Evaluation of serologic responses, lymphocyte proliferative responses, and clearance from lymphatic organs after vaccination of bison with *Brucella abortus* strain RB51

Steven C. Olsen, DVM, PhD; Allen E. Jensen, PhD; Mitchell V. Palmer, DVM, PhD; Mark G. Stevens, DVM, PhD

Objective—To evaluate clearance of the vaccine strain, immunologic responses, and potential shedding of *Brucella abortus* strain RB51 organisms after vaccination of bison calves.

Animals—Fourteen 7-month-old female bison calves.

Procedure—10 bison calves were vaccinated SC with 1.22×10^{10} colony-forming units of *B abortus* strain RB51. Four bison calves were vaccinated SC with 0.15*M* NaCl solution. Rectal, vaginal, nasal, and ocular swab specimens were obtained to evaluate potential shedding by vaccinated bison. The superficial cervical lymph node was biopsied to evaluate clearance of the vaccine strain. Lymphocyte proliferative responses to strain RB51 bacteria were evaluated in lymph node cells obtained from biopsy specimens and also in peripheral blood mononuclear cells.

Results—Strain RB51 was recovered from superficial cervical lymph nodes of vaccinates examined 6, 12, and 18 weeks after vaccination (4/4, 3/4, and 1/4, respectively) but not in vaccinates examined at 24 weeks (0/3) after vaccination or nonvaccinates examined at all sample collection times (n = 1 bison/sample period). Serologic, immunologic, and bacterial culture techniques failed to reveal shedding of strain RB51 by vaccinates or infection of nonvaccinated bison. Lymphocyte proliferative responses were evident in lymph node cells and blood mononuclear cells from strain RB51-vaccinated bison beginning 12 weeks after vaccination.

Conclusion—Strain RB51 was cleared from bison by 18 to 24 weeks after vaccination. Bison vaccinated with strain RB51 did not shed the vaccine strain to nonvaccinated bison housed in close proximity. Strain RB51 did not induce antibody responses in bison that would interfere with brucellosis surveillance tests, but did stimulate cell-mediated immunity. (*Am J Vet Res* 1998;59:410–415)

Brucella abortus infections in elk (*Cervus elaphus nelsoni*) and bison (*Bison bison*) within the Greater Yellowstone Area (Yellowstone National Park, Grand Teton National Park, and adjacent areas) may threaten the ability to eradicate brucellosis from cattle by 1998. Between the years of 1992 and 1996, it is estimated that mean populations of approximately 3,500 bison and 115,000 elk inhabited this area.[a] Seroprevalence for brucellosis in these populations was approximately 50% for the bison population,[a] 37% for adult female elk on feedgrounds,[1] and less than 2% for elk that did not spend the winter on feedgrounds.[b] The ability of brucellosis to be transmitted from bison[2] and elk[3] to cattle has been proven under experimental conditions. The current vaccine used in cattle, *B abortus* **strain 19 (S19)**, has been used in bison, but appears to be more virulent in bison, causing a high number of abortions.[4]

A new brucellosis vaccine for cattle, *B abortus* **strain RB51 (SRB51)**, was recognized by the USDA-Animal Plant Health and Inspection Service for use as an official calfhood vaccine in March 1996. The SRB51 was identified by passage of the *B abortus* strain 2308 on media containing rifampin.[5] The SRB51 vaccine is protective in cattle when administered to calves between 3 and 10 months of age.[6] Because of low expression of the lipopolysaccharide O-side chain,[5] SRB51 offers an advantage over S19 for calfhood vaccination. Cattle vaccinated with SRB51 remain seronegative when evaluated by using routine brucellosis surveillance tests such as the standard tube agglutination, rivanol, or complement-fixation tests,[7] which detect antibodies against the lipopolysaccharide O-side chain.

We have previously evaluated the use of SRB51 in bison. After SC vaccination of bison with 10^{10} **colony-forming units (CFU)** of SRB51, the vaccine strain can be recovered for at least 16 weeks from the lymph node draining the site of injection.[8] Bison vaccinated with SRB51 remain seronegative at all times when tested by use of the card and standard tube agglutination tests, although antibody responses to SRB51 can be detected by using a dot-blot assay[8] that has high sensitivity and specificity in cattle.[9] The purposes of the study reported here were to determine when SRB51 is cleared from the lymph nodes of vaccinated bison, to characterize cell-mediated responses, and to assess the biosafety of this vaccine by determining whether bison shed SRB51 after inoculation.

