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Source: Journal of Wildlife Diseases, 38(1) : 18-26

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-38.1.18

Journal of Wildlife Diseases, 38(1), 2002, pp. 18–26

BRUCELLA ABORTUS STRAIN RB51 VACCINATION IN ELK I. EFFICACY OF REDUCED DOSAGE

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ABSTRACT: Bovine brucellosis is a serious zoonotic disease affecting some populations of Rocky Mountain elk (*Cervus elaphus nelsoni*) and bison (*Bison bison*) in the Greater Yellowstone Area, USA. The fear that elk and/or bison may spread *Brucella abortus* to livestock has prompted efforts to reduce or eliminate the disease in wildlife. *Brucella abortus* strain RB51 (RB51) vaccine has recently been approved for use in cattle. Unlike strain 19 vaccine, RB51 does not cause false positive reactions on standard brucellosis serologic tests. If effective, it may become the vaccine of choice for wildlife. In February 1995, 45 serologically negative female elk calves were trapped and taken to the Sybille Wildlife Research and Conservation Education Unit near Wheatland, Wyoming, USA. In May 1995, 16 of these elk calves were hand-vaccinated with 1×10^9 colony forming units (CFU) of RB51, 16 were vaccinated with 1×10^8 CFU RB51 by biobullet, and 13 were given a saline placebo. The elk were bred in fall of 1996 and they were challenged with 1×10^7 CFU of *B. abortus* strain 2308 by intraconjunctival inoculation in March 1997. Thirteen (100%) control elk aborted, 14 (88%) hand-vaccinated elk aborted, and 12 (75%) biobullet vaccinated elk aborted or produced nonviable calves. These results suggest that a single dose of 1×10^8 to 1×10^9 CFU RB51 does not provide significant protection against *B. abortus* induced abortion in elk. However, the vaccine appears to be safe at this dose and additional study may reveal a more effective RB51 vaccine regimen for elk.

Key words: *Brucella abortus*, brucellosis, *Cervus elaphus nelsoni*, Rocky Mountain elk, strain RB51, vaccination.

INTRODUCTION

Bovine brucellosis is an important disease of domestic and wild animals and occasionally of humans. There is much controversy about this disease in wildlife and its impacts in the Greater Yellowstone Area (GYA), USA and nationally. The GYA consists of Yellowstone National Park, Grand Teton National Park, and the surrounding areas of Montana, Wyoming, and Idaho. This debate has implications for management of wildlife and livestock of the region.

The Cooperative State-Federal Brucellosis Eradication Program began in 1934 and has cost about $3.5 billion to 1997 (Frye and Hillman, 1997). This program, which is administered by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), has been quite successful. When the program began, brucellosis affected one in every eight cattle herds in every state in the country (King, 1997). Today, 45 states, Puerto Rico, and the Virgin Islands are brucellosis free (United States Department of Agriculture, 2000). The number of human cases in the USA has dropped from 6,321 in 1947 to about 100 per year today (Young and Nicoletti, 1997).

Bovine brucellosis causes about 70% of female cattle to abort their first fetus following infection. Abortions typically occur after the 5th month of infection (Nicoletti and Gilsdorf, 1997). Most cattle will only abort once, but 10–20% may abort in sub-

sequent pregnancies. Brucellosis is transmitted by ingestion of *Brucella abortus* from contaminated fetuses, placentas, and associated fluids (Nicoletti, 1986).

Brucellosis affects elk herds that winter on feedgrounds in western Wyoming (Thorne et al., 1978a; 1979). The elk of Yellowstone National Park and the National Elk Refuge in Jackson, Wyoming have been known to be infected since the 1930s (Rush, 1932; Thorne et al., 1978b). There are 23 feedgrounds in western Wyoming, where approximately 25,000 elk are fed in the winter. Brucellosis has been documented in elk on most of these feedgrounds and is assumed to be present on the remaining untested feedgrounds (Thorne et al., 1991). Seroprevalence in adult cow elk is as high as 50% on some feedgrounds (Herriges et al., 1989). The abortion rate among naturally exposed naive elk is about 50% for the first pregnancy after infection, and it is estimated that highly infected herds may lose 12% of their reproductive potential (Thorne et al., 1991).

