3–13 years	0.1	
14 years	0.2	
15 years	1.0	
Social group size		National Park Service
Minimum	24	
Maximum	48	
Disease state		National Park Service and Montana Department of Livestock captures
Susceptible (S)	0.53	
Infected (I)	0.22	[33]
Adult latent (L)	0.25	
Rate of recrudescence	0.05	Review of latency literature
Exposures per infectious event	Poisson ($\lambda = 1$)	National Park Service
Vertical transmission	0.66	[45]
Minimum incubation	35 days	[45]
Social transmission factor (β)	1.5	Fitted parameter
Bison captures at park boundary per year		National Park Service 1985–2005
0–10% of population	0.84	
10–20% of population	0.11	
20–40% of population	0.05	
Bison removals at capture facility		
Removal of infected class	1.0	[32]
Removal of latent class	0.94	
Vaccine efficacy		Modeled over a range of values

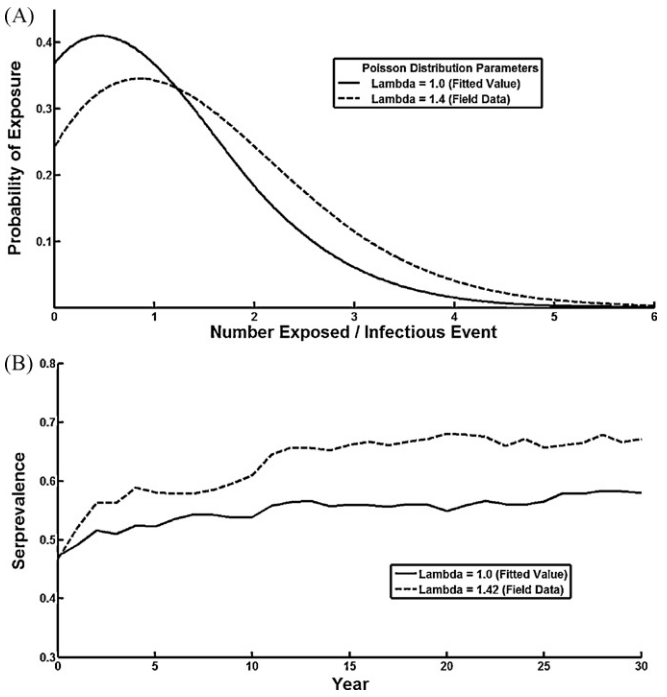

Fig. 3. Estimate of *B. abortus* transmission following an infectious event (abortion or live birth). The number of bison exposed was estimated using a probability (Poisson) distribution fit to field observations of bison making contact with newborns and expelled birth tissues (panel A). The rate parameter (lambda) for the probability of exposure was adjusted to simulate historic seroprevalence ranges (40–60%) for Yellowstone bison (panel B).

calves classified as susceptible), an infectious live birth (i.e., *Brucella* shed; calves classified as susceptible (0.34) or infected (0.66)), or a brucellosis-induced abortion. We treated infectious material from abortions and infectious live births equally with regard to disease transmission. Susceptible bison had a non-infectious live birth unless exposed to *B. abortus* during pregnancy and there was sufficient incubation time (35 days) for *B. abortus* to be shed. If there was insufficient incubation time (<35 days) before parturition, the female did not abort or have an infectious birth. However, the female's newborn calf was infected via vertical transmission with a set probability (0.66). Bison infected with greater than 35 days of incubation prior to parturition aborted their pregnancy at a specified probability (0.96) or infected their newborn calves via vertical transmission (0.66). Pregnant, latent cows had a non-infectious birth unless they relapsed to the infectious state. We assumed 5% of latently infected adult females relapsed in a given year and shed *B. abortus* through infectious live births and infected their calves through vertical transmission.

Based on field observations of bison group members interacting with newborn calves and birth tissues, we assumed cow–calf pairs approached parturition sites and were exposed to *B. abortus* together. Thus, transmission was modeled using the exposure of maternal units that were either cows and their newly born calves or single female bison (≥1-year old) to infectious events (i.e., abortions and infectious live births). Maternal units in the susceptible class became infected when exposed, while the disease status of already infected and latent class bison remained unchanged. The number of maternal units exposed per infectious event was decided by drawing from a Poisson distribution fitting field observations of Yellowstone bison licking newborns or expelled birth tissues. Contact with birth material was treated as a discrete random variable and a Poisson distribution was fit to the frequency of contacts by group members. The rate parameter that best fit the field data ($\lambda = 1.42$) was adjusted ($\lambda = 1.0$) to fit the historical population seroprevalence estimates (Fig. 3).