Materials and Methods

***Brucella abortus* culture**—A master seed stock of *B abortus* SRB51 was obtained.[c] After one passage on tryptose agar, this seed stock was designated ARS/1. For experimental use, SRB51 (ARS/1) bacteria were grown on tryptose agar[b] for 48 h at 37 C. For the dot-blot assay, SRB51 suspensions

Received for publication Apr 7, 1997.
Manuscript passed review Nov 3, 1997.
From the Zoonotic Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, 2300 Dayton Ave, Ames, IA 50010.
The authors thank Richard Hornsby, Randy Capsel, Aileen Duit, Terry Krausman, Howard Cox, Dennis Weuve, Royce Huber, Kathy McPeak, David Wiseman, Bill Berg, Tim Smith, and Kevin Sherman for technical assistance.
Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Unauthenticated | Downloaded 04/22/25 05:06 PM UTC

(1.3×10^{12} CFU/ml) were inactivated by γ-irradiation (1.4×10^6 rads). After irradiation, suspensions were washed in 0.15*M* NaCl solution and stored in 1-ml aliquots at −70 C.

For vaccination of bison, a commercially available product[d] derived from ARS/1 was prepared according to the manufacturer's instructions. The vaccine was then diluted in 0.9% NaCl solution to contain approximately 10^{10} CFU, as determined from standard plate counts on other vials with the same lot number. After dilution, concentration of viable bacteria within the inoculum was determined, using standard plate counts.

Animals and inoculation—Fourteen 7-month-old bison heifers from brucellosis-free herds were donated by the National Park Service. After a 4-week acclimation period, 10 bison were vaccinated SC with 1.22×10^{10} CFU of SRB51 suspended in 4 ml of 0.9% NaCl solution. The remaining 4 bison were given 4 ml of 0.9% NaCl solution, SC. All vaccinations were administered in divided doses (2 ml/side) in the right and left cervical regions, areas drained by the superficial cervical lymph nodes.

Serologic evaluation—Blood samples were collected by jugular venipuncture prior to vaccination and 2, 4, 6, 8, 12, 18, and 24 weeks after inoculation. Blood was allowed to clot for 12 h at 4 C and was then centrifuged. Serum was divided into 1-ml aliquots, frozen, and stored at −70 C.

Serologic titers to *Brucella* organisms were determined by use of a standard tube agglutination test[10] and a previously described antibody dot-blot assay in which γ-irradiated SRB51 is used as antigen.[9]

Bacterial culturing of blood and monitoring of shedding by vaccinates—Rectal, vaginal, nasal, and ocular swab specimens were obtained from all bison 1, 2, 3, 4, 6, 8, and 12 weeks after inoculation. Swabs were plated on tryptose agar containing 5% bovine serum. To determine whether there was prolonged SRB51 septicemia in bison, 20 ml of blood was obtained from all bison 2, 4, 6, and 8 weeks after inoculation and mixed 1:1 with tryptose broth containing 1% sodium citrate. One milliliter of solution from bacterial cultures of blood collected from each bison were directly plated on tryptose agar containing 5% bovine serum. Remaining bacterial cultures of blood samples were stored at −5 C for 24 h and then incubated at 37 C and 5% CO_2 with 1-ml volumes plated onto tryptose agar containing 5% bovine serum 1, 2, 3, 7, 14, 21, and 35 days after beginning of incubation. After incubation of tryptose agar plates at 37 C and 5% CO_2 for 72 h, SRB51 was identified on the basis of colony morphology, growth characteristics, and resistance to rifampin.

Lymph node biopsy—The right or left superficial cervical lymph node of 4 SRB51-vaccinates and 1 nonvaccinated bison was randomly selected for biopsy 6, 12, 18, and 24 weeks after inoculation. After surgical removal,[6,8] each lymph node was divided into proximal, middle, and distal portions. Lymph node sections were weighed, triturated by using a tissue grinder, serially diluted in 0.9% NaCl solution, and placed on tryptose agar plates containing 5% bovine serum.[6] After incubation at 37 C and 5% CO_2, bacterial cell counts were made from each dilution using standard plate counts. The SRB51 organisms were identified on the basis of colony morphology and growth characteristics.[10]

Areas adjacent to samples collected for bacterial culture were placed in neutral-buffered 10% formalin, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with H&E. Formalin-fixed sections were stained with polyclonal rabbit anti-SRB51 antibodies, using an avidin-biotin-peroxidase complex immunoenzyme technique.[11] Control samples processed with each assay included sections of lymph node from nonvaccinated bison and sections of SRB51-infected mouse liver. Buffer-control slides (sections in which the secondary antibody was added without the primary antibody) were also processed concurrently.

