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Bacterial Survival, Lymph Node Pathology, and Serological Responses of Bison (*Bison bison*) Vaccinated with *Brucella abortus* Strain RB51 or Strain 19

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ABSTRACT: From August 1993 to June 1994, 3 month-old bison (*Bison bison*) were vaccinated with *Brucella abortus* strain RB51 (SRB51, $n = 6$), strain 19 (S19, $n = 3$), or with saline ($n = 1$) and serologic responses and persistence of vaccine strains within lymph nodes were monitored. Bison vaccinated with S19 had granulomatous lymphadenitis and greater peak numbers of *B. abortus* than those vaccinated with SRB51. Bison vaccinated with RB51 had similar histological lesions and *B. abortus* were still present in lymph nodes at 16 weeks. Although antibodies against RB51 were produced, standard tube agglutination test responses of RB51-vaccinates remained negative. The histological lesions of *B. abortus* infections in bison were similar to those observed in cattle, but bison did not clear SRB51 as rapidly as cattle.

Key words: bison, *Bison bison*, *Brucella*, RB51, serology, vaccine.

A major complication affecting the eradication of brucellosis from cattle in the United States is the infection of bison (*Bison bison*) and elk (*Cervus elaphus nelsoni*) with *Brucella abortus*, especially in the Greater Yellowstone area of Wyoming (USA). Infections in bison may be transmitted to cattle (Davis et al., 1990). The current cattle vaccine, *B. abortus* strain 19 (S19), has been used in bison, but causes a high incidence of abortion when given to pregnant bison (Davis et al., 1991). In this study, conducted between August 1993 and May 1994, we examined the persistence of infection and immunological responses of bison calves to *B. abortus* strain RB51. Strain RB51 (SRB51), a laboratory-derived mutant of the 2308 strain of *B. abortus* (2308), has very low expression of the lipopolysaccharide (LPS) O-side chain (Schurig et al., 1991). This strain is protective in cattle (Cheville et al., 1993) and has the advantage that vaccinated animals remain seronegative when evaluated using routine surveillance tests such as the standard tube agglutination, rivanol, or complement fixation tests (Stevens et al., 1994a) which detect antibodies against the LPS O-side chain.

Ten female bison calves were obtained from a private, brucellosis-free herd (Wedeking Bison, Clarksville, Iowa, USA) within 24 hr of birth. At 3 mo of age bison were divided into S19 ($n = 3$), SRB51 ($n = 6$) or non-vaccinated control ($n = 1$) groups and inoculated within S19 (2.9×10^{10} CFU), SRB51 ($1.5\text{-}2.3 \times 10^{10}$ CFU), or saline, respectively. The respective vaccine was injected subcutaneously into cervical areas drained by the superficial cervical lymph node with total dosage divided equally between right and left sides.

Bison were randomly assigned for surgical removal of right or left superficial cervical lymph nodes at 1, 2, 4, 6, 10, and 16 wk post-vaccination. Animals were anesthetized with intravenous administration of xylazine (0.1 to 0.2 mg/kg, Haver Lockhart, Mobay Corp, Shawnee, Kansas, USA) and ketamine-HCl (3 to 5 mg/kg, Vetalar, Fort Dodge Labs, Fort Dodge, Iowa). Excision of the superficial cervical lymph node was performed as done for cattle (Cheville et al., 1992).

For bacteriology evaluation, cross sections were taken in three areas of lymph nodes (proximal, middle, and distal), weighed, triturated using a tissue grinder, serially diluted in 0.01% saline, and placed on tryptose agar plates (Difco Laboratories, Detroit, Michigan, USA) containing 5% bovine serum. Mean weight of cross sections were 0.906 gm (±0.083 SEM). Following incubation at 37 C and 5% CO_2,

TABLE 1. Colony forming units of *Brucella abortus* per gram of tissue from the superior cervical lymph node of vaccinated bison.[a]

Brucella abortus strain	Total bison per treatment	Weeks post-vaccination					
		1	2	4	6	10	16
RB51[b]	6	667	12,769	7,337	165	174	9
		104	19,367	7,450	3,001	68	33
S19[c]	3	370,075	131,967	7,746	1,216	324	0
Control	1	0					

[a] Average of three samples taken at proximal, middle and distal portions of lymph node.
[b] Two bison biopsied at each time point.
[c] One bison biopsied at each time point.

bacterial cell counts were made from each dilution by standard plate counts. *Brucella abortus* was identified on the basis of colony, morphology, and growth characteristics (Alton et al., 1988).

