BioOne COMPLETE

IMMUNE RESPONSES OF BISON TO BALLISTIC OR HAND VACCINATION WITH BRUCELLA ABORTUS STRAIN RB51

Authors: Olsen, Steven C., Kreeger, Terry J., and Schultz, Will

IMMUNE RESPONSES OF BISON TO BALLISTIC OR HAND VACCINATION WITH *BRUCELLA ABORTUS* STRAIN RB51

Steven C. Olsen,[1,3] **Terry J. Kreeger,**[2] **and Will Schultz**[2]

[1] Bacterial Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa 50010, USA

[2] Wyoming Game and Fish Department, 2362 Highway 34, Wheatland, Wyoming 82201, USA

[3] Corresponding author (email: Solsen@nadc.ars.usda.gov)

ABSTRACT: From January through July of 2000, a study was conducted to evaluate clearance, immunologic responses, and potential shedding of *Brucella abortus* strain RB51 (SRB51) following ballistic or subcutaneous (SQ) vaccination of 7 mo old bison (*Bison bison*) calves. Ten bison calves were vaccinated SQ with 1.4×10^{10} colony-forming units (CFU) of SRB51 and five calves were inoculated SQ with sterile 0.15 M sodium chloride. An additional 10 bison calves were ballistically inoculated in the rear leg musculature with 1×10^{10} CFU of SRB51 and five calves were ballistically inoculated with an empty Biobullet®. Serologic responses were monitored at 0, 2, 4, 6, 8, 12, 18, and 24 wk using the standard tube agglutination test and a dot-blot assay. Swabs from rectal, vaginal, nasal, and ocular mucosal surfaces, and blood were obtained for culture from all bison at 2, 4, 6, and 8 weeks post-inoculation to evaluate potential shedding by vaccinated bison or persistent septicemia. The superficial cervical lymph node was biopsied in eight ballistic and eight hand vaccinated bison at 6 or 12 wk to evaluate clearance of the vaccine strain from lymphatic tissues. Lymphocyte proliferative responses to irradiated SRB51 bacteria were evaluated in peripheral blood mononuclear cells (PBMC) at 4, 6, 8, 12, 18, and 24 wk after inoculation. Serum obtained from hand or ballistically vaccinated bison demonstrated antibody responses on the dot-blot assay that were greater than control bison (saline or empty Biobullet®) at 2, 4, 6, and 8 wk after vaccination. Antibody titers of ballistically vaccinated bison did not differ ($P>0.05$) from hand vaccinated bison at any sampling time. Blood samples obtained from all bison at 2, 4, 6 and 8 wk after vaccination were negative for SRB51. One colony of SRB51 was recovered from the vaginal swab of one ballistically vaccinated bison at 2 wk after vaccination. All other ocular, vaginal, nasal, and rectal swabs were culture negative for SRB51. Strain RB51 was recovered from superficial cervical lymph nodes of hand and ballistic vaccinated bison at 6 (two of four and two of four bison, respectively) and 12 wk (three of four and one of four bison, respectively). Serologic tests and bacterial culture techniques failed to demonstrate infection of nonvaccinated bison. Peripheral blood mononuclear cells obtained from hand vaccinated bison had greater ($P<0.05$) proliferative responses to strain RB51 bacteria when compared to PBMC from nonvaccinated and ballistically vaccinated bison. Proliferative responses of PBMC from ballistically vaccinated bison did not differ ($P>0.05$) at any sampling time from proliferative responses of PBMC from control bison. Serum α_1-acid glycoprotein concentrations, plasma fibrinogen, and total protein concentrations were not influenced by treatments. Ballistic delivery of SRB51 did not induce adverse effects or influence clearance of the vaccine strain. There were no proliferative responses of PBMC to SRB51 in bison ballistically vaccinated with SRB51; whereas bison inoculated with SRB51 by hand injection had greater proliferative responses than control or ballistically vaccinated bison. Our study suggests that ballistic delivery may require a greater dose of SRB51 to induce cell-mediated immune responses in bison that are comparable to those induced by hand injection, and that ballistic or hand delivery of 1×10^{10} CFU of SRB51 is safe in bison calves.

Key words: bison, *Brucella abortus*, efficacy, RB51, vaccine.