Preparation of SRB51 proteins—Whole-cell lysates from γ-irradiated SRB51 (approx 1.5×10^{11} CFU) were separated into a range of 106- to 18-kDa proteins by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE), eluted into 22 fractions by using a Blotelutor,[e] and concentrated by membrane filtration.[12] A 50-µl aliquot of each protein fraction (representing undiluted fractions and fractions diluted 1:2 and 1:4) was added in duplicate to wells of a 96-well microtiter plate, and plates were stored at −70 C until used. Protein assays[f] of the concentrated fractions indicated that aliquots added to each well contained approximately 0.25 to 1.25 µg of protein.

Preparation of peripheral blood mononuclear cells and lymph node cells for lymphocyte proliferation assays—Five SRB51-vaccinated and 3 control bison were randomly selected at the initiation of the study for evaluation of proliferative responses of peripheral blood mononuclear cells. Blood was obtained from the jugular vein of selected bison 2, 4, 6, 8, 12, and 18 weeks after vaccination and placed into an acid-citrate dextrose solution. Blood mononuclear cells were enriched by density centrifugation, using a polysucrose-sodium diatrizoate gradient.

For preparation of lymph node cells, sections of superficial cervical lymph nodes were placed on a sterile 60-mesh stainless-steel screen, minced with scissors, and processed to form a single-cell suspension, as described previously.[12] Lymph node cells and blood mononuclear cells were diluted in RPMI 1640 medium[g] to a final concentration of 1×10^7 viable cells/ml as determined by use of trypan blue dye exclusion.

Fifty microliters of each cell suspension containing 5×10^5 lymph node cells or blood mononuclear cells was added to each of 2 separate flat-bottom wells of 96-well microtiter plates that contained 100 µl of RPMI 1640 medium only, 1640 medium containing γ-irradiated SRB51 (10^9 to 10^5 bacteria/well), or 1640 medium containing PAGE-separated SRB51 proteins. Cell cultures were incubated for 7 days at 37 C and 5% CO_2. Microtiter plates were shaken for 1 minute on an electronic device every 2 days during the incubations. After incubation for 7 days, 1.0 µCi of [^{3}H]thymidine per well was added, and cell cultures were incubated for an additional 18 h. Cells were harvested onto glass filter mats and counted for radioactivity in a liquid scintillation counter. Cell proliferation results were converted to stimulation indices (counts per minute of wells containing antigen ÷ counts per minute of wells without antigen) for statistical comparisons.

Statistical analysis—Differences between treatments in proliferative responses to γ-irradiated bacteria for each sample collection period were compared by use of a general linear model procedure.[h] Serologic responses for all periods and proliferative responses to protein fractions at each sample collection period were compared, using a two-way ANOVA. Because biopsy specimens from only 1 control bison were available at each time point, responses of lymph node cells from control bison were pooled for all time points for comparison with responses of lymph node cells from SRB51-vaccinated bison. Means for specific treatments were separated by use of a least-significant difference procedure ($P < 0.05$).

Results

Serologic evaluation—Bison vaccinated with SRB51 remained seronegative by use of the standard tube agglutination test at all time points after inoculation. Vaccinated bison had greater ($P < 0.05$) antibody titers on the dot-blot test 2, 4, 6, and 8 weeks after inoculation, compared with titers of control bison (Fig 1).

Unauthenticated | Downloaded 04/22/25 05:06 PM UTC

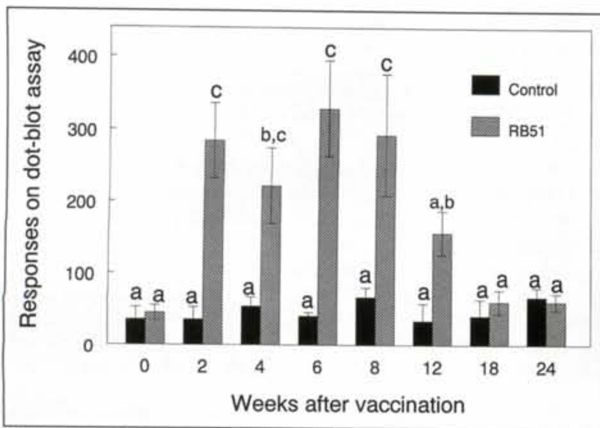

Figure 1—Serologic responses of strain RB51 (SRB51)-vaccinated (n = 10) or control bison (n = 4) to γ-irradiated SRB51 in a dot-blot assay. Responses are titer (mean ± SEM). Means with different superscript letters are significantly ($P < 0.05$) different.

