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## EFFICACY OF SINGLE CALFHOOD VACCINATION OF ELK WITH *BRUCELLA ABORTUS* STRAIN 19

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**Abstract:** Brucellosis has been eradicated from cattle in the states of Wyoming, Montana, and Idaho, USA. However, free-ranging elk (*Cervus elaphus*) that use feedgrounds in the Greater Yellowstone Area (GYA) and bison (*Bison bison*) in Yellowstone and Grand Teton national parks still have high seroprevalence to the disease and have caused loss of brucellosis-free status in Wyoming. Management tools to control or eliminate the disease are limited; however, wildlife vaccination is among the methods currently used by wildlife managers in Wyoming. We conducted a controlled challenge study of single calfhood vaccination. Elk calves, caught in January and February of 1999 and 2000 and acclimated to captivity for 3 weeks, were randomly assigned to control or vaccinate groups. The vaccinate groups received *Brucella abortus* vaccine strain 19 (S19) by hand-delivered intramuscular injection. Calves were raised to adulthood and bred at either 2.5 or 3.5 years of age for 2000 and 1999 captures, respectively. Eighty-nine (44 controls, 45 vaccinates) pregnant elk entered the challenge portion of the study. We challenged elk at mid-gestation with pathogenic *B. abortus* strain 2308 by intraconjunctival instillation. Abortion occurred in significantly more ( $P = 0.002$ ) controls (42; 93%) than vaccinates (32; 71%), and vaccine protected 25% of the vaccinate group. We used *Brucella* culture of fetus/calf tissues to determine the efficacy of vaccination for preventing infection, and we found that the number of infected fetuses/calves did not differ between controls and vaccinates ( $P = 0.14$ ). Based on these data, single calfhood vaccination with S19 has low efficacy, will likely have only little to moderate effect on *Brucella* prevalence in elk, and is unlikely to eradicate the disease in wildlife of the GYA.

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**Key words:** abortion, *Brucella abortus*, *Cervus elaphus*, elk, Greater Yellowstone Area, infection, reproduction, vaccine efficacy.

Brucellosis in GYA bison and elk has been a source of controversy and a focus of the Greater Yellowstone Interagency Brucellosis Committee (GYIBC) for years. Brucellosis had been eradicated from cattle in Wyoming, Montana, and Idaho, and these states were classified as "brucellosis free" with regard to livestock. However, 2 different outbreaks in cattle during 2003 and 2004, linked to feedground elk, resulted in downgrading of Wyoming's brucellosis status to Class A on 24 February 2004 (U.S. Department of Agriculture 2004). Free-ranging elk that use feedgrounds in the GYA and bison in Yellowstone and Grand Teton National Parks still have high sero-

prevalence to the disease and are viewed as a threat to the state-federal cooperative national brucellosis eradication program. The GYIBC, representing the state and federal agencies involved in wildlife and livestock management in the 3 states, has committed to eventual elimination of brucellosis from wildlife. Management tools to control or eliminate the disease are limited; however, wildlife vaccination is among the methods currently employed.

The Wyoming Game and Fish Department has vaccinated >40,000 elk with *B. abortus* S19 vaccine (Kreeger et al. 2002). Earlier studies of S19 efficacy (Herriges et al. 1989) suffered from inadequate controls, culling animals differentially from control and vaccinate groups, unknown causes of fetal losses, small sample sizes, combining results from disparate trials, and use of animals from a known infected herd. This has resulted in considerable controversy and debate regarding the effectiveness of S19 in elk. To address these concerns, we conducted a single-dose S19 calfhood vaccine efficacy study in elk.