INTRODUCTION

Brucella abortus has almost been eradicated from cattle and privately-owned bison (*Bison bison*) within the United States. However, bison and elk (*Cervus elaphus*) within the Greater Yellowstone Area (GYA; Yellowstone National Park, Grand Teton National Park, and adjacent areas) maintain a high seroprevalence for brucellosis (Thorne et al., 1978; Pac et al., 1991) and may threaten the goal for eradication of brucellosis from cattle in the US. Because bison (Davis et al., 1990) and elk (Thorne et al., 1979) are capable of transmitting brucellosis under experimental conditions, there is concern that persistence of the disease in wildlife in the GYA may pose a threat to completion

of the brucellosis eradication program in cattle.

We have previously demonstrated that hand injection of bison with 1×10^{10} colony-forming units (CFU) of *B. abortus* strain RB51 vaccine (SRB51) does not cause clinical illness but does induce immune responses that are similar to responses of cattle (Olsen et al., 1998). Vaccinates did not transmit SRB51 to nonvaccinated bison housed in close association (Olsen et al., 1998).

Development of alternative delivery methods for vaccination of bison and elk are needed because a brucellosis management program is unlikely to be successful if capture is required for hand vaccination. One method, ballistic vaccination, has been used to inoculate free-ranging elk on Wyoming feedgrounds (Herriges et al., 1989), and it presently is the most promising candidate for use in bison. The purpose of this study was to compare immunologic responses of bison to ballistic or hand vaccination with SRB51 and to determine if vaccination induced an acute phase response.

MATERIALS AND METHODS

This study was conducted at the National Animal Disease Center in Ames (Iowa, USA; 42°52′N, 93°63′W) from January through July 2000.

Brucella abortus culture

A master seed stock of *B. abortus* SRB51 was obtained from G. Schurig (Virginia Tech, Blacksburg, Virginia, USA), and after one passage on tryptose agar (Difco Laboratories, Detroit, Michigan, USA), was designated as the ARS/1 seed stock of SRB51. For experimental use, SRB51 bacteria from the ARS/1 seed stock were grown on tryptose agar for 48 hr at 37 C. For the dot-blot assay, SRB51 suspensions (1.3×10^{12} colony-forming units [CFU]/ml) were inactivated by γ-irradiation (1.4×10^{6} rads). Following irradiation, suspensions were washed in 0.15 M sodium chloride (saline) and stored in 1 ml aliquots at −70 C.

For hand vaccination of bison, a commercially prepared SRB51 product (Colorado Serum Company, Denver, Colorado, USA) derived from the ARS/1 seed stock, was prepared according to the product literature. The vaccine was then diluted in saline to approximately 10^{10} CFU based upon standard plate counts on other vials with the same lot number. Following dilution, the concentration of viable bacteria within the inoculum was determined by standard plate counts.

For ballistic vaccination of bison, the lyophilized commercial strain RB51 product was incorporated into a 0.25 caliber, hydroxypropyl cellulose Biobullet® (Ballistic Technologies, Oklahoma City, Oklahoma, USA), and inoculated into the left hip region using an air-powered rifle system (Ballistic Technologies). The concentration of inoculum in the Biobullet® was determined by standard plate counts.

Animals and inoculation

Thirty, approximately 7 mo old bison heifers were obtained from brucellosis-free herds. After acclimation for 4 wk, 10 bison were vaccinated subcutaneously (SQ) with 1.4×10^{10} CFU SRB51 suspended in 2 ml of saline. Five bison were inoculated SQ with 2 ml saline. All inoculations were administered in the left cervical region drained by the superficial cervical lymph node.

Ten bison were ballistically inoculated in the left hip region with a Biobullet® containing 1.0×10^{10} CFU SRB51. Five additional bison were ballistically inoculated in a similar manner with an empty Biobullet®.

Serologic evaluation

Blood samples were collected by jugular venipuncture prior to vaccination, and at 2, 4, 6, 8, 12, 18, and 24 wk postinoculation. Blood was allowed to clot for 12 hr at 4 C and centrifuged. Serum was divided into 1 ml aliquots, frozen, and stored at −70 C.

Antibody titers to *Brucella* were determined by a standard tube agglutination test (STAT; Alton et al., 1988) and a previously described antibody dot-blot assay in which γ-irradiated SRB51 is used as antigen (Olsen et al., 1997b).