Table 1—Number of *Brucella abortus* organisms per gram of superficial cervical lymph node tissue obtained from nonvaccinated and *B abortus* strain RB51-vaccinated bison

Treatment	n	Weeks after vaccination 6	12	18	24
Strain RB51	4	844 ± 248 (4/4)	605 ± 382 (3/4)	21 ± 24 (1/4)	0 ± 0 (0/3)
Nonvaccinated	1	0 ± 0 (0/1)	0 ± 0 (0/1)	0 ± 0 (0/1)	0 ± 0 (0/1)

Results are reported as number of colony-forming units (mean ± SEM).
No. in parentheses are No. of bison culture-positive for strain RB51, compared with No. of bison examined at each sample collection period.

Bacterial culturing of blood samples and monitoring of shedding—The blood sample from 1 SRB51-vaccinated bison was culture-positive for SRB51 at 2 weeks after inoculation. With the exception of this 1 sample, all other blood samples were culture-negative for *B abortus*. *Brucella abortus* was not recovered from fecal, conjunctival, nasal, or ocular swab specimens from any bison at any sample collection time.

Evaluation of tissue from superficial cervical lymph node—*Brucella abortus* SRB51 was cultured from 4/4, 3/4, 1/4, and 0/3 bison at 6, 12, 18, and 24 weeks after inoculation, respectively (Table 1). Lymph node biopsy specimens from all control bison (n = 1 bison/sample period) remained culture-negative for *B abortus*.

At 6 weeks after vaccination, lymph nodes from vaccinated bison were characterized by filling of intermediate sinuses, and to a lesser extent medullary sinuses, by a variable number of macrophages and a lesser number of neutrophils (Fig 2). These infiltrates caused mild to moderate expansion of the deep cortex, which attenuated the superficial cortex in some areas. Most SRB51 vaccinates had a moderate number of germinal centers (0.5 to 1.5 mm in diameter) within the superficial cortex, although the number of lymphocytes in areas between germinal centers was decreased. Lymph node sections obtained 12 and 18 weeks after vaccination were similar, although germinal centers were larger (1 to 2 mm in diameter), more numerous, and were surrounded by larger and more prominent mantle layers of lymphocytes with dark basophilic nuclei. In lymph nodes obtained 6 and 12 weeks after vaccination, and to a lesser extent at 18 weeks, macrophages were the predominant cell type within medullary cords as a result of decreased numbers of lymphocytes. At 24 weeks after vaccination, the cortex was expanded in most areas by numerous and prominent germinal centers and associated lymphoid follicles, although focal areas of attenuation of the superficial cortex remained. Medullary cords in these samples were uniformly filled with lymphocytes, plasma cells, macrophages, and a few neutrophils.

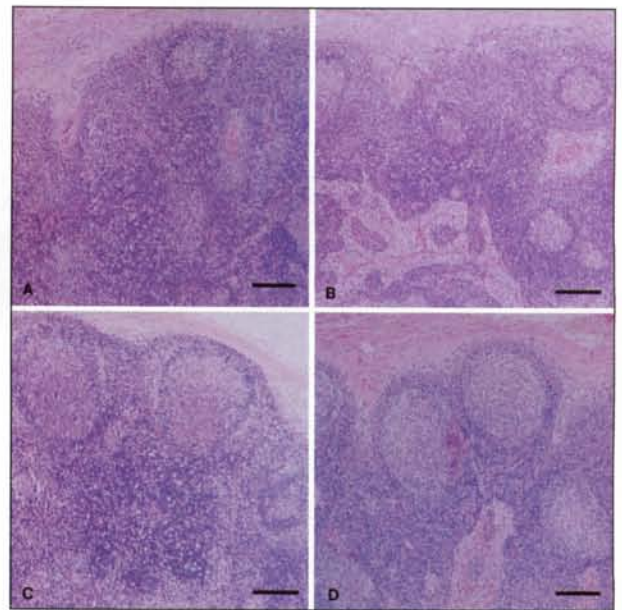

Figure 2—Photomicrograph of sections of prescapular lymph nodes from a nonvaccinated bison (A) and from strain RB51-vaccinated bison at 6 (B), 12 (C), and 18 weeks (D) after inoculation. H&E stain; bar = 200 μm.

Immunohistologic examination of superficial cervical lymph nodes of SRB51-vaccinates 6, 12, 18, and 24 weeks after vaccination revealed positive staining for SRB51 antigens in 1/4, 2/4, 2/4, and 0/3 bison lymph nodes, respectively. Cells staining positive in samples obtained 6 and 12 weeks after vaccination were macrophages in the deep cortex or within germinal centers, whereas positively staining cells were predominantly macrophages within medullary cords in samples obtained 18 weeks after vaccination. Compared to culture results, immunohistologic examination identified 1/4, 1/3, and 1/1 culture-positive lymph nodes in samples obtained 6, 12, and 18 weeks after vaccination, respectively.