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### METHODS

**Capture**  
We captured female calves from free-ranging (n = 25) and Montana (n = 54) in 2000. Captures (n = 1999 (Idaho), 15-1 tana), and 16-18 Febr included land owned by heering and Environme mately 65 km northwe and both private and p 20 km northeast of D. had not previously bee tions by the respective

We captured elk by Each animal was blindfo in a heavy rubberized the staging area. At the tagged with 2 unique weighed using a tripc assigned a unique iden ear-tag number). We c by jugular venipunctu tum with lubricated, ported elk to the Idah Game Wildlife Health Idaho, and released th (4 pens total), design: vaccinate pen. We m: ment and age-segrega

### Monitoring, Handlin

Following capture, facility until March. W vaccine and sterile dil Company (Denver, Co vaccine chilled prior t procedures and recon vaccinate elk per mar 2 ml dose consisted c units (CFU) per dose per dose in 2000, bas pany titrations. We d ular hand injection gauge 3.81-cm need March 2000 while th a cattle chute. Elk w of-age at vaccination. by the same method We provided elk trace mineral salt, o the duration of the

## METHODS

### Capture

We captured female elk as 7- to 8-month-old calves from free-ranging populations in Idaho ( $n = 25$ ) and Montana ( $n = 27$ ) in 1999, and Montana ( $n = 54$ ) in 2000. Capture dates were 19–25 January 1999 (Idaho), 15–16 February 1999 (Montana), and 16–18 February 2000. Capture sites included land owned by the Idaho National Engineering and Environmental Laboratory approximately 65 km northwest of Idaho Falls, Idaho, and both private and public land approximately 30 km northeast of Dell, Montana. Brucellosis had not previously been found in these populations by the respective state wildlife agencies.

We captured elk by helicopter net gunning. Each animal was blindfolded, hobbled, and placed in a heavy rubberized bag for transport back to the staging area. At the staging area, elk were ear-tagged with 2 uniquely numbered metal tags, weighed using a tripod and spring scale, and assigned a unique identification number (the left ear-tag number). We collected samples of blood by jugular venipuncture and feces from the rectum with lubricated, gloved fingers. We transported elk to the Idaho Department of Fish and Game Wildlife Health Laboratory in Caldwell, Idaho, and released them into 2 pens each year (4 pens total), designated as either a control or vaccinate pen. We maintained the elk in treatment and age-segregated pens until challenge.

### Monitoring, Handling, and Sampling

Following capture, elk were acclimated to the facility until March. We procured lyophilized S19 vaccine and sterile diluent from Colorado Serum Company (Denver, Colorado, USA). We kept the vaccine chilled prior to use and during vaccination procedures and reconstituted vaccine as needed to vaccinate elk per manufacturer's directions. Each 2 ml dose consisted of  $4.42 \times 10^9$  colony-forming units (CFU) per dose in 1999 and  $8.58 \times 10^9$  CFU per dose in 2000, based on Colorado Serum Company titrations. We delivered vaccine by intramuscular hand injection with a 3-ml syringe and a 20-gauge 3.81-cm needle on 8 March 1999 and 9 March 2000 while the animals were restrained in a cattle chute. Elk were approximately 9 months-of-age at vaccination. Controls received 2 ml saline by the same method on the same days.

We provided elk with ad libitum alfalfa hay, trace mineral salt, oat supplement, and water for the duration of the project. Periodically, we han-

dled the elk in chutes to obtain blood samples and body weights, trim feet, and administer ectoparasite treatments. We handled animals from both vaccine treatment and control groups identically. We added a large, easily visible plastic ear tag with the animal's identification number to each ear during the project to assist in rapid remote visual identification. Thus, each animal had 4 numbered ear tags, unique to the individual, throughout the project. Lost tags were replaced as needed.

We blood-sampled elk captured in 1999 at capture, vaccination, and 1, 2, 6, 8, 12, 20, 22, and 27 months post-vaccination. Elk captured in 2000 were blood-sampled at capture, vaccination, and 1, 2, 5, 12, and 15 months post-vaccination. Blood was allowed to clot at room temperature, centrifuged, and serum decanted into plastic cryovials. Serum was frozen, transported to Bozeman, Montana, and stored at  $-72^\circ\text{F}$  ( $-57.8^\circ\text{C}$ ) until processing within 3 days.

The Montana Department of Livestock veterinary diagnostic laboratory assayed serum for *Brucella* antibodies using card, standard plate, standard tube, rivanol, complement fixation, and buffered-acidified plate antigen tests (MacMillan 1990). We considered elk positive seroreactors if  $\geq 2$  tests were positive.