Infected elk may co-mingle with cattle on summer ranges, during migrations, and occasionally may be in close contact with cattle on cattle winter feedlines (Thorne et al., 1979). Experimentally infected elk can transmit brucellosis to susceptible cattle when they are confined together under conditions of prolonged close contact (Thorne et al., 1979), although the risk in a free-ranging situation is unknown. There is remote potential for brucellosis transmission from elk to domestic cattle (Cheville et al.,1998). If this occurred, Wyoming could lose its status as a brucellosis free state by the USDA-APHIS which would be very costly to the livestock industry. Because of this threat, efforts are being undertaken to reduce or eliminate the prevalence of the disease in the elk herds of northwestern Wyoming.

One method to reduce the prevalence of the disease is through vaccination. Elk on the state of Wyoming feedgrounds are currently being vaccinated as calves with *B. abortus* strain 19 (strain 19) via biobullet inoculation. Biobullets are made of a hydroxypropylmethylcellulose shell containing 3×10^9 colony forming units (CFU) lyophilized strain 19 and are shot into the large muscle mass of the hind leg (Angus, 1989; Thorne et al., 1997). To date, about 40,000 elk have been vaccinated with strain 19 by biobullet. Although the program is deemed successful (Cheville et al., 1998), there are some problems with strain 19.

The biggest limitation of strain 19 is the potential for it to induce false positive reactions on the standard brucellosis serologic tests (Stevens et al., 1994). This can make it difficult to distinguish some vaccinated animals from those infected with the virulent field strain of the organism.

However, a new vaccine, *B. abortus* strain RB51 (RB51) does not induce false positive reactions and is less pathogenic than strain 19 (Schurig et al., 1991). Strain RB51 has replaced strain 19 as the official calfhood vaccine for cattle by USDA-APHIS (Cheville et al., 1998). If effective in elk, RB51 would become the vaccination of choice for feedground elk as well. There are two dose regimens for cattle: a reduced dose for adults (10^9 CFU) (Olsen, 2000a) and a calfhood dose (10^{10} CFU)(Olsen, 2000b). The purpose of this study was to test the safety and efficacy of reduced dose RB51 in preventing *Brucella* induced abortion in elk vaccinated as calves.

METHODS

In February 1995, 45 6- to 8-mo-old female elk calves were corral trapped at the National Elk Refuge, Teton County, Wyoming (43°30′N, 110°45′W). The calves were bled at the trap site and screened with the card test (United States Department of Agriculture, undated) to insure that they were negative for *Brucella* antibodies. They were then shipped to the Sybille Wildlife Research and Conservation Education Unit, Wyoming Game and Fish Department (41°45′N, 105°42′W), near Wheatland, Wyoming.

Over the next 8 wk, elk were bled five additional times and serologically tested for *Bru-*

cella antibodies using the card test, the standard plate test, the rivanol test, and the complement fixation test (Alton et al., 1988) to be certain that the elk had not been exposed to *Brucella.* Elk were fed free choice alfalfa hay and approximately 1 kg per head per day of pelleted alfalfa and corn. Water and mineral salt blocks were provided ad libitum.

Elk were randomly assigned to one of three groups. On 9 and 10 May 1995 one group ($n = 16$) was vaccinated by intramuscular injection into the left hind hip with 1×10^9 CFU of RB51 and a control group ($n = 13$) was similarly intramuscularly injected with a saline placebo. The final group ($n = 16$) was vaccinated via biobullet with 1×10^8 CFU of RB51 on 23 May 1995. Biobullets were prepared by the Diagnostic Bacteriology Laboratory, Veterinary Services, APHIS (Ames, Iowa, USA). Due to complications in preparing the biobullets, the vaccine dose of the biobullet group was a log lower than the hand-vaccinated group, and the biobullet group was vaccinated almost 2 wk later.