Lymphocyte proliferation assays—At all sample collection periods prior to 12 weeks after inoculation, superficial cervical lymph node cells from SRB51 vaccinates failed to cause proliferative responses to γ-irradiated whole SRB51 bacteria or PAGE-separated SRB51 proteins that were greater than responses of nonvaccinated bison (data not shown). Lymph node cells from SRB51 vaccinates had significantly ($P < 0.05$) greater proliferative responses to irradiated whole SRB51 bacteria 12 and 24 weeks after vaccination, compared with responses of control bison (Fig 3). Responses to SRB51 bacteria by lymph node cells obtained from SRB51-vaccinated bison 18 weeks after inoculation were not greater than responses of cells from nonvaccinates (data not shown). Responses of lymph node cells from vaccinates to PAGE-separated

Unauthenticated | Downloaded 04/22/25 05:06 PM UTC

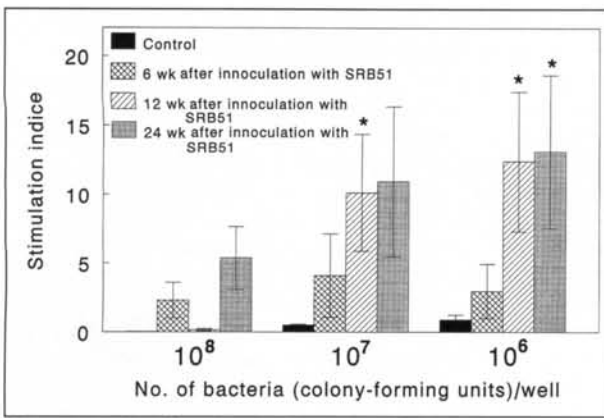

Figure 3—Proliferative responses of superficial cervical lymph node cells from SRB51-vaccinated or nonvaccinated bison to γ-irradiated SRB51. Cells were incubated at 37 C and 5% CO_2 for 7 days and labeled for 18 h with [^{3}H]thymidine. Results are expressed as mean stimulation indexes. *Means are significantly ($P < 0.05$) different from responses at other sample collection periods for that concentration of SRB51. Responses of nonvaccinated bison (n = 4) were pooled for all time periods, whereas data from SRB51-vaccinated bison (n = 4/time period) represent samples obtained 6, 12, and 24 weeks after inoculation.

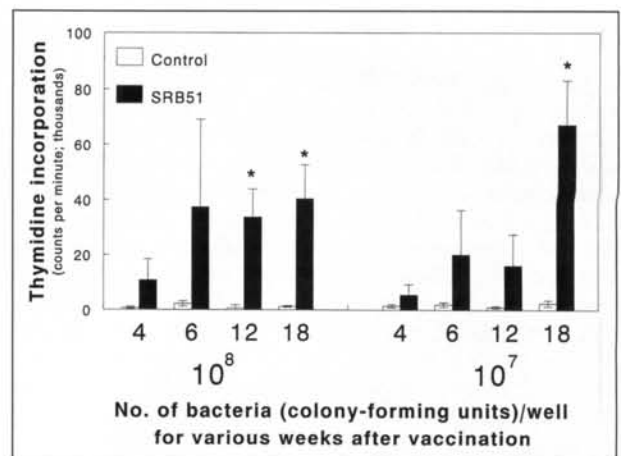

Figure 5—Proliferative responses of peripheral blood mononuclear cells from SRB51-vaccinated (n = 5/time period) and control (n = 3/time period) bison to γ-irradiated SRB51 4, 6, 12, or 18 weeks after innoculation. Cells were incubated at 37 C and 5% CO_2 for 7 days and labeled for 18 h with [^{3}H]thymidine. Results are expressed as counts per minute (mean ± SEM). *Means of SRB51-vaccinates are significantly ($P < 0.05$) different from responses of control bison to that concentration of bacteria at that sampling time.

