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BRUCELLA ABORTUS STRAIN RB51 VACCINATION IN ELK II. FAILURE OF HIGH DOSAGE TO PREVENT ABORTION

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ABSTRACT: *Brucella abortus* strain RB51 is used as a vaccine because it induces antibodies that do not react on standard serologic tests for brucellosis allowing differentiation between vaccination and infection. Strain RB51 was evaluated in captive elk (*Cervus elaphus*) to determine if vaccination protected against abortion following experimental challenge. Thirty elk were vaccinated intramuscularly with 1.0×10^{10} colony-forming units (CFU) of strain RB51 in March 1998. Fourteen of these were given a booster dose of 1.13×10^{10} CFU exactly 1 yr later. All vaccinated elk seroconverted via a modified dot blot assay to strain RB51 with the booster group having higher titers ($P \leq 0.001$). Seventeen other elk served as unvaccinated controls. All elk were bred and determined pregnant using pregnancy-specific protein B analysis. Elk were challenged in March 2000 with 1.1×10^7 CFU of *B. abortus* strain 2308 administered intraconjunctivally and all elk seroconverted to strain 2308. Fifteen of 17 control elk aborted; 16 of 16 elk given a single vaccination aborted ($P = 0.44$); and 13 of 14 elk given a booster aborted ($P = 0.86$). There were two viable calves in the control group and one in the booster group. Strain 2308 was recovered from fetuses and nonviable calves in all groups. Based on the results of this and other studies, the use of strain RB51 to prevent abortion in elk cannot be recommended.

Key words: Abortion, *Brucella abortus*, *Cervus elaphus*, elk, strain RB51, vaccination.

INTRODUCTION

Brucellosis is a zoonotic disease that causes abortion in domestic cattle, elk (*Cervus elaphus*), and bison (*Bison bison*) (Hunter and Kreeger, 1998) and undulate fever in humans (Young and Nicoletti, 1997). State and federal agencies are in the final stages of eradicating brucellosis from cattle, but the disease persists in free-ranging elk and bison inhabiting the Greater Yellowstone Area (Cheville et al., 1998), an ecosystem comprising northeast Wyoming (including Yellowstone and Grand Teton National Parks) and adjacent parts of Montana and Idaho. Brucellosis in elk and bison not only poses a threat to the domestic cattle industry but it also conflicts with state agency mandates to maintain healthy wildlife populations.

Brucellosis vaccines are comprised of living, mutant *Brucella* organisms that infect the host, but are less pathogenic than virulent field strains, yet induce protective immune responses. Probably the most important criterion for assessing vaccine efficacy is its ability to prevent shedding of *Brucella* in aborted fetuses and associated fluids, the major mechanism of brucellosis transmission (Nicoletti, 1986).

The Wyoming Game and Fish Department (WGFD) has vaccinated >40,000 elk on feedgrounds with strain 19 vaccine (Herriges et al., 1989). Strain 19, however, induces antibodies to the lipopolysaccharide (LPS) O-side chain that are detected in most serologic tests used for brucellosis surveillance (Stevens et al., 1995). Thus, serologic differentiation between infection and vaccination cannot be made which makes assessment of a vaccination program difficult.

Strain RB51 is a laboratory-derived rough mutant of virulent *B. abortus* strain 2308. It lacks most of the antigenic LPS O-side chain (Schurig et al., 1991) and it does not induce antibodies that react in particle concentration fluorescence immunoassay, card, tube agglutination, or complement fixation tests (Stevens et al.,

1994). Because of these properties, strain RB51 may be preferable to strain 19 for diagnostic purposes.

Two strain RB51 dosage regimens have been developed for cattle: a reduced dose (10^9 colony forming units [CFU]) for adults (Olsen, 2000a) and a high dose (10^{10} CFU) for calves (Olsen, 2000b). Previously, elk calves were vaccinated one time with $\leq 10^9$ CFU of strain RB51, which failed to protect significantly against abortion (Cook et al., 2002). Two hypotheses were developed to explain this failure: (1) the initial vaccine dosage was insufficient to develop an adequate immune response or (2) strain RB51 parenteral vaccination in elk requires more than one dose to be efficacious.

Herein, we report on the efficacy in elk of (1) a single calfhood vaccination with $\geq 10^{10}$ CFU strain RB51 and (2) a booster vaccination with strain RB51 given 1 yr after the initial dose. The study was approved by the institutional animal care and use committee.

MATERIALS AND METHODS

This study was conducted from February 1998 to July 2000 at the WGFD's Sybille Wildlife Research and Conservation Education Unit (Sybille; Wheatland, Wyoming, USA; 41°45.778′N, 105°22.605′W). In January 1998, female elk calves were captured in corral traps at the National Elk Refuge (Jackson, Wyoming) and transported to Sybille. There, elk were housed in 0.4-ha corrals and fed alfalfa hay supplemented with a pelleted ration. Water and a trace mineral block were provided ad libitum.

