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SAFETY OF REVACCINATION OF PREGNANT BISON WITH *BRUCELLA ABORTUS* STRAIN RB51

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ABSTRACT: From December 1998 through February 1999, a study was conducted in a *Brucella*-infected bison herd to evaluate the safety of booster vaccination of adult bison (*Bison bison*) with 6×10^9 colony forming units (CFU) of *Brucella abortus* strain RB51 (SRB51) that had previously been vaccinated as yearlings with 1×10^{10} CFU of SRB51. Abortions or other adverse effects were not observed after SRB51 booster vaccination. At 10 wk after adult vaccination, pregnant and nonpregnant bison ($n=65$) were randomly selected for bacteriologic sampling of targeted maternal tissues during abattoir processing. Fetal tissues were also sampled in pregnant bison. The SRB51 vaccine was recovered from tissue samples of eight of 48 pregnant bison and none of 17 nonpregnant bison. In three of the eight culture-positive bison, SRB51 was recovered from fetal tissues. In three additional bison, one pregnant and two nonpregnant, *B. abortus* biovar 1 field strain was recovered from internal iliac or supramammary lymphatic tissues. Results of this study suggest the possibility that the SRB51 vaccine can be safely used to booster vaccinate pregnant bison in a *Brucella*-infected bison herd. Our data also reaffirms the potential for *B. abortus* field strains to persist in bison until attainment of reproductive age, despite extensive use of vaccination and serologic testing.

Key words: Bison, *Brucella abortus,* brucellosis, strain RB51, vaccine.

INTRODUCTION

The prevalence of brucellosis in free-ranging bison (*Bison bison*) and elk (*Cervus elaphus nelsoni*) in Yellowstone National Park (Wyoming, Montana, and Idaho, USA) and surrounding areas has been well documented (Thorne et al., 1978; Pac et al., 1991; Rhyan et al., 2001). Following completion of the brucellosis eradication program for cattle, bison and elk in the Greater Yellowstone Area will be the sole remaining reservoirs of *Brucella abortus* in the U.S. Although heavily influenced by winter conditions, mean bison populations within Yellowstone National Park between 1989 and 2000 were estimated at 3,058±192 (Anon., 2000). Based on estimates within the park and demographics of bison exiting the park, this report estimates that females compose approximately 50% of the population, and 70% of the bison are adults (≥3 yr of age or older) prior to spring calving. Therefore, the bison herd in Yellowstone National Park averages 900–1,100 adult females. Although the life span of most bison in Yellowstone National Park is probably 12–15 yr, some may reach the age of 20 (Anon., 2000). In a study of bison killed when they exited the park during the winter of 1988–89, 43% of females tested positive or suspect for exposure to *B. abortus,* with the oldest female estimated to be approximately 15 yr of age (Pac et al., 1991).

At the present time, *B. abortus* strain RB51 (SRB51) vaccine is being considered for use in a brucellosis management plan for free-ranging bison in Yellowstone National Park. Calfhood vaccination of bison heifers with 10^{10} colony forming units (CFU) of SRB51 reduces the incidence of abortion, fetal infection, or maternal mammary gland infection when compared to nonvaccinated bison (Olsen et al., 2003). Because brucellosis is transmitted laterally through fluids associated with birth or abortion of an infected fetus, or vertically to the calf through the ingestion of milk containing *B. abortus,* currently available data suggest that calfhood vaccination with SRB51 will prevent transmission of brucellosis in bison (Olsen et al., 2003). How-

ever, the length of time that a single SRB51 vaccination will protect bison is currently unknown.

Although vaccination of cattle against brucellosis has typically only included heifer calves of less than 12 mo of age, factors such as duration of vaccine immunity, environmental factors such as topography and weather conditions, animal behavior, and policy issues may require that a brucellosis management program for free-ranging bison include vaccination of bison that are greater than 1 yr of age. For instance, calf and yearling populations may need to be targeted to maximize the percent of immature bison receiving brucellosis vaccination. Some female bison may survive for more than 20 yr within Yellowstone National Park. These older bison may require booster vaccination if protective immunity induced by calfhood vaccination with SRB51 diminishes. A previous study found that naive pregnant bison are susceptible to abortion or fetal infection after subcutaneous vaccination with 10^9 CFU of SRB51 (Palmer et al., 1996), whereas another study (Elzer et al., 1998) found that vaccination with 10^9 CFU of SRB51 was safe in pregnant bison from a herd chronically infected with *B. abortus.* The purpose of the study reported here was to evaluate persistence and safety of booster vaccination of pregnant bison which had previously been vaccinated as calves with SRB51.