Eight adult elk bulls (6 commercial and 2 long-term captive wild-caught) were used for breeding from August 2001 to November 2001. Experimental elk were placed in small homogeneous (age and treatment) groups ( $n = 12$ – $13$ ) with a bull for 2 months. Bulls were then rotated to a different group of cows for an additional 2 months. On 8–9 January 2002, we established preliminary pregnancy status by pregnancy-specific protein B (Huang et al. 2000) and transrectal ultrasound. We repeated both tests on the date of *Brucella* challenge, and only elk positive on both tests at challenge were considered pregnant and used in the study.

On 28 February 2002, we challenged all pregnant elk with pathogenic *Brucella abortus* strain 2308 by intraconjunctival instillation of  $1.0 \times 10^7$  CFU as described in Elzer et al. (1998), Cook et al. (2002), and Kreeger et al. (2002). To control for differential exposure to pathogenic *Brucella* during the abortion period, we randomly assigned elk to 3 pens following challenge with roughly 30 elk in each pen, equally distributed by age class and treatment group.

Following the first abortion in mid-March 2002, we monitored elk 7 days/week during daylight

hours. Each fetus was collected as soon as possible after delivery, labeled with date and identification number, double-bagged while in the pens, and placed in a freezer. If known, we included the mother's identity as part of the record of the abortion. Live calves were left in the pens with mothers for at least 5 days before being euthanized by succinylcholine-xylazine remote immobilization followed immediately by intracardiac injection of sodium pentobarbital. Calves that were apparently healthy at the end of 5 days were considered "viable calves." Weak calves that died before the 5-day minimum viable period and still-born full-term calves were included with aborted fetuses as "aborted calves."

### Bacteriology

We shipped frozen and intact aborted calves in biocontainment packages to Louisiana State University (LSU) for bacteriological processing under biolevel 3 security and Centers for Disease Control/Department of Transportation regulations (Code of Federal Regulations, Titles 42 and 49). Viable calves were euthanized in Idaho and tissues (lung, liver, spleen, abomasal fluid, and mesenteric and iliac lymph nodes) were aseptically removed, individually frozen, double-bagged, and shipped to LSU for *Brucella* culture with aborted calves.

At LSU, all tissue samples were thawed and homogenized in a sterile saline (0.9% NaCl) solution and plated onto Farrell's selective medium containing 5% bovine blood (Farrell 1974), which inhibits the growth of non-brucellae organisms. Abomasal samples were plated directly onto Farrell's media. The plates were incubated for 14 days at 37 °C in a 5% CO<sub>2</sub> atmosphere. The limit of detection in the laboratory was 13 CFU/gm or ml.

Potential *B. abortus* isolates were identified on the basis of urease and oxidase reactions, colony morphology, growth rate, and Gram-stain reaction. Representative samples were subjected to dye sensitivity to assure they were *B. abortus* (Alton et al. 1988). To further characterize the *B. abortus* strains used in this study, the brucellae were plated on erythritol containing media to differentiate between vaccine and challenge strains. Strain 19 is sensitive to erythritol, and strain 2308 is resistant.

### Maternity Determination

We mixed control and vaccinate cows after challenge so that both groups had exactly the same exposure to the *Brucella* pathogen. This design required intensive monitoring of the elk during the abortion period. We made multiple daily visits

to pens to observe and identify elk. Aborted fetuses were collected as soon as we were confident of maternal identity or when we decided that we could not determine maternity. Healthy live calves were captured by hand, ear-tagged, and painted with unique markings to ensure identification. We used the following criteria for conclusive assignment of maternity to an aborted or viable calf:

**Abortions.**—(1) Direct observation of cow aborting a fetus, or (2) finding a cow with fresh retained placenta with an aborted fetus in the pen. In the rare occurrence of multiple abortions and multiple cows with placentas at first morning observation, the fetuses were identified as belonging to either cow.

**Viable Births.**—(1) Direct observation of cow delivering a live calf, or (2) consistent daily observation over the 5-day viability test of all maternal behaviors by 1 specific cow. Maternal behaviors included nursing, leading, grooming, and bedding calf away from the herd. Observation of inconsistent behaviors or less than the full complement of maternal behaviors was considered inadequate evidence of maternity.