Long-term group size information for Yellowstone bison [23] was used to divide the population into groups of cows and their calves. Social groups of 24–48 females and calves were assumed to have greater contact with each other than with bison outside their group. Thus, the probability of exposure following an infectious event is expected to be higher within groups than among groups due to the proximity of individuals to infectious birth tissues. However, the mixing of bison social groups and the ability of *B. abortus* to persist on the landscape [41] suggests there is transmission potential to bison outside the social group experiencing the infectious event. The specific maternal units exposed were determined using a biased draw from the population, with parameter β biasing exposures in favor of bison maternal units within the social group where the infectious event occurred. The probability that an exposure will occur in any group, other than the group containing the infectious

Table 2
Annual proportions of bison captured at the boundary of Yellowstone National Park during winters 1985–2005.

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

event, was expressed using Eq (1):

Probability of outside group transmission

$$= \frac{N_i}{\beta(N_k - 1) + \sum_{\substack{j=1 \\ j \neq k}}^{n} N_j} \quad (1)$$

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

Probability of within group transmission

$$= \frac{\beta(N_k - 1)}{\beta(N_k - 1) + \sum_{\substack{j=1 \\ j \neq k}}^{n} N_j} \quad (2)$$

Following the daily processes influencing transmission and exposure, the remaining annual processes were simulated. Social groups and their maternal units were reestablished based on group size criteria. Bison were subjected to natural mortality based on estimated age-specific death rates. Management operations (i.e., test, remove, vaccinate) were modeled for each of the three vaccination alternatives. The portion of the Yellowstone bison moving beyond the park boundary was modeled based on the past 20 years of capture operations. We used a frequency distribution of the portion of the population captured (<0.1, 0.1–0.2, and 0.2–0.3) at the park boundary each winter during 1985–2005 (Table 2) to estimate the number of bison that might be tested in a given year.

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

Elk populations in the greater Yellowstone ecosystem are also infected by *B. abortus* and have been implicated as the source of brucellosis infection to cattle herds in Idaho, Montana, and Wyoming [42]. The pathology of the disease in elk is believed to be similar to bison and cattle. We included elk as a potential source of brucellosis infection for bison and modeled exposure from elk to bison at a low probability (0.01).

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

4. Results

We conducted 10 simulations at intermediate levels of vaccine efficacy (0.5) for each of the three vaccination alternatives: (1) boundary vaccination of female calves and yearlings; (2) combination of boundary and remote vaccination of female calves and yearlings; and (3) boundary and remote vaccination of all females. Under Alternative 1, seroprevalence decreased by 24% from 0.46 to 0.35 over the 30-year period, with 1% of the population vaccinated. Under Alternative 2, seroprevalence decreased by 40% from 0.47 to 0.28 over the 30-year period, with 10% of the population vaccinated. Under Alternative 3, seroprevalence decreased by 66% from 0.47 to 0.16 over the 30-year period, with 29% of the population vaccinated. Thus, combining boundary and remote vaccination of all female bison (Alternative 3) resulted in the greatest seroprevalence decreases over the 30-year simulation period (Fig. 4A).

Alternative 3 resulted in a larger proportion of vaccine-protected bison compared to the other two alternatives (Fig. 4B), and the relationship between seroprevalence and the proportion of the bison population vaccinated over the 30-year period was $y = 2.4x + 0.85$ ($R = 0.92$). Boundary removals resulting from migrations out of the park were stochastic, but there was a reduction of seropositive bison removed at the boundary as the level of vaccine-protected bison increased in the population. The proportion of seropositive-vaccinates (i.e., vaccinated bison that were subsequently exposed to *B. abortus*) was larger under Alternative 3 than Alternatives 1 and 2. Population growth rates increased from $\lambda = 1.02$ (Alternative 1) to $\lambda = 1.05$ (Alternative 3) with greater vaccination effort.

