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Source: Journal of Wildlife Diseases, 36(3) : 484-488

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-36.3.484>

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## SAFETY OF *BRUCELLA ABORTUS* STRAIN RB51 IN BULL ELK

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**ABSTRACT:** Some of the elk (*Cervus elaphus nelsoni*) of the Greater Yellowstone Area (Wyoming, Idaho, Montana; USA) are infected with *Brucella abortus*, the bacterium that causes bovine brucellosis. *Brucella abortus* strain RB51 vaccine is being considered as a means to control *B. abortus* induced abortions in cow elk. However, the most probable vaccination strategies for use in free-ranging elk might also result in some bull elk being inoculated, thus, it is important to insure that the vaccine is safe in these animals. In the winter of 1995, 10 free-ranging bull elk calves were captured, tested for *B. abortus* antibodies, and intramuscularly inoculated with  $1.0 \times 10^9$  colony forming units (CFU) of *B. abortus* strain RB51. Blood was collected for hemoculture and serology every 2 wk after inoculation for 14 wk. Beginning 4 mo postinoculation and continuing until 10 mo postinoculation elk were serially euthanized, necropsied, and tissues collected for culture and histopathology. These elk cleared the organism from the blood within 6 wk and from all tissues within 10 mo. No lesions attributable to *B. abortus* were found grossly and only minimal to mild lymphoplasmacytic epididymitis was found in a few elk on histologic examination. In a separate study, six adult bull elk from Wind Cave National Park (South Dakota, USA) were taken to a ranch near Carrington (North Dakota, USA). Three were orally inoculated with approximately  $1.0 \times 10^{10}$  CFU of RB51 and three were inoculated with corn syrup and saline. Ninety days post-inoculation semen was examined and cultured from these bulls. Strain RB51 was not cultured from their semen at that time. There were no palpable abnormalities in the genital tract and all elk produced viable sperm. Although they contain small sample sizes, these studies suggest that *B. abortus* strain RB51 is safe in bull elk.

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

### INTRODUCTION

Bovine brucellosis is a bacterial disease of cattle that has become established in elk (*Cervus elaphus nelsoni*) herds which winter on feedgrounds in western Wyoming (USA) (Thorne et al., 1978). The artificial concentration of elk on these feedgrounds promotes the spread of brucellosis among elk (Smith et al., 1997) and possibly to bison (*Bison bison*) (Williams et al., 1993). Brucellosis is primarily transmitted by ingestion of *Brucella abortus* from contaminated fetuses, placentas, and associated fluids (Nicoletti, 1986). Infected elk can spread the disease to cattle under experimental conditions when the animals are held in close confinement for prolonged periods (Thorne et al., 1979).

Bovine brucellosis is nearly eradicated

from cattle in the United States (King, 1997). There is a remote potential for the disease to be spread from elk to domestic cattle under free-ranging conditions (Cheville et al., 1998). If this occurred, Wyoming could lose its status as a brucellosis free state by United States Department of Agriculture–Animal and Plant Health Inspection Service (USDA-APHIS). Because of this threat, efforts are underway to reduce or eliminate the prevalence of the disease in these elk herds.

Elk calves on feedgrounds managed by Wyoming Game and Fish Department (Cheyenne, Wyoming, USA) are currently being vaccinated with *B. abortus* strain 19 (strain 19) via biobullet inoculation (Smith et al., 1997). A limitation of strain 19 vaccine is the potential for it to induce false

positive reactions on the standard brucellosis serologic tests (Stevens et al., 1994). Thus, it may be difficult to serologically distinguish some vaccinated animals from those infected with a field strain of *B. abortus*. However, the vaccine *B. abortus* strain RB51 does not induce these false positive reactions (Stevens et al., 1994). Strain RB51 lacks the lipopolysaccharide O side chain (LPS) found in strain 19 and virulent strains; it is this side chain which causes serologic reactions on the standard tests (Schurig et al., 1991). Additionally, strain RB51 protected cattle against experimental challenge with virulent *B. abortus* (Cheville et al., 1993). This vaccine has been approved as a brucellosis calfhooed vaccine by USDA-APHIS and in many states cattle ranchers are required to use strain RB51 in place of strain 19. If an effective vaccination protocol using strain RB51 was developed for elk, it would become the vaccine of choice for feedground elk due to lack of serologic cross-reaction with field strain *B. abortus*.