Elk were bled every 2 wk for serology and hemoculture. In addition to the standard serologic tests mentioned above, indirect enzyme-linked immunosorbant assays (ELISAs) for anti-RB51 antibodies prior to vaccination and on postvaccination (PV) wk 4, 8, 10, 14, 18, and 27 were conducted (Colby, 1997). The indirect ELISA was developed to identify elk with *B. abortus* strain RB51-specific titers. Appropriate wells of 96-well, medium-binding polystyrene plates (Costar, Kennebunk, Maine, USA) were coated with lyophilized, acetone-killed RB51 diluted in bicarbonate phosphate buffer (pH 9.6). Plates were sealed and incubated overnight. On the following day, plates were washed with a phosphate buffered solution containing 0.05% Tween 20 (PBST). Serum samples were diluted in PBST, dispensed into the antigen-coated wells, and then incubated in the RB51-coated polystyrene plates. After incubation, the wells were washed four times. Each well was then incubated with mouse monoclonal anti-bovine IgG_1 (Big715A, Veterinary Medical Research and Development Inc., Pullman, Washington, USA) diluted in PBST. Next, the plates were washed four times and then incubated with polyclonal horseradish peroxidase conjugated goat IgG fraction to mouse IgG whole molecule (Cappel, West Chester, Pennsylvania, USA) diluted in PBST. The plates were washed four times then incubated with a developing solution consisting of ortho-phenylenediamine (Sigma, St. Louis, Missouri, USA), methanol, distilled water, and H_2O_2. The developing reaction in each well was stopped with H_2SO_4. Optical density at 490 nm was determined for each well with a 96-well plate reader (Molecular Devices Corporation, Sunnyvale, California, USA). Optical density readings for each sample were converted into a percent positivity value for analysis. The percent positivity of each sample represents the ratio of RB51 specific antibody in that sample to the amount of RB51 specific antibody in the positive control. A negative cutoff was determined above which a sample was considered to have significantly elevated anti-RB51 antibody level.

Our hemoculture protocol followed Alton et al. (1988): 10–12 ml of blood was placed into a sterile bottle containing soybean-casein digest agar (Trypticase® Soy Agar, Becton Dickinson and Company, Cockeysville, Maryland, USA) at a slant and 20 ml of tryptose broth (Becton Dickinson and Company) with 1% sodium citrate. The blood was aseptically added to the bottle incubated at 37 C with 10% CO_2 atmosphere. Slants were checked for bacterial growth twice weekly. Those with growth were streaked on *Brucella* media (Kuzdas and Morse recipe, Alton et al., 1988). Those without growth were inverted to coat the slant with the blood/broth mixture and returned to the incubator. Hemocultures were kept until they yielded growth or for 6 wk. Hemocultures were performed on all animals until each elk had been negative on two consecutive samples. When hemocultures were no longer positive, blood samples were collected from elk monthly and serum alone tested as above.

In late August, 1996 two mature *Brucella* antibody negative bull elk were introduced to each pen for breeding. All cow elk were rectally palpated on 30 January 1997 to confirm pregnancy. Elk were challenged by intraconjunctival inoculation with 1×10^7 CFU *B. abortus* strain 2308 on 4 and 5 March 1997. This was done by placing half the inoculating dose (in 50 μl of saline) in the bottom of each conjunctival sac.

Elk were bled at challenge and at 2 wk intervals for hemoculture and standard serology as described above. For the next several months elk were closely watched from a distance for evidence of abortion or calving. Additionally, each pen was thoroughly examined daily for fetuses or calves.