MATERIALS AND METHODS

This study was conducted at a privately owned ranch in South Dakota (USA; 44°41′N, 100°48′W) and the National Animal Disease Center in Ames, Iowa (USA; 42°52′N, 93°63′W) from August 1997 through February 1999.

Brucella abortus culture

A master seed stock of *B. abortus* strain RB51 (SRB51) was obtained from Dr. Gerhardt Schurig (Virginia Tech, Blacksburg, Virginia, USA). For experimental use, SRB51 bacteria were grown on tryptose agar (Difco Laboratories, Detroit, Michigan, USA) for 48 hr at 37 C. For the dot-blot assay, SRB51 suspensions (1.3×10^{12} CFU/ml) were inactivated by γ-irradiation (1.4×10^6 rads). Following irradiation, suspensions were washed in 0.15 M sodium chloride (saline) and stored in 1 ml aliquots at −70 C.

For vaccination of bison, a commercially prepared SRB51 product (Colorado Serum Company, Denver, Colorado, USA) was prepared according to the product literature. The vaccine was then diluted in saline based upon standard plate counts on other vials with the same lot number. Following dilution, the inoculum was sent on ice to the National Animal Disease Center via overnight shipping, and the concentration of viable bacteria within the inoculum was determined by standard plate counts.

Animals and inoculation

Bison in this experiment were from a privately owned herd in South Dakota that was under quarantine for brucellosis from 1982–2000. Serologic testing and SRB51 vaccination were being used as part of a herd plan for elimination of brucellosis.

In August of 1997, 496 bison heifers of approximately 12–15 mo old, were vaccinated subcutaneously in the cervical region with 1.08×10^{10} CFU of SRB51 suspended in 2 ml of saline. In December 1998, 68 wk after initial vaccination with SRB51, all remaining bison from this group (n=362) were subcutaneously booster vaccinated with 6×10^9 CFU of SRB51. Rectal palpation indicated that 72 (19.8%) were not pregnant at the time of booster vaccination. For most bison in this group, vaccination occurred during their first pregnancy, at approximately 3–5 mo of gestation.

Serologic evaluation

Serologic testing by regulatory personnel identified 63 females (13%) as reactors in August 1997 for subsequent removal from the herd. In September 1997, and April, July, August, and December 1998, an additional 39 animals were removed from this group of heifers by regulatory personnel due to positive responses on brucellosis surveillance tests (20, 8, 5, 5, and 1, respectively). Serologic testing by regulatory personnel at 6 wk after booster vaccination identified two additional bison with positive responses on brucellosis surveillance tests.

For research purposes, blood samples were collected by jugular venipuncture at the time of booster vaccination in 1998 and 6 wk later in January 1999. Blood was allowed to clot, was centrifuged, and serum shipped to the National Animal Disease Center for serologic evaluation. Upon arrival, serum was stored at −70 C until

evaluated. Serologic titers of animals to *Brucella* were determined by a previously described antibody dot-blot assay in which γ-irradiated SRB51 is used as antigen (Olsen et al., 1997).

Bacteriologic evaluation

In February of 1999, 10 wk after booster vaccination with SRB51, heifers were depopulated as part of a brucellosis herd management plan. Sixty-five heifers were randomly selected for collection of bacteriologic samples during processing at an abattoir. In pregnant and nonpregnant bison, samples of uterus, supramammary lymph node, and internal iliac lymph node were obtained for bacteriologic evaluation. In pregnant animals, samples of fetal liver, fetal spleen, and gastric contents were also obtained. Tissues selected for bacteriologic sampling were based on availability during abattoir processing and tissue localization data from a previous study of a bison herd chronically infected with *B. abortus* (Rhyan et al., 2001).

Samples were packed in dry ice, shipped to the National Animal Disease Center, and held at −70 C until processed. For bacteriologic evaluation, tissue samples were weighed, triturated using a tissue grinder, serially diluted in saline, and placed on tryptose agar plates containing 5% bovine serum (TSA), Kuzdas and Morse media (KM; Kuzdas and Morse, 1953), and plates containing a selective media for SRB51 (RBM; Hornsby et al., 2000). Antibiotics in RBM media minimize growth of contaminants without inhibiting growth of SRB51 and thereby enhance the ability to detect small numbers of SRB51 within samples. For fetal gastric content samples, fluid was plated directly onto TSA and RBM media. Following incubation of plates at 37 C and 5% CO_2 for 7 days, *Brucella* isolates were enumerated and identified on the basis of colony morphology and growth characteristics (Alton et al., 1988). In a similar manner, SRB51 isolates were identified by culture characteristics and resistance to rifampin (Schurig et al., 1991). Isolates were confirmed as *Brucella* using a polymerase chain reaction (PCR) procedure with primers specific for *B. abortus* omp2A (Lee et al., 2002) and SRB51 isolates were identified using a PCR procedure with SRB51-specific primers (Vemulapalli et al., 1999). Results were expressed as mean $\log_{10}$ CFU of *B. abortus* per g tissue ± SEM.