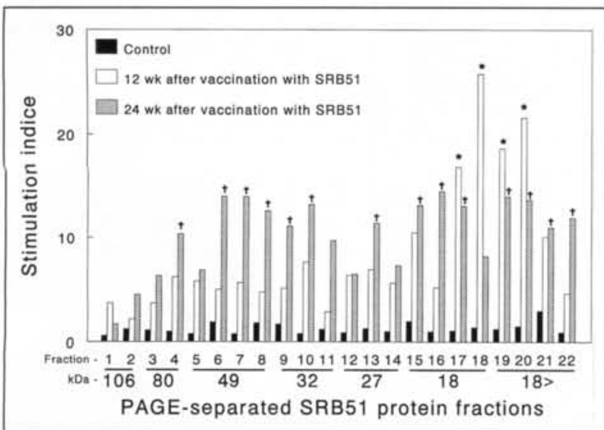

Figure 4—Proliferative responses of superficial cervical lymph node cells from SRB51-vaccinated and nonvaccinated bison to 1D-polyacrylamide gel electrophoresis (PAGE)-separated SRB51 proteins. Cells were incubated at 37 C and 5% CO_2 for 7 days and labeled for 18 h with [^{3}H]thymidine. Results are expressed as mean stimulation indexes. *,†Means for 12 or 24 weeks, respectively, are significantly ($P < 0.05$) different from responses of cells from control bison to that protein fraction. Responses of nonvaccinated bison were pooled for all time periods, whereas data from SRB51-vaccinated bison (n = 4/time period) represent samples obtained 12 and 24 weeks after inoculation.

SRB51 proteins were significantly ($P < 0.05$) greater than cells from control bison 12 and 24 weeks after vaccination, but not 18 weeks after inoculation (Fig 4).

Responses to γ-irradiated whole SRB51 bacteria by blood mononuclear cells isolated from SRB51-vaccinated or control bison prior to 12 weeks after vaccination did not differ. Samples obtained 12 and 18 weeks after vaccination yielded blood mononuclear cells from SRB51-vaccinated bison that had proliferative responses to SRB51 bacteria that were greater ($P < 0.05$) than responses of cells isolated from nonvaccinated bison (Fig 5).

Discussion

Analysis of the results of the study reported here indicated that the SRB51 vaccine was safe for use as a calfhood vaccine in bison. Bison vaccinated in this study did not have vaccine-related clinical signs during the 24-week period after vaccination. Analysis of our data indicated that it was unlikely that SRB51-vaccinated bison will laterally transmit the vaccine strain to unvaccinated bison or shed it from mucosal surfaces into the environment. The lack of transmission to nonvaccinated bison was also supported by serologic and bacterial culture data that failed to indicate exposure or infection in any of the control bison housed in close proximity to the SRB51-vaccinated bison.

Analysis of data from the study indicated that bison will clear the SRB51 vaccine from the lymph node draining the site of inoculation by 18 to 24 weeks after vaccination. This is consistent with results of another study[8] in which investigators found a low number of SRB51 in the superficial cervical lymph node of bison 16 weeks after vaccination. In both studies, clearance of SRB51 from lymph nodes of bison was delayed, compared to results of studies in cattle in which the vaccine strain was cleared between 6 and 12 weeks.[6,13] Results from these studies and data from other studies[4,14] on bison would suggest that, compared to cattle, bison may be more susceptible to *B abortus* infections and may not clear the bacteria as quickly.

Analysis of results of our study also indicated that vaccination of bison with SRB51 stimulated humoral and cell-mediated responses. Lack of serologic responses on brucellosis surveillance tests by SRB51-vaccinated bison in this study and another study[8] suggests that SRB51 will not interfere with identification of bison infected with field strains of *Brucella*. Others have reported similar results after SRB51 vaccination of naive or S19-vaccinated cattle.[7,15] Although a correlation

Unauthenticated | Downloaded 04/22/25 05:06 PM UTC

between greater lymphocyte proliferative responses and protection against *Brucella* infection has not been reported for cattle,[16,17] evaluation of blastogenic responses do provide experimental evidence of stimulation of the cell-mediated component of the immune response. Similar to protective immunity against other intracellular organisms, antibodies are considered to play a minor role in protecting cattle against *B abortus*.[18] Resistance conferred after vaccination of cattle is considered to be mostly attributed to cell-mediated immune responses.[18] The delay in detection of lymphocyte proliferative responses to SRB51 antigens was also similar to that of cattle in which lymph node cells failed to respond to PAGE-separated SRB51 proteins or whole S2308 bacteria before 10 or 12 weeks after vaccination.[12]

We were unable to explain the failure of lymph node cells obtained from vaccinates 18 weeks after vaccination to yield proliferation responses against irradiated SRB51 or PAGE-separated SRB51 proteins that were greater than responses of nonvaccinated bison. Responses of SRB51 vaccinates 18 weeks after vaccination were similar to responses of vaccinates 12 and 24 weeks after vaccination and may have been of importance had responses of more bison been evaluated.