The two most likely methods for vaccinating elk with RB51 would be to use biobullets to individually inoculate calves, or as an oral vaccine for whole herd vaccination. While the goal of a vaccination program is to protect female elk, either of these methods would result in bull elk being exposed to the vaccine as well. The goal of this study was to determine if strain RB51 is safe in bull elk.

#### METHODS

In February 1995, 10 male elk calves were trapped on the National Elk Refuge (Teton County, Wyoming, USA; 43°30'N, 110°45'W) and taken to the Sybille Wildlife Research and Conservation Education Unit near Wheatland (Wyoming). These elk were tested for antibodies to the genus *Brucella* using card, standard plate, rivanol, and complement fixation tests (Jones, 1977; Alton et al., 1988) five times over the next 10 wk to insure they had not been previously exposed to *B. abortus*. All calves were considered serologically negative on these tests by established criteria for elk (Morton et al., 1981; United States Department of Agriculture, 1998).

In May 1995, the calves were inoculated with  $1 \times 10^9$  colony-forming units (CFU) of strain RB51 by intramuscular injection into the left hip. The elk were then bled for serology using the standard tests as previously described, and hemoculture every 2 wk postinoculation (PI) for the first 14 wk PI after which time elk were bled for standard serology monthly. Hemocultures were prepared as per Alton et al. (1988). Briefly, 10–12 ml of blood was placed into a sterile bottle containing soybean-casein digest agar (Trypticase® Soy Agar or TSA, Becton Dickenson and Company, Cockeysville, Maryland, USA) at a slant and 20 ml of tryptose broth (Becton Dickenson and Company, Cockeysville, Maryland, USA) with 1% sodium citrate. The blood was mixed with the broth and the bottle incubated at 37 C with 10% CO<sub>2</sub> 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 swirled to slosh the blood/broth mixture on the slant and returned to the incubator. Hemocultures were kept until they yielded growth or for 6 wk.

Additionally, elk were bled at 2 wk prior to inoculation, on the date of inoculation, and at 4, 8, 10, 14, and 18 wk PI for indirect enzyme linked immunosorbent assay (ELISA) to detect anti-RB51 antibodies (Colby, 1997). This indirect ELISA was developed to identify elk with *B. abortus* strain RB51-specific titers. The ELISA reacts elk serum samples diluted 1:50 in PBST-20 (phosphate buffered saline containing 0.05% Tween 20) with a mouse monoclonal antibody specific for bovine IgG<sub>1</sub>. Optical density (O.D.) 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 value was determined above which a sample was considered to have a significantly elevated anti-RB51 antibody level.

Elk were necropsied as follows: three elk on day 132 PI, one elk on day 145 PI, one elk on day 186 PI, two elk on day 217 PI, and three elk on day 306 PI. Time points were selected such that elk would be necropsied at approximately 4, 7, and 10 PI, except two elk became physically injured and were necropsied earlier than scheduled. Elk were euthanized via gun shot to the neck.

At necropsy, tissues were examined grossly and collected for culture and histologic evaluation. Tissues collected included: mandibular, medial and lateral retropharyngeal, prescapular, prefemoral, popliteal, parotid, mesenteric,