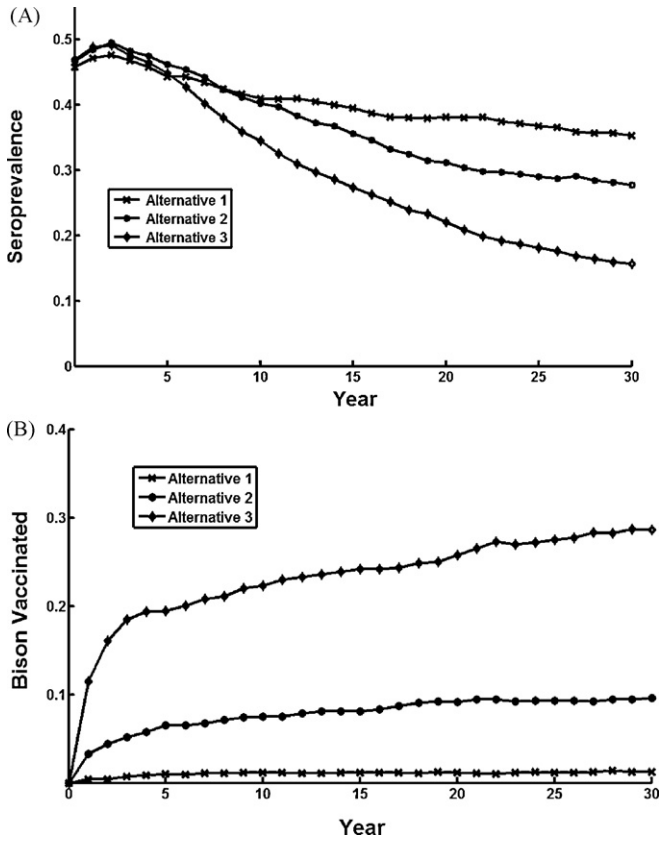

Fig. 4. Simulated declines in brucellosis seroprevalence (panel A) and the proportion of the bison population vaccinated (panel B) for each of the vaccination alternatives.

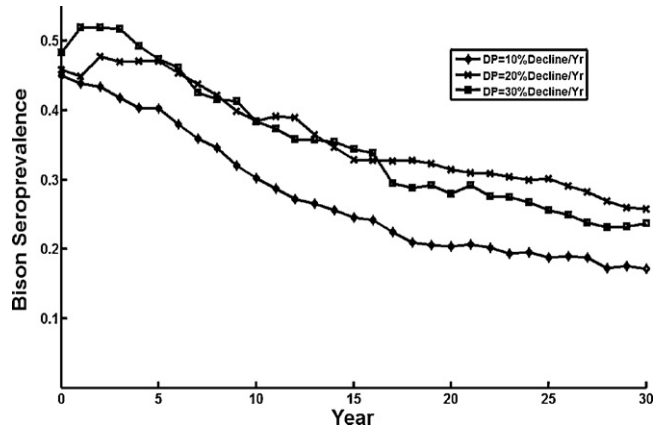

Fig. 5. Simulated declines in seroprevalence for Alternative 3 with waning vaccine protection. Line markers correspond to decreasing vaccine protection (based on the initial vaccine efficacy of 0.5) each year. The initial level of protection was restored if bison were re-vaccinated.

Simulations indicated the effect of decreasing levels of vaccine efficacy (0.10, 0.20, and 0.30 per year) on seroprevalence had the most pronounced effect on Alternative 3 (i.e., the alternative with the most remote vaccination effort, Fig. 5), while model trajectories were more variable in the other two alternatives with less vaccination effort. Exploratory simulations to better understand the response of infection under a short-term (10 years) implementation of Alternative 3, after which all vaccination and management activities ceased, indicated seroprevalence returned to pre-vaccination levels and the rate of return was more sensitive to the level of vaccine efficacy (0.10, 0.30, 0.50, and 0.70) for alternatives with greater vaccination effort (Fig. 6A). The level of vaccinated animals decreased toward zero as individuals were removed based on natural mortality rates (Fig. 6B).

5. Discussion

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

The current vaccine, SRB51, is not expected to provide lifetime protection and female bison may need booster vaccinations [20]. Thus, targeting only young animals for remote vaccination (Alternative 2) would increase the variability in seroprevalence declines because the level of vaccine protection would likely decrease as animals age. However, vaccine SRB51 is safe for multiple immunizations [35], which would reduce the uncertainty of protection in years following vaccination. Targeting all female bison (Alternative 3) allows animals to receive multiple vaccinations that extend the duration of vaccine protection and reduce the potential for latently infected bison to relapse into an infectious state.

The difficulty in monitoring the level of brucellosis infection within the population underscores the need for multiple indicators to evaluate the effectiveness of a vaccination program. Seroprevalence is an attractive indicator of infection because serum is easily obtained, diagnoses are quick and simple, and sampling does not involve killing the animal. However, seroprevalence indicates a history of exposure (i.e., antibody responses) and does not provide a complete picture of how bison may be responding to vaccination because rates of active infection are likely to be much lower than indicated by seroprevalence [33]. Thus, testing bison at boundary capture facilities should combine serologic tests with tissue culture on the seropositive bison that are shipped to slaughter. Because the antibody responses to *B. abortus* are long-lived, the proportion of actively infected bison would be expected to decrease faster in response to vaccination than population seroprevalence. Also, vaccinated bison that are subsequently exposed to field strain *Brucella* will react positively on serologic tests even though they may be protected from further transmission. These bison would be removed during boundary operations, thereby impeding the reduction of brucellosis infection. These bison play an important role in herd immunity by reducing the number of exposures of susceptible bison during an infectious event. Thus, a delay in seroprevalence decrease is expected in the first 10 years of initiating a vaccination program because of high population seroprevalence,