Fetuses were placed in two layers of plastic bags and transported to the Wyoming State Veterinary Laboratory (WSVL, Laramie, Wyoming) for necropsy to verify the cause of abortion. Abomasal contents, heart blood, cerebrospinal fluid, lungs, mandibular lymph nodes, liver, spleen, and rectal swabs were cultured on *Brucella* media (Alton et al., 1988). *Brucella* plates were incubated at 37 C in 10% CO_2 until

growth was noted or for 10 days. Colonies characteristic of *Brucella* were identified by Gram stain and morphology (Gram negative coccobacilli), urease (positive; United States Department of Agriculture, undated), lead acetate (positive; Alton et al., 1988), catalase spot test and oxidase spot test (both positive; DIFCO Laboratories, Detroit Michigan, USA; Alton et al., 1988). When available, cotyledons, intercotyledonary placenta, and allantoic fluid were likewise cultured. Live calves had nasal, pharyngeal, and rectal swabs taken for *Brucella* culture within a few hours of being found. Vaginal swabs were taken on all cow elk within 24 hr of delivery, again after 2–3 days, and when elk were bled, generally about a week after delivery, and then bimonthly; swabs were cultured as above. One hand-vaccinated cow was not identified for several days after aborting so sampling was delayed. Vaginal swabs were taken every 2 wk until the elk had been negative on at least two attempts.

After all elk calved or aborted, elk cows and calves from each group were randomly euthanized and necropsied. Necropsies were performed from 15–20 wk after challenge. At necropsy the animal was examined for gross lesions and tissues taken for *Brucella* culture. Tissues cultured included the mandibular, medial and lateral retropharyngeal, prescapular, prefemoral, popliteal, parotid, mesenteric, hepatic, external and internal iliac, bronchial, and mediastinal lymph nodes; ovaries; uterus; cervix; biceps femoris; spleen; liver; lungs; ileum; rectum; kidney; bone marrow; synovial fluid; cerebrospinal fluid; and urine. Solid tissue samples were sliced and macerated with a scalpel blade and rubbed over the entire surface of *Brucella* media (Alton et al., 1988). Approximately 0.2 ml of cerebrospinal fluid, synovial fluid from the antebrachiocarpal joint, and urine were used to coat *Brucella* media. *Brucella* plates were incubated and colonies identified as stated above. Colonies on each plate were counted and each group was assigned a mean index of infection (Jones et al., 1964). Briefly, an index of infection measures how heavily infected an animal is based on the number of CFUs isolated from all tissues sampled.

Comparisons of rates of abortions between each vaccinated group and the control group were made via a Fisher's exact test with $P < 0.10$ considered significant. Comparisons of indices of infection were made via a one way analysis of variance (ANOVA) with $P < 0.10$ considered significant. All statistical tests were conducted using Number Cruncher Statistical Systems software (NCSS©, 1998, Kaysville Utah, web site: www.ncss.com). Means are reported with standard deviations (SD).

RESULTS

None of the hand-vaccinated elk or controls seroconverted on standard *Brucella* serologic tests after vaccination. However, all but one of the biobullet vaccinates had short term (4–6 wk) weak positive reactions on all serologic tests (Cook, 1999). All hand-vaccinated elk were positive on the RB51 indirect ELISA at 4 wk PV; three of these elk were negative, one was suspect, and the rest were positive at 8 wk PV; by 27 wk PV only seven were still positive. In contrast, it took 8 wk PV for all biobullet elk to seroconvert on RB51 indirect ELISA; at 4 wk 11 were positive, six were negative, and one was suspect. For a complete discussion of the RB51 indirect ELISA results see Colby, 1997. We cultured RB51 from the blood of 13 (81%) of the biobullet inoculated animals, 10 (63%) of the hand-vaccinated elk, and none of the controls. Biobullet inoculated elk cleared the vaccine strain from the blood within 6 wk, as did most of the hand-vaccinates, but one hand-vaccinated animal took 14 wk until cultures were negative. No signs of disease attributable to vaccination were noted.