Elk were examined monthly for *Brucella* antibodies using four standard serologic tests (MacMillan, 1990). Any elk found to be positive according to criteria established by Morton et al. (1981) was removed from the study. In March 1998, elk were divided randomly into three groups: single ($n = 16$), which received a single calfhood dose of strain RB51; booster ($n = 14$), which received a calfhood dose of strain RB51 and a second dose 1 yr after the initial dose; and control ($n = 17$), which received a volume of physiologic saline comparable to the vaccine volume.

On 20 March 1998, the single and booster groups were inoculated intramuscularly with 1.00×10^{10} CFU of strain RB51 vaccine (Colorado Serum Co., Denver, Colorado, USA) administered in the hindquarters. The vaccine dosage was determined by standard plate counts on Columbia agar with 5% sheep blood (Hardy Diagnostics, Santa Maria, California, USA). The control group was given sterile saline administered similarly. On 20 March 1999, the booster group was given 1.13×10^{10} CFU strain RB51. Elk were analyzed periodically for antibody response to strain RB51 via a dot blot assay modified for elk (Kreeger et al., 2000).

Beginning in September 1999, a bull elk was placed with each group for breeding. In January 2000, elk were examined for pregnancy using pregnancy-specific protein B assay (Huang et al., 2000). On 15 March 2000, elk were challenged via the conjunctival sac with 1.1×10^7 CFU of *B. abortus* strain 2308, an established virulent strain in cattle and elk (Elzer et al., 1998; Kreeger et al., 2000). The inoculum, prepared as described in Elzer et al. (1994) and diluted in sterile saline, was administered by dropping 50 μl in each conjunctival sac using a pipette. The challenge dose was verified by serial dilutions and plating as described above for the vaccine dose. Elk were bled periodically for serology. Antibodies to strain 2308 were detected using standard plate agglutination, standard tube, rivanol, and complement fixation tests based on criteria established by Morton et al. (1981).

Following challenge, elk were observed twice daily for abortion or other indications of reaction to the challenge. Calving was assisted if signs of labor exceeded 12 hr or if an abnormal presentation was observed. Aborted fetuses were collected immediately and frozen, then necropsied and cultured at later date. The primary culture medium consisted of *Brucella* agar (Difco Laboratories, Detroit, Michigan, USA) with the addition of 7,500 International Units (IU) bacitracin, 1,800 (IU) polymixin B, 30 mg cyclohexamide, and 0.000125% crystal violet per liter and Columbia agar with 5% sheep blood (CBA; Hardy Diagnostics). Culture swabs from the abomasal content were streaked across one quadrant of the plate, and plates were then streaked for isolation. Tissues were aseptically processed by slicing longitudinally, mincing the cut tissue surface. The minced surface was then smeared across one quadrant of a plate, and the plate streaked for isolation. All specimens were plated in duplicate on *Brucella* and CBA, inverted and incubated at 37 C under 10% CO_2, and atmospheric O_2 for a minimum of 7 days. Suspect bacterial colonies were removed and streaked for isolation on MacConkey agar plates (Hardy Diagnostics). Isolates that were unable to grow on MacConkey agar were identified using standard

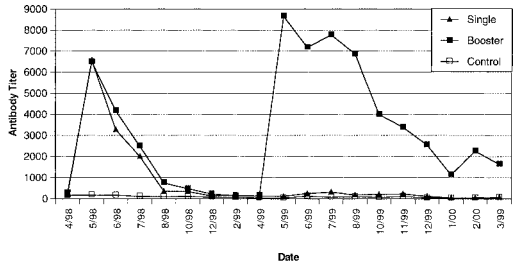

FIGURE 1. Mean dot blot antibody titers to *Brucella abortus* strain RB51 in elk vaccinated one time with 10^{10} colony forming units (CFU) strain RB51 (single), two times with 10^{10} CFU strain RB51 (booster), or with saline (control).

bacteriologic methods (Quinn et al., 1994) and *Brucella* species differentiated by carbon utilization panels (Biolog, Inc., Hayward, California).

The null hypothesis tested was that there was no difference in abortion rates between elk vaccinated with *B. abortus* strain RB51 and controls. For the purposes of this comparison, abortion was defined as delivery of a calf infected with strain 2308 or a fetus too contaminated or autolytic for culture. This included both pre-term, non-living fetuses as well as calves that survived for <7 days, but which succumbed to infection. Differences in abortion rates among single, booster, and control groups were compared by Fisher's exact test for two proportions at a significance level of $P \leq 0.05$. Antibody titers to strain RB51 vaccine were compared by one-way ANOVA at a significance level of $P \leq 0.05$. Means are reported with standard errors.

RESULTS

None of the elk seroconverted to field strain *Brucella* after capture. Sample sizes for single, booster, and control groups were 16, 14, and 17, respectively. All elk were pregnant. Strain RB51 antibody titers of vaccinated elk were higher than controls ($P \leq 0.001$) by 1 mo postvaccination and remained so through March 1999 (Fig. 1). The booster group had higher strain RB51 titers than either the single or control groups ($P \leq 0.001$) 1 mo after the second vaccination and they remained higher through challenge in March 2000 (Fig. 1). All elk seroconverted to strain 2308 2 wk postchallenge and had positive titers until conclusion of the study in July.