Statistical analysis

For statistical comparisons, dot-blot titers and colonization data (CFU/g) were analyzed as the logarithm of their value. Due to conversion to logarithm, titers or colonization data with a value of 0 were analyzed with a value of 1. Statistical differences between treatments were determined by an analysis of variance procedure (SAS Institute Inc., Cary, North Carolina, USA), and significant differences were reported when $P \leq 0.05$. Means for each treatment were compared by use of a least significant difference.

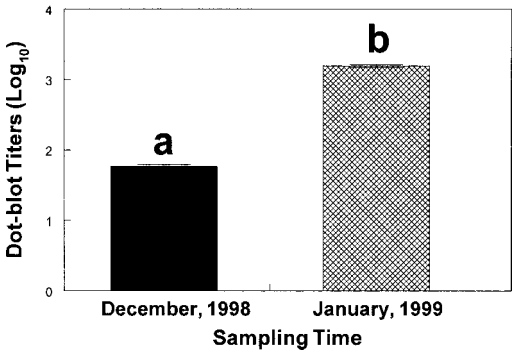

FIGURE 1. Serologic responses of bison before and after booster vaccination with 6×10^9 CFU SRB51 as adults to γ-irradiated SRB51 in a dot blot assay. Responses are presented as mean titer±SEM. Means with different superscripts are significantly different ($P<0.05$).

RESULTS

No abortions or adverse reactions were observed after booster vaccination. Serum samples were obtained from 353 female bison. Serum obtained from bison 6 wk after booster vaccination had a greater ($P<0.001$) dot-blot titer to SRB51 when compared to titers of paired samples obtained immediately prior to booster vaccination (Fig. 1).

Of the 65 bison randomly selected for sampling at the abattoir, 48 were pregnant. The SRB51 vaccine strain was recovered from maternal or fetal tissues obtained from eight pregnant bison, but was not recovered from any samples obtained from 17 nonpregnant bison. Strain RB51 was recovered from fetal tissues in three of the eight culture-positive bison. Tissues from which SRB51 were recovered included supramammary lymph node ($n=3$), placentome ($n=2$), internal iliac lymph node ($n=2$), fetal spleen ($n=3$), fetal liver ($n=2$), and fetal gastric contents ($n=1$).

Mean SRB51 colonization in tissue was 28.6±27.4 CFU/g.

In samples from three bison, one pregnant and two nonpregnant, *B. abortus* field strain was recovered from internal iliac ($n=2$) and supramammary ($n=2$) lymph nodes. Mean colonization of tissue was 710±277 CFU/g. Isolates were biotyped as *B. abortus* biovar 1 at the National Veterinary Services Laboratory.

DISCUSSION

Results of this study suggest bison that have been vaccinated with calfhood doses of SRB51 may be safely booster vaccinated with SRB51 during the first pregnancy. Although tissue sampling was limited in the current study, the SRB51 vaccine strain was recovered from only 17% of pregnant bison at 10 wk after booster vaccination. The tissues sampled in the present study were based on a previous study of bison naturally infected with virulent *B. abortus* in which *Brucella* was recovered from the supramammary and internal iliac lymph nodes of 83% and 58%, respectively, of culture-positive bison (Rhyan et al., 2001). It is apparent from our data that SRB51 is cleared more rapidly during anamnestic responses of adult bison as compared to clearance of SRB51 after calfhood vaccination. In bison calves vaccinated once with 10^{10} CFU, SRB51 was recovered from multiple lymphatic tissues of all bison ($n=4$ vaccinates/time) sampled at 14 and 18 wk after vaccination (Roffe et al., 1999). However, as compared to the study of bison infected under field conditions (Rhyan et al., 2001), the supramammary and internal iliac lymph nodes were not as predictive of culture status after calfhood vaccination. After calfhood vaccination, SRB51 was recovered from the supramammary lymph node of 50% and 75% of bison at 14 and 18 wk, respectively, whereas *B. abortus* was recovered from the supramammary lymph node of 83% of bison culture-positive for field strains of *B. abortus* (Rhyan et al., 2001). In addition, SRB51 was not recovered at either sampling time from the internal iliac lymph node of any calfhood vaccinated bison; whereas this lymph node was culture-positive in 58% of naturally infected bison. The possibility cannot be excluded that tissue localization after vaccination with SRB51 may differ from natural exposure to virulent field strains of *B. abortus*. Therefore, the possibility cannot be excluded that the limited tissues sampled in the present study may have underestimated the number of bison remaining colonized with SRB51 at 10 wk after booster vaccination.