hepatic, external and internal iliac, bronchial, and mediastinal lymph nodes; seminal vesicles; ampulla; prostate; testes; biceps femoris; spleen; liver; lungs; ileum; rectum; kidney; bone marrow; brain; synovial fluid; cerebral spinal fluid; and urine. Solid tissue samples were sliced in half and then macerated with a scalpel blade and rubbed over the surface of *Brucella* medium (Alton et al., 1988). Approximately 0.2 ml of cerebrospinal fluid, synovial fluid, and urine were used to coat *Brucella* medium. *Brucella* plates were incubated at 37 C in 10% CO<sub>2</sub> until growth was noted or for 10 days. Colonies characteristic of the genus *Brucella* were positively identified by Gram stain and morphology (negative coccobacilli), urease (positive) (United States Department of Agriculture, undated), lead acetate (positive) (Alton et al., 1988), catalase spottest (positive), and oxidase spottest (positive) (DIFCO Laboratories, Detroit, Michigan, USA) (Alton et al., 1988). Colonies were identified as RB51 by rough morphology (verified by agglutination in acriflavin and uptake of crystal violet dye) and rifampin resistance (Colby, 1997). The number of CFU on each plate was counted and recorded. Samples of each tissue were placed in 10% buffered formalin, embedded in paraffin, sectioned at 6 µm, stained with hematoxylin and eosin, and examined by light microscopy.

In a separate experiment, six sexually mature, brucellosis card test negative, bull elk were obtained from Wind Cave National Park (South Dakota) in March 1995 and transported to a ranch near Carrington (North Dakota). In June, 1995 the animals were randomly divided into two groups. The control group was restrained in a cattle chute and 5 ml of a corn syrup/saline mixture (1:1) was poured into their mouths. For the treatment group lyophilized strain RB51 was reconstituted with saline and this suspension was added to corn syrup at a ratio of 1:1. The treatment group was restrained and each animal received approximately  $1 \times 10^{10}$  CFU of strain RB51 orally. All elk were allowed to breed cow elk in the 1995 and 1996 rut.

In September 1995, each adult bull elk was anesthetized with an intramuscular injection of carfentanil citrate (Wildnil®, Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA; 3 mg/ml) (0.01 mg/kg) and xylazine hydrochloride (Rompun®, Miles Laboratory Inc., Shawnee, Kansas, USA; 100 mg/ml) (0.1 mg/kg) and physically evaluated, rectally palpated, bled, and electroejaculated. Semen was cultured on *Brucella* medium at 37 C with 10% CO<sub>2</sub> for 5 days and was examined for sperm viability and motility.

Serum was tested using the standard card

test and western blot analysis for specific antibodies against strain RB51 (Schurig et al., 1991; Colby, 1997). Briefly, the western blot consisted of acetone killing RB51, suspension in 10 mM Tris, centrifugation, resuspension of the pellets in 10 mM Tris and storage at -40 C. Prior to use, aliquots were mixed with Laemmli 2X sample buffer (Sigma, St. Louis Missouri, USA), boiled, centrifuged, and the supernatant used to load gel strips. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDA-PAGE) was performed as per Laemmli (1970). Gels were transferred to nitrocellulose sheets and the sheet stained and destained. Sheets were cut into strips corresponding to the strips of transferred antigen and agitated in elk serum diluted 1:50 in Tris buffered saline. Strips were washed, agitated in monoclonal mouse anti-bovine IgG<sub>1</sub>, washed, agitated with a horseradish peroxidase-conjugated goat anti-mouse IgG solution, washed again, and agitated in developing solution until positive control strips developed.

## RESULTS

Table 1 summarizes the important results. All intramuscularly inoculated elk were serologically negative on the standard brucellosis tests throughout the study. All these elk were negative on the indirect RB51 ELISA prior to and on the date of inoculation but were positive at 4, 8, 10, and 14 wk PI. Eight of ten (80%) elk were still positive at 18 wk PI, but two (#3 and #22) were negative.

Three bull calves had positive hemocultures for strain RB51 2 wk PI, and four elk were positive 4 wk PI. All elk were hemoculture negative by 6 wk PI and remained negative through 14 wk after which hemoculture was no longer performed.

All tissues from two elk necropsied on day 132 PI were negative for *B. abortus*, but a third elk necropsied on the same day had >300 CFU in the left seminal vesicle, one CFU in the ampulla, and six CFU in the prostate. Remaining tissues were culture negative.

All tissues from the two elk necropsied on days 145 and 186 PI were negative. All tissues were negative from one elk necropsied day 217 PI. However, the second animal necropsied on day 217 PI had 244

TABLE 1. Findings for male elk intramuscularly injected with  $1 \times 10^9$  CFU<sup>a</sup> of *Brucella abortus* strain RB51.

