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ABSTRACT: To determine the safety of *Brucella abortus* strain RB51 (SRB51) vaccine in American bison (*Bison bison*), 31 animals from a herd with brucellosis were used. In October 1996, 10 adult bison males and seven calves were vaccinated with the standard calfhood cattle dose of 1.8×10^{10} colony forming units (CFU) of SRB51 subcutaneously while the adult females received the standard adult cattle dose of 1×10^9 CFU. Western immunoblot indicated the presence of SRB51 antibodies following vaccination. To evaluate prolonged bacterial colonization of tissues, the adult males, calves, and three adult females were divided into two groups which were slaughtered at either 13 or 16 wk post-vaccination. At necropsy, tissue samples were obtained for *B. abortus* culture from the liver, spleen, lymph nodes, and reproductive tract of each animal. While *B. abortus* field strain was cultured from one adult bull, no SRB51 was isolated from any of the animals. Seven pregnant females were monitored until parturition for signs of abortions and fetal lesions. Six cows delivered healthy calves and one delivered a dead full-term calf that was brucellae negative. Based on these results, administration of SRB51 to bison did not cause prolonged bacterial colonization of tissues in calves, adult males, or adult females. Furthermore, SRB51 did not induce abortions following vaccination in the second month of gestation.

Key words: American bison, *Bison bison*, *Brucella abortus*, safety, strain RB51, vaccine.

A current problem confounding the effort to eradicate brucellosis from the United States is a reservoir of *Brucella abortus* infected bison (*Bison bison*) and elk (*Cervus elaphus*) in Yellowstone and Grand Teton National Parks (Wyoming, USA). These free-ranging animals may contact domestic cattle on drive trails or on surrounding pasture lands. This is important because bison abortions due to *B. abortus* infection have been documented under both field (Williams et al., 1993; Rhyan et al., 1994) and experimental conditions (Davis et al., 1990). Also, infected bison transmitted brucellosis to cattle pastured together on a mixed species ranch in North Dakota (Flagg, 1983). Furthermore, bison to cattle transmission has been observed under experimental conditions (Davis et al., 1990). Therefore, a brucellosis vaccine for bison is needed. One possible vaccine candidate was smooth *B. abortus* strain 19 (S19), but vaccination of pregnant bison with this strain induced abortions, chronic infections, and serologic responses to the lipopolysaccharide (LPS) O-side chain (Davis et al., 1991). The standard serologic methods used to detect brucellosis test for O-side chain specific antibodies making vaccine titers induced by S19 indistinguishable from field strain infections.

A second possibility is the cattle vaccine *B. abortus* strain RB51 (SRB51) which is a stable, rough, rifampin-resistant, variant of *B. abortus* strain 2308 (S2308) (Schurig et al., 1991). Compared to S19 in cattle, SRB51 provides significant protection, decreased virulence and does not induce LPS O-side chain specific antibodies (Cheville et al., 1993; Palmer et al., 1997). Previously uninfected bison vaccinated with SRB51 had increased colonization, lesions, and fetal infection when compared to vaccinated cattle (Palmer et al., 1996; Olsen et al., 1997). Therefore, the purpose of this study was to evaluate the colonization and virulence of SRB51 in a bison herd with a history of brucellosis.

In this study 31 American bison were obtained from a *B. abortus* infected herd

in Strong City (Kansas, USA). In September 1996, the animals were shipped to The Veterinary Medical Park, Texas A&M University (College Station, Texas, USA) where they were housed throughout the experiment. The herd consisted of 10 adult males, seven calves, and 14 adult females. Of the 31 animals, two adult bulls had antibodies to field strain *B. abortus* based on the card test (Alton et al., 1975). Due to the inherent difficulty in working with untamed bison, pregnancy was not evaluated by rectal palpation. Instead, jugular blood samples were obtained from each adult female in October 1996 for analysis of progesterone levels by the Texas A&M Diagnostic Lab using the Immulite Progesterone kit (Diagnostic Products, Corp., Los Angeles, California, USA) which is a chemiluminescent enzyme immunoassay. Seven of the adult females were identified as pregnant based on a progesterone level of 4.0 or higher.

In October 1996, ten adult males and seven calves (five bulls and two heifers) were vaccinated subcutaneously (SC) with 1.8×10^{10} colony forming units (CFU) of SRB51 which is the standard calfhood dose and route of inoculation in cattle. The vaccine was rehydrated according to instructions provided by the manufacturer (Colorado Serum Company, Denver, Colorado, USA). At the same time 12 adult females received SRB51 in the standard adult cattle dose of 1×10^9 CFU SC. Both vaccine doses were verified as correct by standard serial dilution and culture on the appropriate media (Alton et al., 1975). Two non-pregnant females died due to injuries sustained from other bison during shipment and introduction into the containment center. Since both deaths occurred prior to vaccination, the cows were not necropsied. Also, two additional vaccinated, nonpregnant adult cows were reserved for future breeding studies and were not sacrificed.