Plasma protein and fibrinogen

Plasma protein and fibrinogen were determined (Clinical Pathology Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA) using a refractometer. Concentration of fibrinogen was determined by heating plasma to 58 C for 3 min (Coles, 1980), centrifugation to clear agglutinated protein, and determination of protein concentration as described above. Fibrinogen concentration was defined as the difference in total protein concentrations of plasma before and after heating at 58 C.

Serum α_1-acid glycoprotein

Concentration of α_1-acid glycoprotein (AGP) was determined by radial immunodiffusion in accordance with manufacturer's instructions for a commercially available kit to measure bovine AGP (Cardiotech Services, Inc., Louisville, Kentucky, USA). All samples obtained over time from an individual bison were run concurrently on a single immunodiffusion plate.

Blood cultures and monitoring of shedding by vaccinates

Rectal, vaginal, nasal, and ocular swabs (American Scientific Products, McGaw Park, Illinois, USA) were obtained from all bison at 2, 4, 6, and 8 wk after inoculation. Swabs were plated on a selective media (RBM) for SRB51 containing rifampicin and other antibiotics (Hornsby et al., 2000). Antibiotics in RBM media minimize growth of contaminants without inhibiting growth of SRB51, thereby enhancing the ability to detect small numbers of SRB51. To determine if prolonged SRB51 septicemia occurs in bison, 15 ml of blood was obtained from bison at 2, 4, 6, and 8 wk after inoculation and mixed 1:1 with tryptose broth (Difco Laboratories, Detroit, Michigan, USA) containing 1% sodium citrate. One ml of this mixture was directly plated on RBM media. The remaining blood was held at -5 C for 24 hr and then placed at 37 C in 5% CO_2 with 1 ml volumes plated onto RBM media after 7, 14, 21, and 28 days incubation. Following incubation of plates at 37 C in 5% CO_2 for 72 hr, SRB51 was identified on the basis of colony morphology, growth characteristics, and resistance to rifampicin (Schurig et al., 1991), and confirmed by a polymerase chain reaction procedure using primers specific for identification of SRB51 (Vemulapalli et al., 1999).

Lymph node biopsy

Eight hand-vaccinated and eight ballistically-inoculated bison were randomly selected for surgical removal of the left superficial cervical lymph node at 6 or 12 wk after inoculation. After surgical removal as previously described (Cheville et al., 1992;

Olsen et al., 1997a, 1998), the lymph node was divided into proximal and distal portions. Lymph node sections were weighed, triturated using a tissue grinder, serially diluted in saline, and placed on tryptose agar plates containing 5% bovine serum. Following incubation at 37 C in 5% CO_2, bacterial cell counts were made from each dilution by standard plate counts. Strain RB51 was identified as described above.

Areas of lymph nodes adjacent to samples collected for bacteriology were placed into neutral, buffered, 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Lymphocyte proliferation assays

At 4, 6, 8, 12, 18, and 24 wk after vaccination, blood was obtained from the jugular vein of all bison and placed into an acid-citrate dextrose solution. Peripheral blood mononuclear cells (PBMC) were enriched by density centrifugation using a Ficoll-sodium diatrizoate gradient (Sigma Diagnostics, Inc., St. Louis, Missouri, USA). Peripheral blood mononuclear cells were diluted in RPMI 1640 medium (GIBCO Laboratories, Grand Island, New York, USA) to 1×10^7 viable cells per ml as determined by trypan blue dye exclusion.

Fifty μl of each cell suspension, containing 5×10^5 cells, was added to each of two separate flat-bottom wells of 96-well microtiter plates that contained 100 μl of RPMI 1640 medium only, or 1640 medium containing γ-irradiated SRB51 (10^5–10^9 bacteria per well). Microtiter plates were prepared prior to initiation of the study and maintained at -70 C until use. Cell cultures were incubated for 7 days at 37 C in 5% CO_2. Microtiter plates were placed on a shaker (Dynatech Laboratories Inc., Alexandria, Virginia, USA) every 2 days during the incubation and mixed at an instrument setting of 3.5 for 1 min. After 7 days incubation, cell cultures were pulsed with 1.0 μCi of [^{3}H]thymidine (Amersham Biosciences, Piscataway, New Jersey, USA) per well for 18 hr. Cells were harvested onto glass filter mats and count-

ed for radioactivity in a liquid scintillation counter. Cell proliferation results were converted to stimulation indices (counts per minute [cpm] of wells containing antigen/cpm in absence of antigen) for statistical comparisons.