Areas adjacent to all bacteriology samples were placed in neutral, buffered, 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Formalin-fixed sections were also stained with polyclonal rabbit anti-SRB51 antibodies in an avidin-biotin-peroxidase complex immunoenzyme technique (Meador et al., 1986). Controls ran with each assay included sections of lymph node from the nonvaccinated bison, and RB51- or S19-infected mouse liver. Buffer control slides, sections in which the secondary antibody was added without the primary antibody, were also run concurrently.

Blood was collected by venipuncture prior to vaccination and at 2, 4, 10, 12, 16, 20, and 40 wk post-vaccination. Serum was stored at −70 C until use. Titers of animals vaccinated with SRB51 and S19 were determined with a standard tube agglutination test (STT; Alton et al., 1988), a Brucella card test (Becton Dickinson, Cockeysville, Maryland, USA), and an antibody dot-blot assay in which γ-irradiated SRB51 is used as antigen (Cheville et al., 1993). Based on preliminary experiments, the peroxidase-conjugated rabbit anti-bovine immunoglobulin-G antibody, heavy and light chain-specific, (Jackson Immunoresearch Labs, West Grove, Pennsylvania, USA) cross-reacted with bison antibodies. Results were expressed as the highest serum titer that produced a visible color reaction that was greater than the reaction for a negative serum sample.

Superficial cervical lymph nodes of SRB51-vaccinates had maximal numbers of *B. abortus* present at 2 wk post-vaccination (Table 1). Numbers of *B. abortus* declined in all samples thereafter but remained present in both biopsies from SRB51-vaccinates at 16 wk post-vaccination. Recovery of *B. abortus* from S19-vaccinates declined after 1 wk post-vaccination. When compared to SRB51-vaccinates, numbers of *B. abortus* recovered from the S19-vaccinated bison in week 1 and 2 were greater (approximately 1,000-fold and seven-fold higher, respectively). Results were similar for both vaccine groups for the remaining times, with the exception that *B. abortus* was not recovered from the biopsy taken from a S19 vaccinate at 16 wk post-vaccination.

At 1 to 2 wk post-vaccination, lymph nodes from bison infected with SRB51 were characterized by variable filling of subcapsular and intermediate sinuses with large, lightly eosinophilic, vacuolated macrophages and lesser numbers of neutrophils (Fig. 1A). Lymph nodes had moderate numbers of small (0.5 to 1 mm) lymphoid follicles and germinal centers at week 1 which were larger (1 to 2 mm) and more numerous by week 2. Lymphoid follicles and germinal centers were large and numerous throughout the remainder of the experiment. Subcapsular sinuses of SRB51-vaccinates were normal, and con-