### Determining Vaccine Efficacy

The ability of a vaccine to protect against unwanted pathogen effects is a measure of efficacy. With brucellosis, the most important unwanted effect is abortion because this is the primary means for disseminating the pathogen in the environment and transmitting the disease to other animals. However, *Brucella* vaccines that inhibit colonization and replication in the host (infection) can benefit host populations and disease eradication efforts; we therefore included a calculation on the efficacy of the vaccine at preventing infection. We first analyzed the data for significant vaccine effect. If we found significant vaccine effect, we calculated the magnitude of that effect (referred to simply as "efficacy").

We used a 1-tailed Fisher's exact test to compare abortion and infection rates in control and vaccinated elk based on the assumption that vaccination would either decrease abortion/infection rates or have no effect. This statistic was used to determine whether the vaccine had produced a significant reduction in abortion or infection.

We calculated efficacy against abortion as the proportion of viable calves born to vaccinated elk that would otherwise have been expected to abort in absence of vaccine protection. The production of viable calves in the control group was an estimate of viable calf production that would have occurred in the vaccine group if no protec-

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tion had been afforded calves protected from abortion that were viable in proportion that were expected abortions, were the proportion

$$E (\text{efficacy}) = \left[ \frac{(\% \text{ viable in controls})}{(\% \text{ viable in controls})} \right]$$

Calculating efficacy ("ventable fraction") was the successful calving in vaccine estimate of true (the proportion of can grossly underestimate controls demonstrate 100% of vaccine effect, a subtraction of 50%. Using the vaccine efficacy was example. That is, of the population, the

### RESULTS

All elk were seronegative at capture and on month after vaccination seroconverted in prevalence decline challenge, no vaccine seropositive. control elk remained negative for the duration of the study until challenged. One month following challenge, of control and vaccinated elk had seroconverted response to *B. abortus* strain 2308.

Eighty-nine elk (control and 45 vaccinated) 3-yr-old and 42 were successful and entered challenge portion of study. Abortions occurred during March (April ( $n = 22$ ), May 30), and June (2002). All viable

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... had been afforded by vaccination. Thus, calves protected from abortion were the proportion that were viable in the vaccinates minus the proportion that were viable in the controls. Total proportion that were viable in the controls, expected abortions, in absence of protection, were the proportion of abortions in controls:

$$E(\text{efficacy}) = [(\% \text{ viable in vaccinates}) - (\% \text{ viable in controls})] / (\% \text{ abortions in controls}).$$

Calculating efficacy (also known as "preventable fraction") was preferable to subtracting the successful calving in controls from the successful calving in vaccinates because it gave a better estimate of true vaccine effect. Subtraction (the proportion of the study sample protected) can grossly underestimate vaccine effect. For example, during an experiment in which 50% of controls demonstrated no disease effect and 100% of vaccinates demonstrated no disease effect, a subtraction would suggest vaccine efficacy of 50%. Using the calculations outlined above, vaccine efficacy would measure 100% in this example. That is, of the 50% expected disease in the population, the vaccine protected 100%.

RESULTS

All elk were seronegative for *Brucella* antibodies at capture and on the day of vaccination. One month after vaccination, 100% of vaccinates had seroconverted in response to S19. *Brucella*-positive prevalence declined over time (Fig. 1) so that by challenge, no vaccinates were seropositive. Control elk remained seronegative for the duration of the study until challenged. One month following challenge, 100% of control and vaccinate elk had seroconverted in response to *B. abortus* strain 2308.

Eighty-nine elk (44 control and 45 vaccinate; 47 3-yr-old and 42 2-yr-old) were successfully bred and entered the challenge portion of the project. Abortions occurred during March (n = 2), April (n = 22), May (n = 30), and June (n = 21) 2002. All viable births

occurred in May (n = 11) and June (n = 4). We collected 90 aborted or viable calves from 89 adult elk. We observed Cow #156 delivering 2 fetuses. We found no difference in abortion rates among the 3 pens housing challenged elk (chi-square, P = 0.467) or between the 2 age classes (Fisher's exact test, P = 0.778).