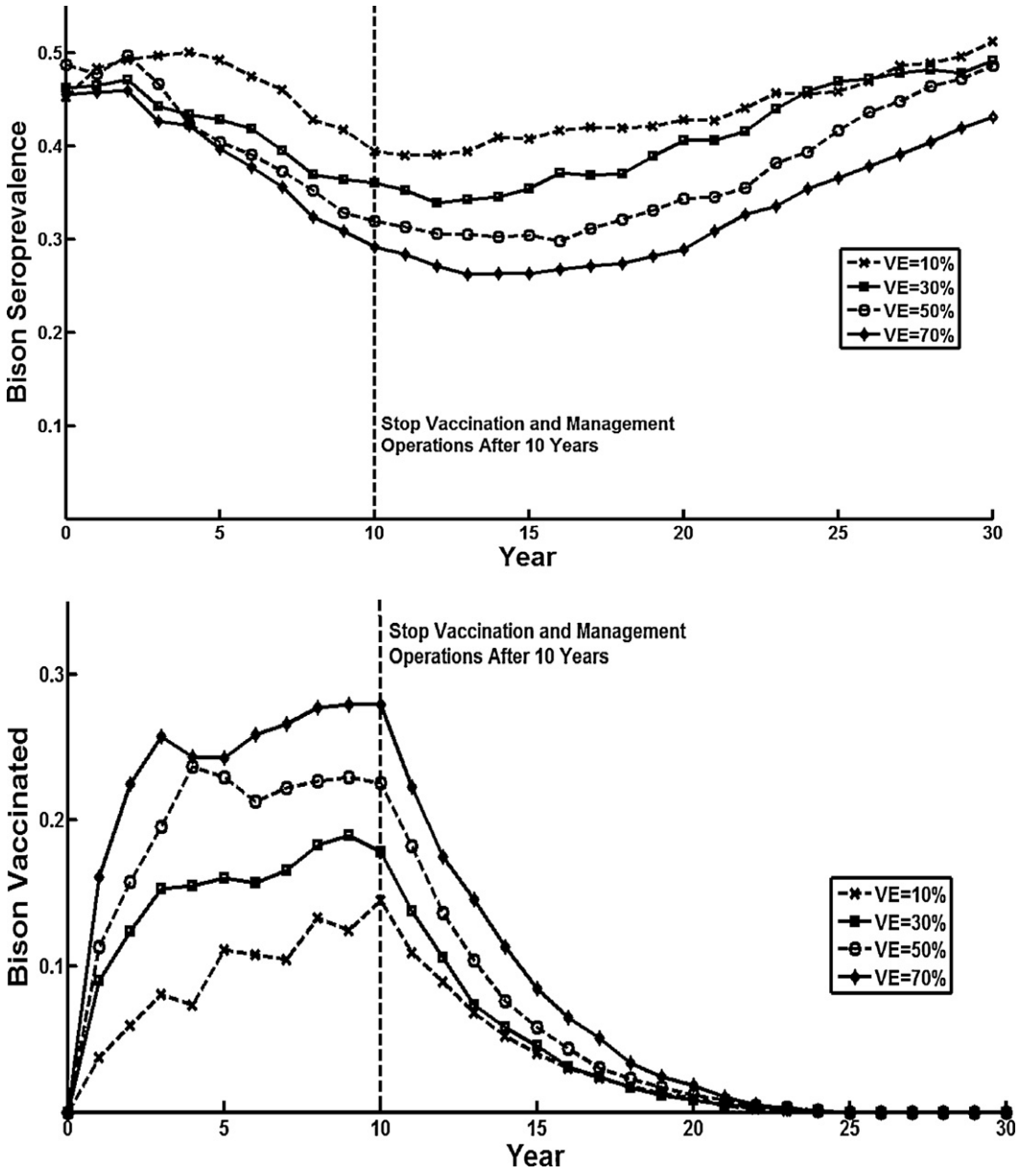

Fig. 6. Simulations of short-term (10 years) vaccination and boundary management for Alternative 3. Line markers correspond to the level of vaccine efficacy influencing seroprevalence declines (panel A) and the proportion of the bison population vaccinated (panel B).

long-lived antibodies, and the removal of vaccinated, seropositive bison.

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

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

unable to compensate for vaccine efficacy if bison are difficult to vaccinate.

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

Conflict of Interest Statement

All authors declare they have no conflict of interest

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