Statistical analysis

Serologic, AGP, total protein, and fibrinogen data were analyzed as the logarithm of their value and compared over all times using a two-way analysis of variance model. Differences between treatments in proliferative responses to γ-irradiated bacteria at each sampling time were compared by a general linear model procedure (SAS Institute Inc., Cary, North Carolina, USA). Means for individual treatments were separated by use of a least significant difference procedure ($P<0.05$). Fisher's exact test was used to compare recovery of SRB51 from superficial cervical lymph node samples.

RESULTS

Two bison, one inoculated with 0.15 M NaCl and one hand vaccinated with SRB51, were removed from the study for reasons unrelated to the experimental treatments. Data from both bison were excluded from the statistical analysis.

Serologic evaluation

Bison hand or ballistically inoculated with SRB51 remained negative on the STAT at all times postinoculation. Bison vaccinated with SRB51 by hand injection or Biobullet® had greater ($P<0.05$) antibody titers on the dot-blot test at 2, 4, and 6 wk, but not 12 or 18 wk after vaccination, when compared to bison inoculated with saline or an empty Biobullet® (Fig. 1). Antibody titers of hand or ballistically vaccinated bison did not differ ($P>0.05$) at any sampling time.

Serum AGP and plasma protein and fibrinogen

Plasma total protein and fibrinogen concentrations did not differ ($P>0.05$) between bison vaccinated with SRB51 by hand injection or Biobullet® (data not shown). Serum concentrations of AGP were not influenced by ballistic or hand vaccination with SRB51 ($P>0.05$) but did decline in all treatments ($P<0.05$) with increasing time after vaccination (Fig. 2).

Blood cultures and monitoring of shedding

One colony of SRB51 was recovered from the vaginal swab of a ballistically vaccinated bison at 2 wk postinoculation. Remaining swabs were negative for SRB51. Blood obtained from all bison was negative for SRB51 at 2, 4, 6, and 8 wk after inoculation.

Superficial cervical lymph node biopsies

Strain RB51 was recovered from superficial cervical lymph nodes of hand and ballistic vaccinated bison at 6 (two of four and two of four bison, respectively) and 12 wk (three of four and one of four, respectively). Strain RB51 colonization (CFU/g) of superficial cervical lymph nodes (Table 1) and rate of recovery did not differ ($P>0.05$) between bison ballistically or hand inoculated with SRB51.

Lymphocyte proliferation assays

When compared to all other treatments, bison vaccinated with SRB51 by hand injection had greater proliferative responses ($P<0.05$) to γ-irradiated whole SRB51. Beginning at 6 wk after vaccination, PBMC from bison hand inoculated with SRB51 had greater proliferative responses ($P<0.05$) to the highest concentration of irradiated SRB51 bacteria (10^9 CFU) when compared to responses of PBMC from bison in all other treatments (representative data in Fig. 3). Proliferative responses of PBMC from bison ballistically inoculated with SRB51 did not differ ($P>0.05$) at any sampling time from responses of PBMC from bison inoculated with saline or an empty Biobullet®.

Histology

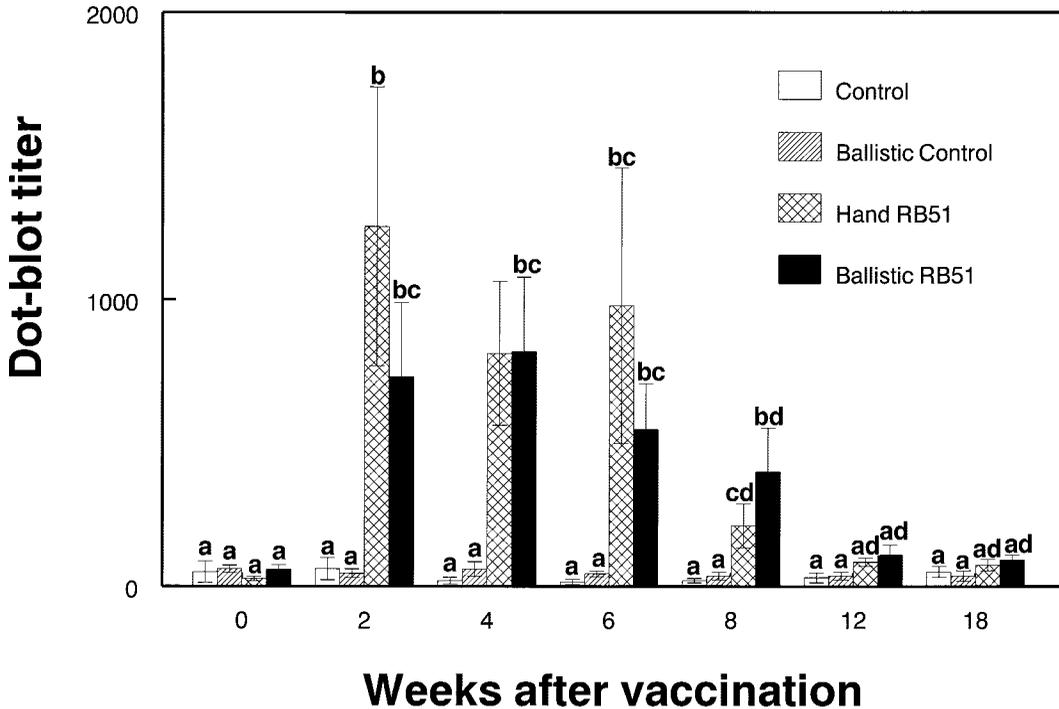Sections of superficial cervical lymph node at 6 wk after SRB51 vaccination were