TABLE 1. Abortion and culture results in elk vaccinated one time with 10^{10} colony-forming units (CFU) strain RB51 (single), two times with 10^{10} CFU strain RB51 (booster), or with saline (control).

Group	*n*	Abortions	*P* value[a]	Fetuses cultured[b]	*Brucella* strain isolated
Single	16	16	0.44	14	2308
Booster	14	13	0.86	13	2308
Control	17	15		11	2308

[a] Values compared to control by Fisher's exact test.
[b] Not all fetuses cultured due to contamination or autolysis.

All elk in the single group aborted, including one nonviable calf. Thirteen of 14 elk in the booster group aborted, including four nonviable calves (Table 1). There was one viable calf in the booster group. Fifteen of 17 control elk aborted; two calves remained viable throughout the study period. There was no difference in abortion rates among the groups ($P \geq 0.44$). Strain 2308 was recovered from fetuses and nonviable calves in all groups (Table 1).

DISCUSSION

The pathogenesis of strain 2308 infection in elk vaccinated with strain RB51 has been previously described (Cook et al., 2002) and was not repeated for this study. Thus, we did not euthanize elk after the study in order to culture tissues and calculate indices of infection. The purpose of the study was to simply determine if a higher initial dose than used by Cook et al. (2002) or a booster dose would result in reduced abortion rates compared to unvaccinated elk. However, data from this study and Cook et al. (2002) suggest that parenteral vaccination with strain RB51 will not protect elk against abortion caused by infection with *B. abortus*.

In a third study, 1.03×10^{10} CFU strain RB51 also failed to reduce abortions in pregnant elk challenged with 9.8×10^{6} CFU strain 2308 40 days post vaccination (Kreeger et al., 2000). The failure of strain RB51 to reduce abortion rates in these three studies combine to cast doubt on its efficacy in elk.

The failure of strain RB51 to protect against abortion could be due to (1) insufficient vaccine; (2) excessive challenge dose; (3) route of vaccination; or (4) insufficient development of cell-mediated immunity (CMI). It is doubtful that the vaccine dose was insufficient. The dose used in this study was the same efficacious dose used in cattle calves (Cheville et al., 1996; Olsen, 2000b). Several elk had strain RB51 antibody titers >1:10,240 in both the single and booster groups indicating a strong humoral response to the vaccine. Although it cannot be excluded that higher dosages of strain RB51 may induce greater protection, the high antibody titers recorded in this study suggest robust stimulation of the immune system and make it unlikely that higher dosages would enhance protection.

Although high, we doubt that the challenge dose was excessive because the unvaccinated control group produced two viable calves. If the challenge dose was too high, we would have expected all of the control animals to have aborted. Reducing the challenge dose may have increased vaccine efficacy in elk, however. Studies with cattle have shown that reducing the challenge dose from 5.2×10^7 CFU strain 2308 to 9.4×10^6 CFU increased vaccine efficacy from 0–20% to 89–90% (Nicoletti, 1990). The challenge dose used in this study was chosen because it was consistent with other studies so that comparisons could be made (Manthei and Carter, 1950; Kreeger et al., 2000; Olsen, 2000b; Cook et al., 2002).

More importantly, the challenge dose may not represent a realistic exposure dose in the field. Cook et al. (2002) calculated that the number of *Brucella* bacteria on a piece of aborted elk fetal skin 10 cm in diameter contained approximately 4.1×10^6 CFU. His observations also indicated that elk made only brief contact with an aborted fetus and likely were exposed to even fewer bacteria. Thus, the challenge dose was almost a log higher than a probable field exposure dose.

The route of vaccination with strain RB51 may be a factor. Although parenteral vaccination of elk with strain RB51 has essentially failed to provide protection against abortion, oral vaccination of elk appeared to provide some protection. Elzer and Davis (1997) orally vaccinated elk with 10^{10} CFU strain RB51. Twelve of 15 controls and five of nine vaccinates ($P = 0.42$) aborted according to the criterion used in this study. Although not significant, elk orally vaccinated with strain RB51 numerically had more viable calves than this or other studies where strain RB51 was administered parenterally. Further work needs to be done to evaluate vaccine efficacy relative to routes of vaccination.

Currently, we believe that the failure of strain RB51 to protect against abortion in elk was most likely due to insufficient development of CMI. Protection from infection and elimination of *B. abortus* most likely requires CMI (Nicoletti and Winter, 1990). Our data indicate a strong humoral response to the strain RB51 vaccine (Fig. 1) confirming antigenic recognition. We are also confident that sufficient time (>51 wk) had elapsed between vaccination and challenge to allow CMI to develop. In cattle, it took 10 to 12 wk after vaccination with strain RB51 for lymph node cells to proliferate when incubated with strain 2308 (Stevens et al., 1995; Olsen, 2000b).

Based on the results of this study combined with those of Cook et al. (2002) and Kreeger et al. (2000), we cannot recommend the parenteral use of strain RB51 vaccine to reduce abortions in elk. Further work should be conducted to elucidate CMI response to *Brucella* in elk.

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