A previous study found that naive pregnant bison ($n=10$) were susceptible to abortion or fetal infection after subcutaneous vaccination with 10^9 CFU of SRB51 (Palmer et al., 1996). However, six female bison from a *Brucella*-infected herd vaccinated during gestation with 1×10^9 CFU of SRB51 did not abort (Elzer et al., 1998). Although SRB51 was not recovered from tissues obtained from a calf that died from dystocia or the milk of its dam, bacteriologic samples were not obtained from cows or remaining calves to determine if SRB51 could be recovered at the time of parturition. In six non-pregnant females, SRB51 was not recovered from tissue samples obtained at necropsy 13 or 16 wk after vaccination with 1×10^9 CFU (Elzer et al., 1998). One explanation for the disparity between the two studies is that bison in a herd chronically infected with *Brucella* may develop immunologic responses that prevent adverse effects caused by SRB51 vaccination during pregnancy. The disparity between the results of the two studies suggested the need for additional data. As adult vaccination is a management procedure that might be used control brucellosis in a bison herd in the absence of test and removal procedures, it is important to determine if previous vaccination with SRB51 could prevent adverse effects associated with vaccination during pregnancy.

Although adverse effects, such as abortion, were not observed in this group of

bison, management of the bison after vaccination may have hindered observation of abortion. Also, estimates of stage of gestation suggest that some bison were processed at an abattoir before the third trimester, the time when *Brucella*-induced abortions are most likely to occur. If abortions did occur in the interval between vaccination and euthanasia, the small size of the fetus at 4–6 mo of gestation may also have hindered observation. However, the similarity between the estimated 81% pregnancy rate at the time of vaccination and the 74% pregnancy rate in bison randomly selected during abattoir processing, combined with the low incidence of SRB51 colonization of fetal tissues in sampled bison, suggests that significant numbers of abortions did not occur.

The time interval between initial and booster vaccinations was minimal in the current study and it is possible results may differ with greater time intervals between initial and booster vaccination. Abortions and fetal infections can occur following subcutaneous vaccination of 3–10 yr old bison with 10^9 CFU of SRB51 during pregnancy (Palmer et al., 1996). Data from reports using *B. abortus* strain 19 (S19) in pregnant cattle suggests that adverse effects of brucellosis vaccination may vary between studies. In one study of *Brucella*-infected cattle herds, subcutaneous vaccination between 1 and 9 mo of gestation with 1.2×10^7 CFU of S19 induced abortions in 14.3% of cattle (Moore et al., 1950). Others reported that 22% of pregnant cattle aborted after vaccination with 5.8×10^9 CFU of S19 (Corner and Alton, 1981). However, a large field study involving over 10,000 cattle reported less than 1% abortions after vaccination with approximately 1×10^{11} CFU of S19 (Nicoletti, 1976). Although calfhood vaccination status in two of the studies was not clarified, the herds included in the large field study routinely utilized calfhood vaccination with the standard dose of S19 as a brucellosis management tool.

Results of our study also reaffirm the potential for bison to remain latently infected with virulent *B. abortus* field strains until attainment of reproductive age. Although infection during calfhood remains the most likely source of the field strains isolated during this study, herd management of these calves does not eliminate the possibility that they were exposed to an abortion event after weaning. As management of this herd included extensive use of vaccination and serologic testing, the bacteriologic detection of field strains of *B. abortus* in three (5%) of 65 bison tested suggests the need for protracted testing of bison in *Brucella*-infected herds until after reproductive maturity, even in herds in which extensive brucellosis management practices are utilized.

Although additional studies will be required to further characterize the safety and efficacy of SRB51 booster vaccination of adult bison, lack of adverse effects and low incidence of fetal infection in the present study suggest consideration of SRB51 as a booster vaccine for pregnant bison in *Brucella*-infected herds. However, as with the use of any live vaccine in pregnant animals, thorough assessment of risks and potential benefits should be carefully evaluated prior to use.

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