FIGURE 1.   Serologic responses of control or SRB51-vaccinated bison to γ-irradiated SRB51 in a dot-blot assay. Bison were vaccinated by ballistic ($n=10$/treatment) or hand injection ($n=9$/treatment) with 10^{10} CFU of SRB51, whereas control bison received hand inoculation with 0.15 M NaCl ($n=4$/treatment) or ballistic inoculation with an empty Biobullet® ($n=5$/treatment). Responses are presented as mean antibody titer$\pm$SEM. Means with different superscripts are significantly different ($P<0.05$).
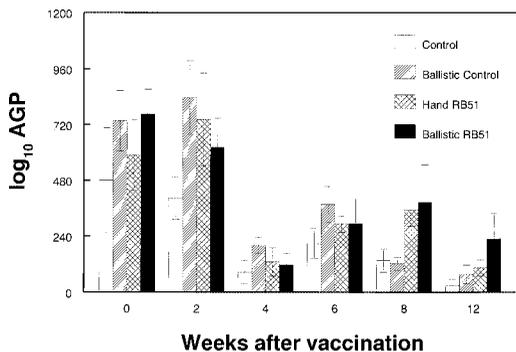

FIGURE 2.   Serum concentration of α_1 acid glycoprotein (AGP) in serum from bison vaccinated by ballistic ($n=10$/treatment) or hand injection ($n=9$/treatment) with 10^{10} CFU of SRB51, or control bison inoculated with 0.15 M NaCl ($n=4$/treatment) or ballistically inoculated with an empty Biobullet® ($n=5$/treatment). Concentration was determined by a radial immunodiffusion assay. Data are presented as mean AGP concentration$\pm$SEM.

characterized by filling of intermediate sinuses, and to a lesser extent medullary sinuses, by variable numbers of macrophages and occasional, mild, multifocal infiltrates of neutrophils. These infiltrates were associated with mild expansion of the deep cortex. Moderate numbers of germinal centers (three to seven per low power field, 0.5–1.5 mm in diameter) within the superficial cortex were found in nodes from most SRB51 vaccinates. At 12 weeks after vaccination, vaccinates had greater numbers of germinal centers within the superficial cortex (five to 12 per low power field) that tended to be larger in size (0.5–1.5 mm in diameter) with more prominent mantle layers than germinal centers observed 6 wk after vaccination. The histiocytic and neutrophilic infiltrates in the intermediate sinuses and medullary sinuses were similar to infiltrates observed in sections obtained 6 wk after vaccination. His-

TABLE 1.   Number of *Brucella abortus* colony forming units (CFU) per gram of tissue from the superficial cervical lymph node of bison vaccinated with *B. abortus* stain RB51 by Biobullet® or hand inoculation.

| | | Weeks after vaccination[a] | |
Delivery of vaccine	Number	6	12
Ballistic	4	42.2 ± 41.9 (2/4)	7.1 ± 7.1 (1/4)
Hand injection	4	15.7 ± 10.7 (2/4)	3.2 ± 1.2 (3/4)

[a] Results are presented as CFU±SEM (number positive/number tested).

tologic lesions did not differ between ballistic or hand inoculated bison. With exception of a section obtained from a culture-negative, ballistically vaccinated bison 6 wk after vaccination which had substantially reduced histiocytic and neutrophilic infiltrates, culture status did not appear to influence histologic lesions.