The adult males, calves, and three adult females were randomly divided into two groups which were euthanized at 13 and

16 wk post-vaccination to evaluate prolonged bacterial colonization of tissues. Tissue samples were obtained from the liver, spleen and entire reproductive tract including testicles, epididymides, seminal vesicles, and prostate from the males. Samples also were obtained from the parotid, mandibular, mediastinal, prescapular, prefemoral, internal iliac, and inguinal lymph nodes. Samples collected from the females were liver, spleen, reproductive tract, and various lymph nodes including the parotid, mandibular, mediastinal, prescapular, prefemoral, internal iliac, and supramammary. Samples were frozen at -20 C and thawed before grinding. Lymph nodes from each animal were pooled, ground in sterile distilled water and plated on Farrell *Brucella* selective media (Farrell, 1974). The samples from each animal were pooled as liver and spleen, lymph nodes, and reproductive tissues. Samples were combined to evaluate general bacterial colonization instead of individual tissue colonization.

The seven pregnant females were monitored until parturition for signs of abortion or fetal lesions. Live calves were allowed to stay with the cow while aborted fetuses and dead calves were frozen at -20 C. Calves were frozen by field technicians since immediate necropsy was not possible due to the distance between the isolation facility (College Station, Texas, USA) and the laboratory (Baton Rouge, Louisiana, USA). As a result, histology could not be performed on the tissue samples. At necropsy lung, liver, spleen, and abomasal fluid from each calf were processed as described above. The cows were kept with the bulls for 16 wk post-vaccination.

Jugular blood samples were obtained prior to vaccination and at necropsy from all but one animal in the prolonged bacterial colonization study. Sera was not obtained from one adult cow due to difficulties encountered during jugular bleeding. Sera were analyzed by the card test (Alton et al., 1975) and western immunoblot

(Schurig et al., 1991). These two tests were selected because the card test is an accepted field agglutination test and the western immunoblot is the most sensitive direct binding assay available. While unfeasible for field use, the western immunoblot is an excellent research tool which is highly specific as compared to the card test. Western immunoblot analysis was performed using cell lysates from rough SRB51 and S2308 containing LPS O-side chain. Cell lysates of SRB51 and S2308 were obtained by sonication and dilution in Laemmli sample buffer (Schurig et al., 1991). Electrophoresis and transfer to nitrocellulose were performed according to Schurig et al. (1991). Briefly, electrophoresis was performed with a BioRad Mini-Protean II unit (Hercules, California, USA) utilizing the Laemmli discontinuous SDS-PAGE method with 200 µl of cell lysate in a 450 µl well. Nitrocellulose transfer was performed for 1 hr with 100V in a Mini Trans-Blot Electrophoretic Transfer Cell (BioRad). Following transfer the blots were blocked with a 5% nonfat milk buffer for 30 min followed by washing with tris-buffered saline containing 0.5% Tween-20. The membrane was cut into separate strips and incubated overnight at room temperature with a 1:40 dilution of the test serum. Subsequently, the blots were washed and incubated with rabbit anti-bovine IgG horseradish peroxidase conjugate (Sigma Chemical Company, Saint Louis, Missouri, USA) at a dilution of 1:800 for 45 min. After washing, the substrate 4-chloro-1-naphthol (Sigma) was added for color development. The number of bands observed with pre-immunization sera was compared to those bands obtained with post-immunization sera and the addition of new banding patterns in post-immunization serum was considered a response to vaccination.

In the prolonged bacterial colonization study, no SRB51 was cultured 13 or 16 wk post-vaccination from the bulls, calves, or three adult females. However, a smooth, rifampin-sensitive field strain *B. abortus* was cultured from an adult bull 13 wk post-vaccination. Pooled samples from both the reproductive tissues and lymph nodes of this bull were culture positive for field strain. At necropsy one adult female was found to be carrying a 2-mo-old fetus indicating that she had become pregnant following vaccination. Tissue samples from placenta, lung, liver, spleen, and abomasal fluid of the calf were culture negative for *B. abortus*.

Of the seven pregnant females vaccinated in the second month of gestation, five produced live, healthy calves. A sixth female gave birth to a large bull calf that died in the birth canal due to an apparent dystocia. The calf was full term with no gross lesions; and upon necropsy, *B. abortus* was not cultured from fetal liver, spleen, lung or abomasal fluid samples. Also, post-partem milk samples from the cow were culture negative for *B. abortus*. One adult animal that was assumed pregnant based on serum progesterone levels did not actually become pregnant until 4 mo post-vaccination. This animal produced a live calf that was left with the mother. All seven cows and their healthy calves were allowed to remain in the herd following parturition and were not necropsied.