All control elk, 88% (14/16) of the hand-vaccinates, and 75% (12/16) of the biobullet vaccinates had abortions or produced nonviable calves attributable to *B. abortus* strain 2308. Additionally, one biobullet cow delivered a live calf which died within 12 hr and from which *B. abortus* strain 2308 was isolated. There were no differences in abortion rates between controls and hand-vaccinates ($P = 0.55$) or biobullet vaccinates ($P = 0.15$). Mean indices of infection were 8.87 ± 6.80 (controls), 7.91 ± 7.08 (hand), and 7.81 ± 7.74 (biobullet). There were no differences in the indices of infection ($P = 0.91$). *Brucella abortus* strain 2308 was isolated from vaginal swabs of all control elk, 12 of 16 (75%) biobullet elk, and 14 of 15 (93%) hand-vaccinated elk. Most elk only shed *B. abortus* strain 2308 vaginally for a few days after abortion. However, three controls

shed for 16, 26, and 27 days post abortion; one biobullet elk shed for 27 days, and three hand-vaccinates shed for 15, 17, and 22 days. *Brucella abortus* strain 2308 was isolated from blood of all but three elk (two biobullet, one hand-vaccinate). *Brucella abortus* strain 2308 was cultured from numerous tissues in each group (Table 1).

At necropsy, pyometras and abscesses were found in each group. One control elk had multiple uterine abscesses from which *Arcanobacterium pyogenes* was isolated. One hand-vaccinate had a subcutaneous abscess on the right front leg from which *B. abortus* strain 2308 was isolated. One hand-vaccinate had pyometra containing *B. abortus* strain 2308. Another hand-vaccinate had a single uterine abscess from which *Escherichia coli* was isolated. A hand-vaccinate had a sterile abscess of the right ovary. One biobullet vaccinate had mild sterile pyometra; another had a sterile firm abscess adjacent to the left ovary.

Brucella abortus strain 2308 was isolated from five of the six calves born live, only one calf born to a biobullet-vaccinated elk was not infected in any of the tissues sampled. This calf's dam was also one of the few elk from which *B. abortus* was not isolated from vaginal swabs, however, this dam had several infected lymph nodes at necropsy.

No *Brucella* was grown from swabs taken at birth from three calves from biobullet vaccinated cows. *Brucella abortus* strain 2308 was cultured from nasal, pharyngeal, and rectal swabs from the other live calf of a biobullet cow. Likewise, *B. abortus* strain 2308 was cultured from the pharyngeal swab only from one calf of a hand-vaccinated cow and the pharyngeal and nasal swab of the other.

All but one elk, a biobullet vaccinate, were seropositive on standard tests by 2 wk after challenge. All elk were seropositive at 4 wk after challenge and remained positive for the remainder of the study.

DISCUSSION

The results of this study indicate that RB51 is safe in female elk inoculated as calves. However, as used in this study it may not be as effective in preventing abortion in elk as strain 19. Strain 19 is about 60% effective in preventing abortion in elk vaccinated as calves (Thorne et al., 1981; Herriges et al., 1989). However, in these strain 19 studies 30% of control elk delivered live calves. Our challenge dose was slightly higher than used in those experiments (1×10^7 CFU *B. abortus* strain 2308 vs. 7.5×10^6 CFU) and the dose was split and given in both conjunctival sacs while in the strain 19 studies it was only placed in one conjunctival sac. Studies with cattle have shown that reducing the challenge dose of *B. abortus* strain 2308 from 5.2×10^7 CFU to 9.4×10^6 CFU increased vaccine efficacy from 0–20% to 89–90% while only increasing live births in control cattle from 0% to 11% (Nicoletti, 1990).

It is also known that about 18% of cattle are naturally resistant to brucellosis and do not abort unless given a very high dose (Templeton and Adams, 1990). Studies on elk indicate that 20–30% of elk are probably likewise resistant (Thorne et al., 1978b, 1981). Based on these studies, we would not expect 100% of our control elk to abort unless we gave them an overwhelming dose. If we had given the elk a challenge dose that only caused 70–80% of controls to abort, protection of vaccinated elk might have been higher. Calculations of the number of bacteria harvested from actual aborted elk fetuses indicate that there are about 4.1×10^6 CFU on a piece of aborted elk fetus skin 10 cm in diameter (Cook, 1999). Observations of elk under natural conditions indicate that most elk simply make brief contact with a fetus and then walk away (Cook, 1999). Most elk probably contact an area 10 cm diameter or less, and thus they would be exposed to 4.1×10^6 CFU or less. The dose we gave elk was nearly a log higher than a realistic field exposure.