DISCUSSION

Results of this study suggest that a 10^{10} CFU dose of SRB51 is more immunogenic in bison when delivered by hand injection, as compared to ballistic inoculation. Although ballistically vaccinated bison developed antibody titers to SRB51 that were greater than bison in control groups, they failed to develop significant proliferative responses to γ-irradiated whole SRB51 bacteria. In comparison, hand vaccinated bison had greater proliferative responses to SRB51 bacteria. Although not statistically significant, peak dot-blot titers of hand vaccinated bison tended to be higher than ballistically vaccinated bison.

Because antibodies play a minor role in protecting cattle against *B. abortus* (Nicoletti, 1990), the antibody responses induced by ballistic vaccination with SRB51 are unlikely to correlate with resistance to brucellosis. However, the possibility that cell-mediated responses were stimulated in vivo that were not detected in PBMC under in vitro conditions cannot be eliminated. Therefore, conventional challenge exposure with a virulent strain of *B. abortus* during pregnancy will be required to determine if protective immune responses were elicited by ballistic vaccination.

Failure of ballistic delivery of SRB51 to stimulate cell-mediated immune responses that are similar to those induced by hand injection may be due to a number of reasons. Local inflammatory responses to ballistic vaccination may be associated with nonspecific immune activation, which induced more rapid clearance of the vaccine strain and a corresponding reduction in stimulation of cell-mediated responses. Although SRB51 colonization of the superficial cervical lymph node was similar for ballistic and hand vaccinated bison at 6 wk after vaccination, SRB51 was recovered from more of the hand vaccinated bison sampled at 12 wk after vaccination when compared to ballistically vaccinated bison Because mild hemorrhage occurred at the site of ballistic vaccination in some bison, this may have reduced the dosage of SRB51 actually delivered in vivo. The possibility cannot be eliminated that intramuscular delivery of a Biobullet®, as compared to SQ delivery for hand vaccination, may have influenced immunologic responses. Other unidentified factors may also have influenced immunologic responses to ballistic vaccination. Ballistic inoculation with dosages of SRB51 greater than 1×10^{10} CFU might stimulate immune responses that are comparable to those induced by 1×10^{10} CFU of SRB51 delivered by hand injection.

Although previous studies have suggested that recommended calfhood dosages of SRB51 are clinically safe in bison calves, additional data obtained in the current study supports this hypothesis. Serum concentrations of AGP, an acute phase reactant protein associated with acute inflammation, tissue damage, or neoplastic dis-
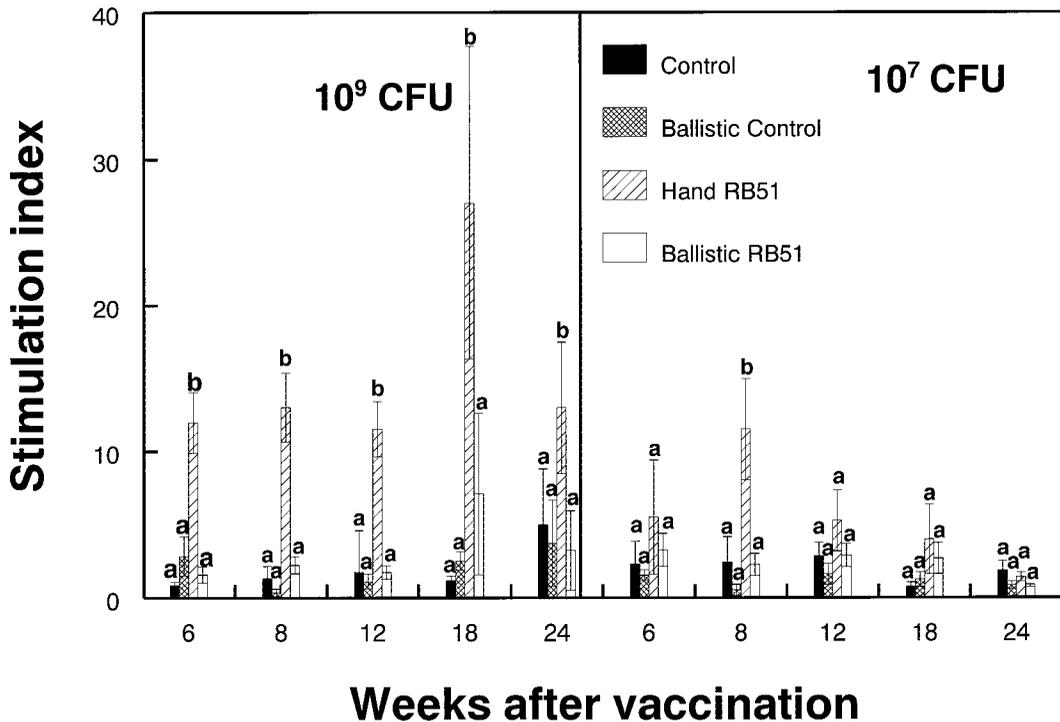
FIGURE 3. Proliferative responses of peripheral blood mononuclear cells from bison vaccinated by ballistic (n=10/treatment) or hand injection (n=9/treatment) with 10^{10} CFU of SRB51, or control bison inoculated with 0.15 M NaCl (n=4/treatment) or ballistically inoculated with an empty Biobullet® (n=5/treatment), to 10^9–10^7 CFU of γ-irradiated SRB51. Cells were incubated at 37 C in 5% CO_2 for 7 days and pulsed for 18 hrs with [^{3}H]thymidine. Results are expressed as mean stimulation indexes. Means within a SRB51 concentration and sampling time with different superscripts are significantly different (P<0.05).