Western immunoblot serologic analysis found 16 of the 19 animals tested in the colonization study were positive for SRB51 specific antibodies following vaccination (Table 1). Positive antibody response to *B. abortus* field strain was detected in three adult males, including the animal culture positive for a field strain isolate (Table 1). Serologic detection of field strain antibodies made the identification of SRB51 specific antibodies in these three bulls unfeasible because SRB51 shares the same internal antigens as field strain *B. abortus*. Only two of these three bulls were positive on the card test for agglutinating antibodies, demonstrating the higher sensitivity of the western immunoblot direct binding assay (Table 1).

Previous studies by others have shown

TABLE 1. Western immunoblot analysis of serum samples taken at slaughter using cell lysates from *Brucella abortus* smooth strain 2308 (S2308) and rough strain RB51 (SRB51) showing results of test samples from ten bison bulls, seven calves and two adult females vaccinated subcutaneously with SRB51.

Animal number	S2308	SRB51	Sex	Age
1	−[a]	+ + +	M	Adult
3[b,c]	+ + +[d]	qv[e]	M	Adult
5[f]	+ + +[d]	qv	M	Adult
8	−	+ +	F	Adult
9	−	+ +	M	Adult
11	−	+ +	M	Adult
13[c]	+ + +[d]	qv	M	Adult
16	−	+ + +	M	Adult
19	−	+ + +	F	Adult
20	−	+ + +	M	Calf
21	−	+ + +	F	Calf
22	−	+ + +	M	Calf
24	−	+ +	M	Calf
25	−	+ + +	F	Calf
26	−	+ +	M	Calf
28	−	+ +	M	Calf
29	−	+ + +	M	Adult
30	−	+	M	Adult
31	−	+	M	Adult

[a] − = negative for SRB51 antibodies; + = 1–3 new SRB51 antibody bands in post-vaccination immunoblot, + + = 4–6 new bands, + + + = > 6 new bands.

[b] Culture positive for *B. abortus* field strain.

[c] Card test positive for field strain antibodies prior to and following vaccination.

[d] Western immunoblot positive for *B. abortus* field strain antibodies prior to and following vaccination causing inability to distinguish SRB51 antibodies from field strain specific antibodies.

[e] qv = equivocal.

[f] Card test negative but western immunoblot positive for field strain antibodies.

that SRB51 can cause increased colonization, lesions, and possible fetal infections when compared to vaccinated cattle. Olsen et al. (1997) recently demonstrated that six 3-mo-old bison vaccinated SC with 1.5–2.3 × 10^{10} CFU of SRR51 had microscopic lymph node lesions similar to those seen with three S19 vaccinated bison. Also, these researchers were able to culture an average of 121 CFU and 21 CFU per gram of tissue of SRB51 at 10 and 16 wk post-vaccination, respectively, from the superficial cervical lymph node which drained the vaccination site. In our study, SRB51 was not cultured from the calves at 13 and 16 wk post-vaccination. We may have experienced a dilution effect since all the lymph nodes including the draining lymph node were combined making isolation of this low number of organisms difficult at 13 and 16 wk post-vaccination. A second significant difference between these two studies is that while the calves used by Olsen et al. (1997) were 3-mo-old bison, the calves in our study ranged in age from 6 to 8 mo. The bison calves used in our study may have had a more mature immune system resulting in a quicker clearance SRB51. Other minor differences included the location of vaccination (cervical verses rump) and stresses on the herd. While the animals used in this study were untamed, the animals used by Olsen et al. (1997) had been hand raised since 24 hr following birth.

Palmer et al. (1996) demonstrated two SRB51 induced abortions following SC vaccination of eight pregnant female bison with 1 × 10^{9} CFU of SRB51. These authors found lesions similar to those seen by Olsen et al. (1997). In the present study, seven pregnant females vaccinated SC with the same vaccine dose produced six healthy calves and one *B. abortus*-negative dystocia. However while six of the seven bison were vaccinated in the second month of gestation, the two aborting bison in the study by Palmer et al. (1996) were vaccinated at 4.5 and 6.5 mo of gestation, respectively. Hence, the difference in abortion may have resulted from the differences in gestation at vaccination. Also, while the animals used in this study were vaccinated in the rump, Palmer et al. (1996) vaccinated the animals in the cervical area.

Based on the results of this study, it appears that SRB51 may be safe for vaccination of pregnant adult female bison in the first few months of gestation. Also, SRB51 did not induce prolonged tissue colonization in seven bison calves, ten adult males, or three adult females. Currently, our laboratory and others are investigating the efficacy of SRB51 in bison

following challenge with virulent S2308. These studies will be important to determine if SRB51 could be useful in the control and eradication of *B. abortus* in wild bison.

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