With no pen or age effects, we combined results from all pens and age groups to determine the effect of treatment (vaccination). Vaccinates produced significantly more viable calves (13 of 45 = 29%) compared to controls (2 of 44 = 5%; Fisher's exact test, P = 0.002). Because vaccination provided a significantly increased chance of producing a viable calf, we calculated the magnitude of vaccine protection. Vaccine efficacy at producing viable calves (preventing abortion) in our study was 25%.

Bacterial culture for *Brucella* was negative in 15 fetuses/calves, including 8 vaccinates, 5 controls, and 2 of unknown maternity. The remaining 75 fetuses/calves were culture positive for *Brucella*. The maximum effect of vaccination for protection against infection would include the 2 culture negative unknowns as vaccinates. Under this assumption, vaccinate infection rate (35/45 = 78%) and control infection rate (39/44 = 89%) were not statistically different (P = 0.138).

DISCUSSION

In our controlled challenge experiment, we found that the vaccine produced low protection against abortion and no protection from infec-

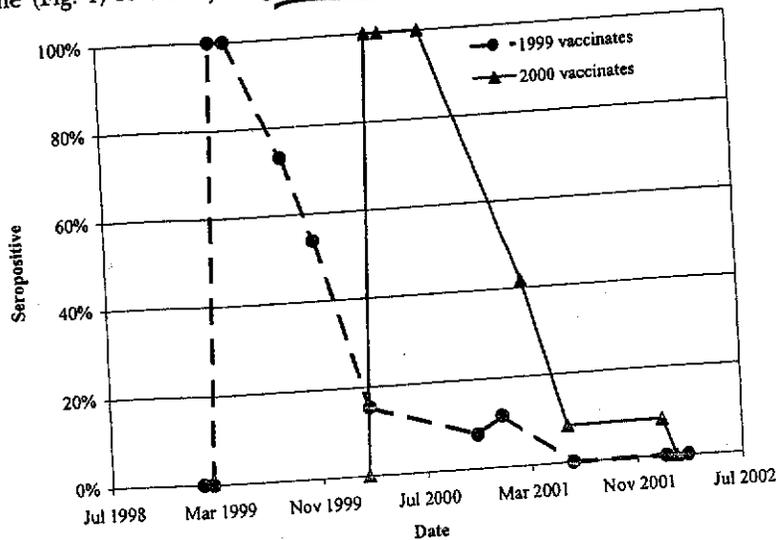


Fig. 1. *Brucella* serologic response of 2 groups of elk captured in the Greater Yellowstone Area, USA, and vaccinated with Strain 19 in a vaccine efficacy trial from capture, through vaccination and breeding, to date of challenge with pathogenic *B. abortus*.

tion. Our methods were similar to but controlled for more factors than those used by Kreeger et al. (2002) and Cook et al. (2002), wherein *Brucella* vaccine RB51 was considered to be ineffective at protecting elk from abortion. As recently as 2002, S19 vaccine was purported to be about 60% effective in preventing abortion in elk vaccinated as calves (Cook et al. 2002). Misperceptions about the efficacy of *Brucella* vaccines may be rooted in inappropriate inferences from abortion or survival rates, numbers, comparisons, or poorly designed experiments.

The most important question about controlled challenge vaccine studies is how realistic they are to wildlife management. Kreeger et al. (2002) summarized the reasons why RB51 may have failed to provide protection against abortion. Our assessment parallels their findings in that we do not think insufficient vaccine, excessive challenge, or route of vaccination were the causes for the poor protection provided by S19. The concern about excessive challenge in these experiments is valid. Studies in cattle have demonstrated a marked effect of challenge dose on *Brucella* vaccine efficacy (summary in Nicoletti 1990). However, Nicoletti (1990) also stated a common acceptance of 90% efficacy to be effective for management. Under experimental conditions similar to ours, S19 has been found 65–75% effective at preventing abortion in cattle (Adams 1990).