Proteins that mediate protective immunity against *B abortus* have not been adequately characterized, and the number of recombinant *Brucella* proteins available for characterizing cell-mediated responses is limited. Therefore, to characterize cell-mediated responses of bison after vaccination, we monitored lymphocyte proliferative responses to crude *B abortus* protein fractions that were separated by electrophoresis. The SRB51-PAGE fractions contained groups of proteins with similar molecular masses, rather than a single protein; therefore, adjustment of fractions so that each contained similar protein concentrations would not have been of benefit in characterizing proliferative responses. Because antigen concentrations could not be equalized within a fraction, it cannot be excluded that all SRB51 proteins of immunologic importance were of sufficient quantities to induce proliferative responses. We attempted to resolve this problem by dramatically increasing the concentration of bacteria used for protein isolation per well when compared to the concentration of whole bacteria that stimulated the largest proliferative responses in a study in cattle.[12]

Protein antigens separated from SRB51 by one-dimensional PAGE that stimulated proliferative responses in bison encompassed a greater range of molecular weights than has been reported for SRB51-vaccinated or S2308-infected cattle.[19,20] This may have been influenced by a number of factors, including time of sample collection, lymph node that was evaluated, differences in persistence of infection, or state of activation of lymph node cells. The greatest proliferative responses by bison lymph node cells were in the range (18 kDa or less) that stimulated the greatest responses by cattle lymph node cells. This may suggest that SRB51 proteins that stimulate the strongest cell-mediated responses are similar in bison and cattle.

In a previous study conducted by members of our laboratory,[8] we found that bison vaccinated with SRB51 at 3 months of age had lesions of granulomatous lymphadenitis and cortical thinning in the superficial cervical lymph node. Although there was evidence of lymphadenitis in the lymph nodes examined in the bison reported here, histologic lesions were not as severe or persistent. The cause of the difference is unknown, although age at vaccination may have had an influence, because bison were 7 months old at time of vaccination in the current study. Because the source of the SRB51 vaccine differed between the study reported here and our previous study, this or other undetermined factors may have also caused differences in histologic lesions. Analysis of the results of the current study also indicated that immunohistochemical analysis of 1 lymph node section has low sensitivity, compared with use of bacterial culture techniques, for identification of SRB51-infected tissues. Analysis of data from the study reported here indicated that this technique had a particularly low sensitivity when used on tissue obtained at the earlier sample collection periods. Therefore, for maximum sensitivity to identify tissues that have been or currently are infected with SRB51, a combination of bacterial culture and immunohistochemical techniques may be necessary.

Although we are not aware of published data documenting its efficacy, the S19 vaccine has traditionally been used to vaccinate bison against brucellosis. However, S19 is highly abortogenic in pregnant bison[a] and, therefore, may be more virulent in bison, compared with cattle. Because S19 also induces antibody titers that cannot be differentiated on brucellosis surveillance tests from titers resulting from natural infections, use of that vaccine in bison may impede surveillance and eradication efforts. Therefore, development of an alternative brucellosis vaccine for bison would be beneficial.

Although efficacy data for use of SRB51 in bison is also lacking, analysis of results of several studies[13,21] have indicated that this vaccine protects cattle against abortion and infection after experimental challenge with virulent *B abortus*. Analysis of currently available data would suggest that SRB51 is not as virulent in bison as S19; however, it can induce abortions in pregnant bison.[22] Analysis of data from the study reported here and other studies[4,22] suggested that use of calfhood vaccines, rather than adult vaccination, may be more appropriate for development of brucellosis eradication or control programs for bison. Alternative methods for delivery of a vaccine of proven efficacy may also need to be developed to address brucellosis problems in free-ranging bison.

[a] Mack J, National Park Service, Yellowstone National Park, City, Wyo: Personal communication, 1997.

[b] Kreeger T, Wyoming Fish and Game, City, Wyo: Personal communication, 1997.

[c] Provided by Schurig G, College of Veterinary Medicine, Virginia Tech, Blacksburg, Va.

[d] Colorado Serum Co, Denver, Colo.

[e] Biometra, Göttingen, Germany.

[f] Biorad, Richmond, Calif.

[g] Life Technologies Inc, Grand Island, NY.

[h] SAS Institute Inc, Cary, NC.

References

1. Herriges JD, Thorne ET, Anderson SL, et al. Vaccination of elk in Wyoming with reduced dose strain 19 Brucella: controlled studies and ballistic implant field trials, in *Proceedings*. US Anim Health Assoc 1989;93:640–655.

Unauthenticated | Downloaded 04/22/25 05:06 PM UTC

2. Davis DS, Templeton JW, Ficht TA, et al. *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis, and transmission to cattle. *J Wildlife Dis* 1990;26:360–371.