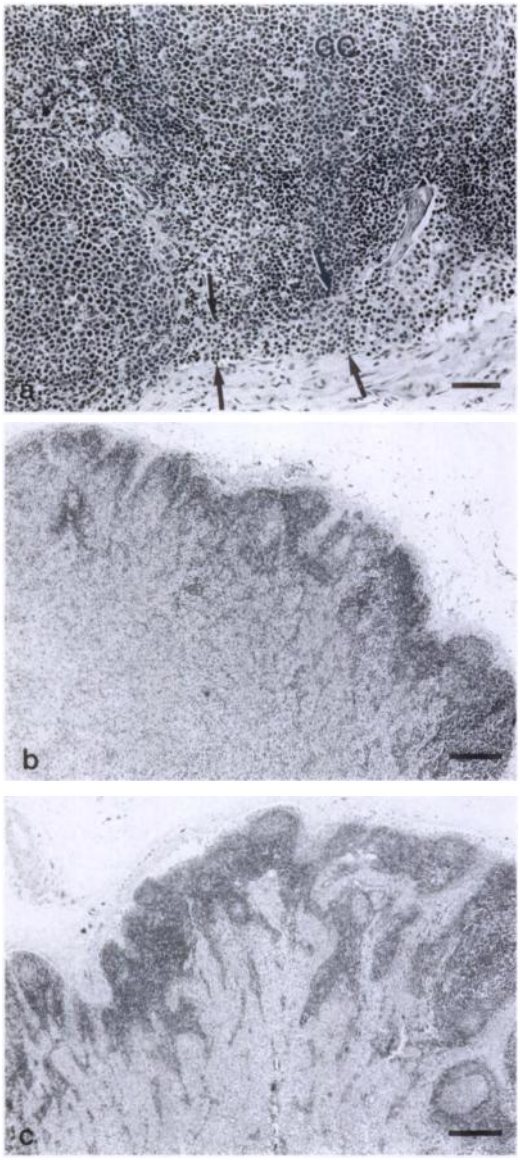

FIGURE 1. Bison superficial cervical lymph node at 2 wk (A, scale bar = 100 μm) and 16 wk (B, scale bar = 350 μm) following vaccination with *B. abortus* strain RB51 or 16 wk post-vaccination with *B. abortus* strain S19 (C, scale bar = 350 μm). Note germinal center (GC) and subcapsular sinus (arrows) in A.

tained only low numbers of reticular cells and lymphocytes by week 4 of the experiment. However, intermediate sinuses of the deep cortex, and to a lesser extent the medullary sinuses, were filled with macrophages and lesser numbers of neutrophils. These infiltrates resulted in expansion of the deep cortex causing the outer cortex to be attenuated in some regions. This attenuation persisted through week 16 (Fig. 1B).

Lymph nodes from S19-infected bison at 1 to 2 wk post-vaccination had more extensive infiltrates of macrophages and neutrophils filling intermediate sinuses of the deep cortex than SRB51-infected bison. These infiltrates expanded the normal deep cortex and attenuated the outer cortex in a similar manner to that seen in SRB51-vaccinates at later time periods. Low numbers of small (0.5 mm) lymphoid follicles were present. By 4 wk post-vaccination, cortical lymphoid follicles were larger and more numerous; however, infiltrates of macrophages persisted in the deep cortex. Infiltrates of macrophages expanding the deep cortex and to a lesser degree the medullary sinuses, persisted through week 16 (Fig. 1C). The lymphoid follicles and germinal centers (1 to 2 mm) remained prominent at wk 16.

Based on immunoperoxidase staining, we observed *B. abortus* antigens in the cytoplasm of macrophages in intermediate sinuses of the deep cortex, in medullary sinuses, and within cortical germinal centers of lymph nodes infected with either S19 or SRB51. Roughly equal numbers of cells stained positively for *B. abortus* antigen in S19- and SRB51-infected lymph nodes. Strain RB51 antigen was detected in SRB51-vaccinates at all time points post-infection; however, there was a trend of decreased numbers of antigen-positive cells after week 6 with very low numbers observed at week 16. Strain 19 antigen was detected in lymph nodes of S19-vaccinates at 1 to 10 wk, but not at 16 wk post-infection, with the greater number of antigen-positive cells seen at week 10.

Sera obtained from all bison were negative in the STT and dot-blot tests prior to vaccination, and SRB51-vaccinates remained seronegative on the STT in all sampling periods (Fig. 2). Bison vaccinated with S19 had STT titers which peaked

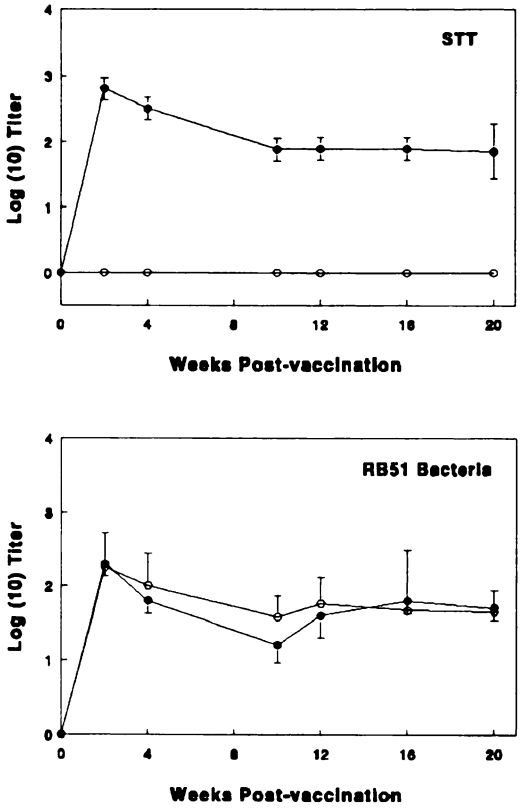

FIGURE 2. Serological responses of SRB51-vaccinated (–○–, n = 6) or S19-vaccinated (–●–, n = 3) bison in the standard tube agglutination test (STT), or to γ-irradiated *B. abortus* strain RB51 in a dot-blot test (RB51 Dot-blot). Responses are presented as the mean titer $\log^{10}$.