ease in cattle (Itoh et al., 1990a, b, 1997; Oonaru et al., 1990), were not influenced by ballistic or hand vaccination of bison with SRB51. Similarly, serum concentrations of fibrinogen, a serum protein which increases in cattle with inflammatory or traumatic conditions (Hirvonen et al., 1998), were not influenced by SRB51 vaccination.

Preliminary data suggests that administration of 10^{10} CFU SRB51 to bison calves protects against abortion and fetal infection (Olsen et al., unpubl. data). Because bison appear to be more susceptible than cattle to abortion when parenterally vaccinated with 10^9 CFU SRB51 during pregnancy (Palmer et al., 1996, 1997), vaccination of calves rather than adult vaccination may be more appropriate for use in bison. Appropriate safe and efficacious delivery systems need to be developed for

free-ranging bison or other wildlife that will allow vaccination without restraint or capture. However, as the data presented here emphasize, efficacy of alternative delivery systems needs to be evaluated, even if the vaccine has proven to be acceptable in that species when delivered by SQ injection.

LITERATURE CITED

ALTON, G. G., L. M. JONES, R. D. ANGUS, AND J. M. VERGER. 1988. Serologic methods. *In* Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris, France, pp. 17–136.

CHEVILLE, N. F., A. E. JENSEN, S. M. HALLING, F. M. TATUM, D. C. MORFITT, S. G. HENNAGER, W. M. FRERICHS, AND G. G. SCHURIG. 1992. Bacterial survival, lymph node changes, and immunologic responses of cattle vaccinated with standard and mutant strains of *Brucella abortus.* American Journal of Veterinary Research 53: 1881–1888.

COLES, E. H. 1980. Erythrocytes. *In* Veterinary clinical pathology. W.B. Saunders Co., Philadelphia, Pennsylvania, p. 90.

DAVIS, D. S., J. W. TEMPLETON, T. A. FCHT, J. D. WILLIAMS, J. D. KOPEC, AND L. G. ADAMS. 1990. *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis, and transmission to cattle. Journal of Wildlife Diseases 26: 360–371.

HERRIGES, J. D., E. T. THORNE, S. L. ANDERSON, AND H. A. DAWSON. 1989. Vaccination of elk in Wyoming with reduced dose strain 19 *Brucella*: Controlled studies and ballistic implant field trials. Proceedings of the United States Animal Health Association 93: 640–655.

HIRVONEN, J., AND S. PYORALA. 1998. Acute-phase response in dairy cows with surgically-treated abdominal disorders. Veterinary Journal 155: 53–61.

HORNSBY, R. L., A. E. JENSEN, S. C. OLSEN, AND C. O. THOEN. 2000. Selective media for isolation of *Brucella abortus* strain RB51. Veterinary Microbiology 73: 51–60.