3. Thorne ET, Morton JK, Ray WC. Brucellosis, its effect and impact on elk in western Wyoming. In: Boyce MS, Hayden-Wing LO, eds. *North American Elk; ecology, behavior, and management*. Laramie, WY: University of Wyoming, 1979;212–220.

4. Davis DS, Templeton JW, Ficht TA, et al. *Brucella abortus* in bison. II. Evaluation of strain 19 vaccination of pregnant cows. *J Wildlife Dis* 1991;27:258–264.

5. Schurig GG, Roop RM, Bagchi T, et al. Biological properties of RB51; a stable rough strain of *Brucella abortus*. *Vet Microbiol* 1991; ;28:171–188.

6. Cheville NF, Olsen SC, Jensen AE, et al. Effects of age at vaccination on efficacy of *Brucella abortus* strain RB51 to protect cattle against brucellosis. *Am J Vet Res* 1996;57:1153–1156.

7. Stevens MG, Hennager SG, Olsen SC, et al. Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with *Brucella abortus* 19 or RB51. *J Clin Microbiol* 1994;32:1065–1066.

8. Olsen SC, Cheville NF, Kunkle RA, et al. Bacterial survival, lymph node changes, and immunologic responses of bison (*Bison bison*) vaccinated with *Brucella abortus* strain RB51. *J Wildlife Dis* 1997;33:146–151.

9. Olsen SC, Stevens MG, Cheville NF, et al. Experimental use of a dot-blot assay to measure serologic responses of cattle vaccinated with *Brucella* abortus strain RB51. *J Vet Diagn Invest* 1997; 9:363–367.

10. Alton GG, Jones LM, Angus RD, et al. *Techniques for the brucellosis laboratory*. Paris, France: Institut National de la Recherche Agronomique, 1988;17–136.

11. Meador VP, Tabatabai LB, Hagemoser WA, et al. Identification of *Brucella abortus* in formalin-fixed, paraffin-embedded tissues of cows, goats, and mice with an avidin-biotin-peroxidase complex immunoenzymatic staining technique. *Am J Vet Res* 1986; 47:2147–2150.

12. Stevens MG, Olsen SC, Cheville NF. Comparative analysis of immune responses in cattle vaccinated with *Brucella abortus* strain 19 or strain RB51. *Vet Immunol Immunopathol* 1995;44:223–235.

13. Cheville NF, Jensen AE, Halling SM, et al. Bacterial survival, lymph node changes, and immunologic responses of cattle vaccinated with standard and mutant strains of *Brucella abortus*. *Am J Vet Res* 1992;53:1881–1888.

14. Davis DS, Templeton JW, Ficht TA, et al. *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis, and transmission to cattle. *J Wildlife Dis* 1990;26:360–371.

15. Olsen SC, Evans D, Hennager SG, et al. Serologic responses of calfhood-vaccinated cattle to *Brucella abortus* strain RB51. *J Vet Diagn Invest* 1996;8:451–454.

16. Chukwu CC. Comparison of the brucellin skin test with the lymphocyte transformation test in bovine brucellosis. *J Hyg Lond* 1986;96:403–413.

17. Confer AW, Hall SM, Faulkner CB, et al. Effects of challenge dose on the clinical and immune responses of cattle vaccinated with reduced doses of *Brucella abortus* strain 19. *Vet Microbiol* 1985; 10:561–575.

18. Nicoletti P. Vaccination. In: Nielsen K, Duncan JR, eds. *Animal brucellosis*. Boca Raton, Fla: CRC Press Inc, 1990;283–299.

19. Stevens MG, Olsen SC, Cheville NF. Lymphocyte proliferation in response to *Brucella abortus* RB51 and 2308 proteins in RB51-vaccinated or 2308-infected cattle. *Infect Immun* 1996;64: 1007–1010.

20. Stevens MG, Olsen SC, Cheville NF. Lymphocyte proliferation in response to immunodominant antigens of *Brucella abortus* 2308 and RB51 in strain 2308-infected cattle. *Infect Immun* 1994;62: 4646–4649.

21. Cheville NF, Stevens MG, Jensen AE, et al. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus*. *Am J Vet Res* 1993;54:1591–1597.

22. Palmer MV, Olsen SC, Gilsdorf MJ, et al. Abortion and placentitis in pregnant bison (*Bison bison*) induced by the vaccine candidate, *Brucella abortus* strain RB51. *Am J Vet Res* 1996;54:1604–1607.

Unauthenticated | Downloaded 04/22/25 05:06 PM UTC