TABLE 1. Number and percent of elk from groups not vaccinated, biobullet vaccinated with RB51, and hand-vaccinated with RB51 positive at necropsy for *B. abortus* strain 2308 after challenge.

Tissue	Number positive controls[a] (%), $n = 13$	Number positive biobullet vaccinates[b] (%), $n = 16$	Number positive hand-vaccinates[c] (%), $n = 16$
R[d] mandibular LN[e]	5 (39)	6 (38)	3 (19)
L[f] mandibular LN	5 (39)	6 (38)	4 (25)
R medial retropharyngeal LN	9 (69)	10 (63)	7 (44)
L medial retropharyngeal LN	5 (39)	7 (44)	6 (38)
R lateral retropharyngeal LN	3 (23)	2 (13)	3 (19)
L lateral retropharyngeal LN	3 (23)	5 (31)	5 (31)
R parotid LN	3 (23)	5 (31)	3 (19)
L parotid LN	2 (15)	5 (31)	5 (31)
R prescapular LN	4 (31)	3 (19)	5 (31)
L prescapular LN	4 (31)	4 (25)	3 (19)
R prefemoral LN	1 (8)	7 (44)	3 (19)
L prefemoral LN	4 (31)	5 (31)	2 (13)
R external iliac LN	6 (46)	4 (25)	7 (44)
L external iliac LN	6 (46)	4 (25)	3 (19)
R internal iliac LN	3 (23)	4 (25)	5 (31)
L internal iliac LN	5 (39)	6 (38)	4 (25)
R popliteal LN	3 (23)	4 (25)	9 (56)
L popliteal LN	6 (46)	6 (38)	2 (13)
Mediastinal LN	3 (23)	2 (13)	3 (19)
Mesenteric LN	0	0	2 (13)
Hepatic LN	2 (15)	3 (19)	2 (13)
Supramammary LN	7 (54)	9 (56)	8 (50)
Bronchial LN	2 (15)	2 (13)	5 (31)
Synovial fluid	0	0	0
Cerebrospinal fluid	0	0	0
Lung	0	0	0
Ileum	0	0	0
Rectum	0	0	0
Feces	0	0	0
Urine	1 (8)	0	0
Mammary gland L front quarter	5 (39)	4 (25)	4 (25)
Mammary gland L hind quarter	4 (31)	4 (25)	2 (13)
Mammary gland R front quarter	1 (8)	4 (25)	1 (6)
Mammary gland R hind quarter	4 (31)	5 (31)	3 (19)
Uterus	3 (23)	5 (31)	5 (31)
Cervix	4 (31)	4 (25)	7 (44)
R ovary	0	1 (6)	0
L ovary	0	0	1 (6)
Milk	2 (15)	2 (13)	1 (6)
R kidney	0	1 (6)	1 (6)
L kidney	0	0	1 (6)
Bone marrow	0	0	0
Biceps femoris	0	0	0
Spleen	2 (15)	2 (13)	2 (13)
Liver	1 (8)	2 (13)	3 (19)

[a] Controls = pregnant elk not vaccinated but challenged with 1×10^7 CFU of *B. abortus* strain 2308.

[b] Biobullet vaccinates = pregnant elk previously vaccinated with 1×10^8 CFU *B. abortus* strain RB51 and challenged with 1×10^7 CFU of *B. abortus* strain 2308.

[c] Hand-vaccinates = pregnant elk previously vaccinated with 1×10^9 CFU *B. abortus* strain RB51 and challenged with 1×10^7 CFU of *B. abortus* strain 2308.

[d] R = right.

[e] LN = lymph node.

[f] L = left.