at 2 wk post-vaccination and gradually declined. All S19-vaccinated bison were seronegative when evaluated using the STT at 40 wk post-vaccination. Antibody titers of SRB51- and S19-vaccinated animals were similar when evaluated using the dot-blot test (Fig. 2). Dot-blot titers peaked at 2 wk post-vaccination but were still present in two SRB51-vaccinates at 40 wk post-vaccination. All bison vaccinated with S19 were positive on the card test at 2 and 4 wk post-vaccination, but not prior to vaccination or at 10 wk after vaccination. In comparison, all SRB51-vaccinates remained negative at these time points.

Based on our results, the SRB51 strain of *B. abortus* may persist longer in bison following vaccination than in cattle. There also may be differences in time for removal of S19 and SRB51 in bison. Although the numbers of bison vaccinated with SRB51 or S19 in this experiment were small (six and three, respectively), SRB51 was recovered from all lymph node samples from SRB51-vaccinated bison, including both biopsies taken at 16 wk post-vaccination. When four age-matched cattle were vaccinated with SRB51 (1.5×10^{10} CFU), *B. abortus* were isolated from biopsy samples up to 4 wk but not at 6 or 10 wk post-vaccination (N. F. Cheville, unpubl.). Clearance of the bacteria from bison also was delayed when compared to similar data from 10-mo-old cattle in which SRB51 (1.2×10^{10} CFU) was cleared from draining lymph nodes by 6 wk (Cheville et al., 1992). The difference in vaccine dosages between cattle and bison (1.2 to 1.5×10^{10} and 1.5 to 2.3×10^{10} CFU, respectively) were relatively minor. However, this difference may have influenced the clearance of *B. abortus* from lymph nodes.

Despite the low numbers of S19-vaccinated bison used in this study, the bacterial numbers present within lymph nodes were 2- to 72-fold higher at all times when compared to the highest bacterial counts recorded from matching post-vaccination biopsies of cattle vaccinated with S19 (1 to 1.9×10^{10} CFU) at 3, 5 (N. F. Cheville, unpubl.), or 10 mo (Cheville et al., 1992) of age. However, as only one bison was examined at each time point in this study, additional work using more S19-vaccinated bison will be required to characterize clearance of S19 in bison.

Serological responses by bison to S19 were similar to those seen in cattle (Cheville et al., 1992) except that peak titers were lower and titers persisted longer after vaccination in bison as compared to cattle. Davis (1993) reported that S19 vaccination of bison calves may cause persistent titers which may increase the incidence of ani-

mals which give positive reactions as adults on brucellosis surveillance tests. Although data from the three bison in our study did not concur, the reported tendency for S19 to cause persistent titers and chronic infections in adult bison (Davis et al., 1991) could make it undesirable for use in bison herds.

The persistence of SRB51 in vivo and the similarity of histological lymph node lesions induced by SRB51 as compared to S19 were unexpected. Perhaps bison evoke a stronger granulomatous response to SRB51 than previously reported in other species. Lymph nodes obtained from SRB51-vaccinated cattle have only slight granulomatous changes in the deep cortex in comparison to more severe reactions induced in the deep cortex and superficial medulla by vaccination with S19 (Cheville et al., 1992). Spleens of mice vaccinated with SRB51 had fewer histiocytes, larger and more prominent germinal centers, and little to no lymphoid depletion when compared to spleens of mice vaccinated with S19 (Stevens et al., 1994b). Our results with S19-vaccinates also concurs with Davis et al., (1990) who reported that histological lesions of *B. abortus* in bison are similar to lesions in cattle.

Although the clinical and histological appearance of *B. abortus* infection in bison can mimic the infection in cattle, these data further support the idea there are some differences in responses of bison and cattle to the SRB51 and S19 vaccine strains, and possibly other *Brucella* strains. Further work will be required to characterize persistence of SRB51 in bison and to determine if SRB51 vaccination will protect against *B. abortus* infection and abortion. If it produces a protective immune response in bison, SRB51 would offer an advantage over S19 for a combined vaccination-surveillance program due to its negative serological responses using common diagnostic surveillance tests.

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No endorsements are herein implied. Brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the products. The use of the names by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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