Elk num-ber	Days of necropsy	Hemoculture results <sup>c</sup>	Bacteriologic results <sup>d</sup>	Histologic lesions
22	132	28	Amp <sup>e</sup> = 1; LSV <sup>f</sup> > 300; Pro <sup>g</sup> = 6	Mild inflammation: SVs <sup>h</sup> , Pro, Amp, testis
10	132	Negative	Negative	Mild inflammation: SVs
36	132	14	Negative	Mild inflammation Pro, Epid <sup>i</sup>
3	145	Negative	Negative	None
32	186	Negative	Negative	None
84	217	Negative	Negative	Mild inflammation: Epid
20	217	14, 28	Amp > 300; LSV = 244; Pro = 25	Moderate inflammation: Left Epid
23	306	14, 28	Negative	Mild inflammation: Epid, Svs, testes, Amp
85	306	Negative	Negative	None
6	306	28	Negative	Mild inflammation: Epid, testes

<sup>a</sup> Colony Forming Units.

<sup>b</sup> Postinoculation.

<sup>c</sup> Days PI that elk was hemoculture positive for RB51.

<sup>d</sup> Number of CFU of RB51 cultured from identified tissues at necropsy.

<sup>e</sup> Ampulla.

<sup>f</sup> Left Seminal Vesicle.

<sup>g</sup> Prostate.

<sup>h</sup> Seminal Vesicles.

<sup>i</sup> Epididymides.

CFU cultured from the left seminal vesicle, >300 CFU from the ampulla, and 25 CFU from the prostate. Tissues from three elk necropsied day 306 PI were culture negative.

No gross lesions attributable to brucellosis were found. Histologically, a mild lymphoplasmacytic epididymitis was present in three elk. Several elk had small aggregates of lymphocytes in the interstitium of the prostate, ampulla, seminal vesicles, and/or testicles. One of these was the elk necropsied 217 days PI which was culture positive in the left seminal vesicle, ampulla, and prostate.

In the oral inoculation study, elk were negative on the standard card test at 90 days PI. All inoculated elk showed marked increases in antibody response to *B. abortus* strain RB51 antigens 90 days PI as measured by western blot analysis. Palpation revealed no difference in size or texture of the epididymides, testicles, or seminal vesicles. There were no apparent gross physical differences between the two groups. All elk produced viable and motile sperm. Strain RB51 was not cultured from

the semen of any of the elk. All bulls bred *Brucella* naive cow elk over the following 2 yr.

## DISCUSSION

Male elk calves intramuscularly inoculated with strain RB51 can become bacteremic and may remain infected in the secondary sex glands for an extended period of time. But, it appears that most, if not all, elk will eventually clear the organism from all tissues. Strain RB51 did not cause significant lesions in bull elk inoculated as calves, and did not induce serological reactions on the standard brucellosis serologic tests. Furthermore, the vaccine was not shed in the semen 90 days PI when adult bull elk were orally inoculated with  $1 \times 10^{10}$  CFU of strain RB51. It did not have an affect on libido, nor did it induce serologic reactions on standard tests in adult bull elk inoculated orally.

If RB51 were used in biobullet form, elk calves and possibly cows would be the target for vaccination. Since bull calves can not be distinguished from heifer calves, bull calves would be vaccinated. Addition-

ally, it is possible that a few biobullets would not properly hit the intended target and would end up on the ground where bull elk might ingest them. On the other hand, if oral vaccination with RB51 were conducted, all age and sex classes of elk would end up ingesting the vaccine. Although the sample sizes used in these trials were small, the results suggest that RB51 is safe should nontarget bull elk be inoculated in the course of managing brucellosis on elk feedgrounds.

#### 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. W. Rotenberger helped obtain elk from Wind Cave National Park and assisted in inoculating them. Technical assistance was provided by S. Pistono, S. Hagijs, J. Walker, N. Persichini, D. Zieler, S. Smith, D. Abendroth, S. Kilpatrick, H. Dawson, V. Welch, H. Edwards, and E. Nelson.

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Received for publication 15 December 1998.