In a previous study, pregnant elk were orally vaccinated with 2×10^{10} CFU RB51 and challenged about 2 mo later. In this study, four of nine oral RB51 vaccinates produced live calves while none of 15 controls produced live calves; RB51 was considered to be partially protective (Elzer and Davis, 1997). When animals are exposed to *B. abortus* the bacteria generally invade through the mucosa (Enright, 1990). It may be that mucosal immunity is important in determining protection against *B. abortus.* Oral vaccination may better stimulate mucosal immunity and may therefore be more effective than intramuscular vaccination. In the present study elk were challenged nearly 2 yr PV while in the above study it was 2 mo after vaccination. Perhaps vaccine induced immunity had waned over the extended period.

Results of the indices of infection were very similar from each group. There was little difference in the percent of animals from each group which were infected in each tissue (Table 1). Additionally, there was little difference in hemoculture results. This, combined with the abortion rates of each group, indicates that the vaccine provided little protection. It may be that a higher dose of RB51 would have stimulated greater resistance. However, one elk died 27 days after vaccination and tissues were cultured for RB51 (Cook, 1999). The vaccine strain was cultured from many lymph nodes and a few other tissues. Thus, the vaccine dose was sufficiently high for the vaccine strain to colonize multiple sites. It seems unlikely that a higher dose would stimulate greater immunity.

We also identified tissues most likely to be infected when elk are intraconjunctivally infected with *B. abortus* strain 2308 (Table 1). Lymph nodes of the head and the iliac and supramammary lymph nodes as well as the uterus, cervix, and mammary glands are the tissues most likely to be infected. Results of vaginal swab cultures indicate that a few elk shed *B. abortus* strain 2308 vaginally for extended periods of time. Seven elk shed the bacteria for a minimum of 15 days. It is likely that several elk shed the bacteria longer than was documented because elk were only sampled every 2 wk. It is not known if these elk were shedding adequate amounts of bacteria to infect other elk because the number of bacteria was not quantified.

It is unknown why most of the biobullet vaccinates developed short term weak positive reactions on the standard brucellosis serologic tests. One possibility is that the biobullets were contaminated with strain 19. Unused biobullets from the same batch were cultured and revealed only pure strain RB51. Additionally, all hemoculture *B. abortus* isolates recovered PV were 100% RB51 with no reversions to the smooth bacterial phenotype (Colby, 1997).

It is possible that in the process of firing biobullets into the muscles other bacteria which might cause seroconversion entered into the wound. *Yersinia enterocolitica, Salmonella urbana, E. coli* O:116, and *Pseudomonas maltophilia* may induce reactions on standard brucellosis serology (MacMillan, 1990). However, it seems unlikely that 17 of 18 biobullet elk would have become infected with one of these bacteria. It is also possible that the process of firing the biobullet changed the characteristics of RB51 in some way. Again this seems unlikely because, as stated above, hemoculture isolates did not show any reversion to smooth characteristics.

Regardless of the cause of the short term serologic reactions of the biobullet vaccinated group it would not be a concern if the vaccine were used in a field situation. If it were used in biobullet form, the vaccine would be administered in late winter. Serologic testing of elk occurs at two times. First, hunter harvested elk are tested; second, elk are trapped and tested on feedgrounds prior to vaccination. In both cases elk would be vaccinated 6 to 12 mo prior to being tested, well beyond the time antibody titers induced by vaccination would no longer be detected.

In cattle, RB51 offers protection similar to strain 19 (Cheville et al., 1993, 1996). Thus, it may still be possible that a vaccination program using RB51 could be developed for elk. Elk may require a higher vaccine dose than we used, the oral route maybe more effective than parenteral inoculation, the challenge dose used here may have been unrealistically high, or perhaps elk require a booster dose of RB51 in order to develop adequate protection.

ACKNOWLEDGMENTS

This project was funded by the Wyoming Game and Fish Department and the United States Department of Agriculture–Animal and Plant Health Inspection Service. Technical assistance was provided by D. Zieler, T. Christinck, N. Persichini, S. Pistono, S. Hagius, J. Walker, S. Smith, D. Abendroth, S. Kilpatrick, H. Dawson, E. Nelson, V. Welch, and P. Jaeger.

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Received for publication 22 January 2001.