So what constitutes a realistic challenge for elk? Cook (1999) suggested that under natural conditions, most elk make only brief contact with an abortion then depart. He calculated that a 10-cm diameter area of skin contained about  $4.1 \times 10^6$  organisms and suggested that this amounted to a "realistic field exposure." If so, our experiment and those of others would have approximately doubled that number of bacteria in the challenge dose. How Cook's (1999) figure translates into elk on feedgrounds with feed contaminated by infected tissues and fluids is unknown, but likely under-represents true exposure. Single abortions on feedgrounds may expose many elk (Thorne et al. 1997), and individual elk could receive multiple exposures from  $\geq 1$  fetuses. Further, because aborted material typically has billions of *Brucella* organisms per gram of tissue (Enright 1990), real world challenge doses for exposed animals could easily and realistically be higher than our experimental challenge dose. Alexander et al. (1981) reported  $10^9$  to  $10^{13}$  CFU from a single gram of tissue or milliliter of fetal fluid from 2 naturally infected bovine cows.

The goal of a controlled experiment is to use realistic, but uniform, challenge and induce disease in most of the controls so as not to overwhelm the immune system. This provides the best comparison between control and vaccinated groups. A poor response in the control group effectively decreases the sample size in the experiment. Because our control animals produced 5% viable calves, we do not consider the pathogen challenge to be excessive.

Environmental conditions also may affect the outcome of experiments and the applicability to management situations. Nicoletti (1990) suggested that duration of immunity under some field conditions may not be as long as that in controlled environments. We believe that our measured efficacy is likely to be the maximum derived from field application of a parenteral vaccination program to management problems using current technology. Our vaccine was a fresh lot obtained directly from the manufacturer, reconstituted on-site, and delivered by hand via intramuscular injection without trauma. This methodology prevented the use of poor potency vaccine and ensured 100% vaccination success. Remote delivery in the field would likely be <100% effective. Olsen et al. (2002) reported that ballistic delivery of *Brucella* vaccine produced a poorer cell-mediated immunity (CMI) response in bison as compared to hand injection. Elk may respond similarly. Cell-mediated immunity is the part of the immune response essential for protection against *Brucella* infection (Nicoletti and Winter 1990). Elk in our study were also provided ad libitum feed, nutritional supplementation, and shelter—nutritional and stress conditions not likely realized by free-ranging elk, including those on feedgrounds. Our elk entered captivity as calves, and their behavior, growth, and reproduction suggested they adapted reasonably well to confinement. We conclude that the observed efficacy of 25% is likely a maximum for real-world application of a single calffood dose of S19 in elk.

Models are frequently used to predict outcomes (costs and/or benefits) of management actions, but models are highly dependent upon model construct, estimated parameters, and inputs. Peterson et al. (1991), modeling brucellosis in Jackson, Wyoming, bison, found that low vaccination efficacy had little impact on brucellosis prevalence. Brucellosis prevalence was predicted to stabilize after declining 23% (from 61 to 47%) with a 24% efficacious vaccine with 20 years of vaccinating.

While Peterson et al. (1991) suggested that it may be affected elk herds which co-mingles with other elk herds. The model also had a parameter for an outside source of infection. Peterson et al. (1998) modeled elk under a variety of challenge scenarios. In one scenario, a vaccine was administered to calves or just calves. The model predicted a reduction in prevalence by 40–50% with 20 years of vaccinations, such as that might be achieved. Peterson et al. (1998) argued that vaccination was necessary to significantly reduce brucellosis seroprevalence in a herd of S19 since 1985, in prevalence (50–59%) for

In the United States, for several decades, but the prevalence of brucellosis in elk. Although uncommon in bison, brucellosis is a variety of species (Thorne et al. 1997). In humans and possibly in elk, the vaccine (Nicoletti 1990) results in positive surveillance test results. It is difficult to accurately measure prevalence using serological tests that positive—by 2 years after vaccination.

Aside from animal health costs are a life vaccination program. The logistics, habitus, and a therapeutic approach to brucellosis. This last item is a wildlife management tool useful in many situations. It is highly adaptable to long-term vaccination programs to eventually manage brucellosis.