ITOH, H., Y. MOTOI, K. TAMURA, H. MURATA, T. CHIBA, AND S. TAKEDA. 1990a. Serum α_1-acid glycoprotein in bovine leukosis and its effects on blastogenesis. Journal of the Japanese Veterinary Medicine Association 42: 39–43.

———, K. TAMURA, Y. MOTOI, K. TAKASE, AND T. NAKAMURA. 1990b. Serum α_1-acid glycoprotein in cattle with inflammatory disease and that after operation. Japanese Journal of Veterinary Science 52: 1293–1296.

———, Y. MOTOI, M. HARITANI, M. KOBAYASHI, K. TAMURA, K. TAKASE, AND S. OIKAWA. 1997. Immunohistochemical localization of α1-acid glycoprotein in liver tissues of bovine fetuses, newborn calves, and sick or healthy adult cattle. American Journal of Veterinary Research 58: 725–728.

NICOLETTI, P. 1990. Vaccination. *In* Animal brucellosis, K. Nielsen and J. R. Duncan (eds.). CRC Press, Inc., Boca Raton, Florida, pp. 283–299.

OLSEN, S. C, N. F. CHEVILLE, R. A. KUNKLE, M. V. PALMER, AND A. E. JENSEN. 1997a. Bacterial survival, lymph node changes, and immunologic responses of bison *(Bison bison)* vaccinated with *Brucella abortus* strain RB51. Journal of Wildlife Diseases 33: 146–151.

———, M. G. STEVENS, N. F. CHEVILLE, AND G. G. SCHURIG. 1997b. Experimental use of a dot-blot assay to measure serologic responses of cattle vaccinated with *Brucella abortus* strain RB51. Journal of Veterinary Diagnostic Investigation 9: 363–367.

———, A. E. JENSEN, M. V. PALMER, AND M. G. STEVENS. 1998. Vaccination of bison with *Brucella abortus* strain RB51: Serology, lymphocyte proliferative responses, clearance, and biosafety. American Journal of Veterinary Research 59: 410–415.

OONARU, K., K. TAKAHASHI, T. KUROSAWA, S. KAWAMOTO, M. SONODA, AND K. TAMURA. 1990. Serum α_1-acid glycoprotein in cattle with neoplastic and inflammatory diseases. Journal of the Japanese Veterinary Medical Association 43: 19–23.

PAC, H. I., AND K. FREY. 1991. Some population characteristics of the Northern Yellowstone bison herd during the winter of 1988–1989. Montana Department of Fish, Wildlife, and Parks, Bozeman, Montana. pp. 1–29.

PALMER, M. V., S. C. OLSEN, M. J. GILSDORF, L. M. PHILO, P. R. CLARKE, AND N. F. CHEVILLE. 1996. Abortion and placentitis in pregnant bison *(Bison bison)* induced by the vaccine candidate, *Brucella abortus* strain RB51. American Journal of Veterinary Research 54: 1604–1607.

———, ———, AND N. F. CHEVILLE. 1997. Safety and immunogenicity of *Brucella abortus* strain RB51 vaccine in pregnant cattle. American Journal of Veterinary Research 58: 472–477.

SCHURIG, G. G., R. M. ROOP, T. BAGCHI, S. BOYLE, D. BUHRMAN, AND N. SRIRANGANATHAN. 1991. Biological properties of RB51; A stable rough strain of *Brucella abortus.* Veterinary Microbiology 28: 171–188.

THORNE, E. T., J. K. MORTON, AND G. M. THOMAS. 1978. Brucellosis in elk I. Serologic and bacteriologic survey in Wyoming. Journal of Wildlife Disease 14: 74–81.

———, ———, AND W. C. RAY. 1979. Brucellosis, its effect and impact on elk in western Wyoming. *In* North American elk; Ecology, behavior, and management, M. S. Boyce and L. O. Hayden-Wing (eds.). University of Wyoming, Laramie, Wyoming, pp. 212–220.

VEMULAPALLI, R., J. R. MCQUISTON, G. G. SCHURIG, N. SRIRANGANATHAN, S. M. HALLING, AND S. M. BOYLE. 1999. Identification of an IS711 element interrupting the *wboA* gene of *Brucella abortus* vaccine strain RB51 and a PCR assay to distinguish strain RB51 from other *Brucella* species and strains. Clinical and Diagnostic Laboratory Immunology 6: 760–764.