While Peterson et al.'s (1991) model was for bison, it may be applicable to some brucellosis-affected elk herds, such as the Jackson herd, which co-mingles with the Jackson bison herd. The model also has the advantage of incorporating a parameter mimicking constant exposure to an outside source of brucellosis, such as the Jackson elk herd faces from infected bison. Gross et al. (1998) modeled brucellosis prevalence in elk under a variety of conditions. Over a 100-year time-frame, a vaccine with a 25% efficacy did not eliminate brucellosis regardless of whether cows and calves or just calves were vaccinated. However, the model predicted a reduction in brucellosis prevalence by 40-50%. Combined with other treatments, such as test and slaughter, eradication might be achieved in 20-30 years. Others have argued that vaccination alone will simply not eliminate brucellosis, and other controls will be necessary to significantly reduce prevalence (Fairright and Nicoletti 1997). Current data on brucellosis seroprevalence at the Gray's River feed-ground, a herd of elk that has been vaccinated with S19 since 1985, indicate increasing and high prevalence (50-59%) for the last 4 years (Scurlock 2004).

In the United States, S19 has been used for decades, but the vaccine has some biological costs. Although uncommon, post-vaccinal orchitis, anaphylactic reactions, endotoxic shock, anorexia, abortion, and arthritis have been reported in a variety of species in response to S19 vaccination (Thorne et al. 1981, Adams 1990, Nicoletti 1990, Davis et al. 1991). Strain 19 also is pathogenic in humans and poses a risk to those administering the vaccine (Nicoletti 1990). In addition, S19 results in positive *Brucella* serology on standard surveillance tests and may interfere with the ability to accurately estimate effects on disease prevalence using serologic methods. Our data show that positive—but low—titers may occur up to 2 years after vaccination.

Aside from animal health issues, other significant costs are associated with conducting a wildlife vaccination program, including expense, logistics, habituation of wildlife, and focusing on a therapeutic—rather than an ecological—approach to solving wildlife disease problems. This last item is perhaps the biggest dilemma for wildlife management. Clinical approaches, while useful in many field situations, are not universally adaptable to free-ranging wildlife. Even if long-term vaccination was part of a successful program to eventually eliminate brucellosis, this type of management could contribute to maintenance

or spread of other diseases. Persisting with this management paradigm could severely hamper our ability to respond to new wildlife disease incursions, especially those for which effective vaccines are nonexistent.

## MANAGEMENT IMPLICATIONS

Vaccination of wildlife is an expensive and logistically difficult operation to conduct. Our results will be used by wildlife management agencies to determine whether the benefits of such operations are worth the costs incurred. Our data suggest that a single calftooth vaccination of elk with S19 produces a very low level of immunity in vaccinated elk. The low level of immunity provided is, in our opinion, highly unlikely to lead to significant reduction or eradication of brucellosis from feedground elk, and we cannot recommend its use to wildlife managers. Further, the operational programs required to vaccinate feedground elk perpetuate management paradigms that exacerbate brucellosis and other wildlife diseases (Dunkley and Cattet 2003). To the degree that vaccination is used as a justification for those management actions, use of vaccination is counterproductive to the elimination of brucellosis. Management alternatives that are at least ecologically neutral (i.e., do not enhance disease transmission) should be sought.

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EFFECTS OF ELK I  
PROCESSES

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**Abstract:** We used 35-year and 4-year above-ground and below-ground biomass (AGB) data from a 35-year study in short and tall willow (*Salix* spp.) communities to estimate nitrogen (N) fluxes (litter decomposition, soil N availability, and herbaceous, shrub and root N) and seasonal movement of N by elk in a willow community ( $P = 0.07$ ) and tall willow sites. Elk N removal in 35-year exclosures, and 66% reduction in N yield of willow vegetation associations and for mixed conifer, mesic meadow sites. Elk herbivory and total time in willows than mesic sites. We recommend management of exclosures, as negative effects from

**Key words:** *Cervus elaphus*, Coexistence, production, Rocky Mountain

Elk have become increasingly abundant in the Rocky Mountain ecosystem of the United States and Canada since the 1940s. Large predators, disruptive hunting routes, and the creation of towns and developed areas have reduced elk numbers in some areas. In the RMNP, elk were extirpated in 1911 and reintroduced to the area in 1947. The population of elk steadily grew to 1944 to 1996. By 1993, concerns over elk management policies and the potential overabundance of elk resulted in criticism of elk management policies and the creation of interagency elk

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