Immune Responses and Safety after Dart or Booster Vaccination of Bison with *Brucella abortus* Strain RB51

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One alternative for management of brucellosis in Yellowstone National Park bison (*Bison bison*) is vaccination of calves and yearlings. Although *Brucella abortus* strain RB51 vaccination protects bison against experimental challenge, the effect of booster vaccinations was unknown. This study characterized immunologic responses after dart or booster vaccination of bison with *Brucella abortus* strain RB51. In two studies, 8- to 10-month-old female bison were inoculated with saline (n = 14), hand vaccinated with 1.1 × 10¹⁰ to 2.0 × 10¹⁰ CFU of RB51 (n = 21), or dart vaccinated with 1.8 × 10¹⁰ CFU of RB51 (n = 7). A subgroup of hand vaccinates in study 1 was randomly selected for booster vaccination 15 months later with 2.2 × 10¹⁰ CFU of RB51. Compared to single vaccinates, booster-vaccinated bison had greater serologic responses to RB51. However, there was a trend for antigen-specific proliferative responses of peripheral blood mononuclear cells (PBMC) from booster vaccinates to be reduced compared to responses of PBMC from single vaccinates. PBMC from booster vaccinates tended to have greater gamma interferon (IFN-γ) production than those from single vaccinates. In general, dart vaccination with RB51 induced immunologic responses similar to those of hand vaccination. All vaccinates (single hand, dart, or booster) demonstrated greater (P < 0.05) immunologic responses at various times after vaccination than nonvaccinated bison. Booster vaccination with RB51 in early gestation did not induce abortion or fetal infection. Our data suggest that booster vaccination does not induce strong anamnestic responses. However, phenotypic data on resistance to experimental challenge are required to fully assess the effect of booster vaccination on protective immunity.

The high prevalence of brucellosis in bison (*Bison bison*) in Yellowstone National Park (YNP) remains a source of controversy due to the historic nature of this herd and its association with what is arguably the most publicly visible national park in the United States. Yellowstone National Park, the first national park in the United States, was established in 1872. Although estimated to number in the hundreds when the park was established, continued poaching had reduced the park bison population to 25 by 1901 (18). Additional bison, 18 cows and 3 bulls, were purchased in 1902. As the bison herd expanded to over 1,000 animals in later years, the population was reduced by shipping of bison to parks, zoos, and private estates (18). The population was estimated at 397 bison in 1967 when new park regulations which allowed populations to be controlled by environmental conditions were established (18).

Since 1967, the bison population in Yellowstone has at times increased to levels approaching 4,500 animals. Brucellosis was first reported in YNP bison in 1917 (9). Based on serology data, prevalence of brucellosis in the herd is approximately 50% at this time. During the winter months, bison migrate from the park to lower elevations outside the park where they can potentially have contact with cattle. As bison have been demonstrated to be capable of transmitting brucellosis to cattle under experimental conditions (6), there is concern for reintroduction of brucellosis into cattle from this wildlife reservoir.

Within the last year, the Department of Interior has released a bison remote vaccination environmental impact statement (EIS) for public comment (http://parkplanning.nps.gov/yell). This EIS includes the following options: option 1, hand vaccination at a capture facility; option 2, expanding option 1 to include remote vaccination of young bison; and option 3, expanding option 2 to add remote vaccination of adult females. There is a need to improve brucellosis vaccines for bison, as overall data from our laboratory suggest that the RB51 vaccine is not as efficacious in protecting bison against experimental challenge when compared to similar data from vaccinated cattle. Any vaccination program for YNP bison will most likely be of long duration and require commitment of significant financial and human resources. Therefore, this study was initiated to characterize the safety, immunogenicity, and efficacy of a second dose of RB51 vaccine in bison and evaluate pneumatic darts as an option for remote delivery of brucellosis vaccines.

**MATERIALS AND METHODS**

*Brucella abortus* cultures. For immunologic assays, RB51 suspensions (1 × 10¹² CFU/ml) were inactivated by gamma irradiation (1.4 × 10⁶ rads), washed in 0.15 M sodium chloride (saline), and stored at −70°C.

Animals and inoculation. In two separate studies, 8- to 10-month-old bison heifers were obtained from a brucellosis-free herd. After acclimation for 4 weeks, bison were randomly assigned to treatment groups. In study 1, treatment included saline (control; n = 7) or single intramuscular vaccination with RB51 (hand RB51; n = 14). Half of the hand vaccinates (n = 7) were randomly assigned for booster vaccination (booster RB51) with RB51 at 15 months after the initial vaccination (approximately 1 month into breeding season). In study 2, treatment (n = 7/treatment) included saline (control), single hand vaccination with RB51 (hand RB51), or dart vaccination with RB51 (dart RB51), administered at 8 to 10 months of age.
A commercial RB51 vaccine was obtained in lyophilized form (Colorado Serum Company, Denver, CO) and diluted in accordance with the manufacturer’s recommendations. All hand inoculations were 2 ml in volume and administered intramuscularly in the cervical region drained by the superficial cervical lymph node. Dart vaccinations were delivered by a cartridge fired gun (model 389 rifle; Pneudart, Williamsburg, PA) and pneumatic darts. The volume of vaccine within each dart was also 2 ml, and delivery of the darts was targeted to the cervical region. Following vaccination, the concentration of viable bacteria within inocula was determined by standard plate counts.

**Serological evaluation.** In both studies, blood samples were collected by jugular venipuncture prior to vaccination and at 4-week intervals up to 24 weeks postvaccination. Blood was also obtained after the booster vaccination at 4-week intervals beginning at 4 weeks postbooster. Blood was allowed to clot for 12 h at 4°C and centrifuged. Serum was divided into 1-ml aliquots, frozen, and stored at −70°C.

Serological responses of bison after vaccination were determined by a previously described enzyme-linked immunosorbent assay (ELISA) procedure using whole RB51 bacteria as the antigen (11).

**Preparation of peripheral blood mononuclear cells and lymph node cells for lymphocyte proliferation assays.** At all sampling times after the initial or booster 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, MO). PBMC were diluted in RPMI 1640 medium to 1×10^6 cells/ml and adjusted to 1 ml of only RPMI 1640 medium or 1640 medium containing gamma-irradiated RB51 (10^9 bacteria per ml). Cells were harvested onto glass filter mats and counted for radioactivity in a liquid scintillation counter.

**Gamma interferon production.** *In vitro* production of gamma interferon (IFN-γ) by peripheral blood mononuclear cells was measured at all sampling times after vaccination. Briefly, PBMC from each animal were isolated and adjusted to 1×10^6 viable cells per ml as described previously. Fifty microliters of each cell suspension, containing 5×10^6 cells, was added to each of two separate flat-bottom wells of 96-well microtiter plates that contained 100 μl of only RPMI 1640 medium or 1640 medium containing gamma-irradiated RB51 (10^9 bacteria per well). Wells containing 1 μg/ml pokeweed mitogen (Sigma Chemical Company) were used as positive controls for proliferative responses. Microtiter plates were prepared prior to initiation of the culture and stored at −70°C until use. Cell cultures were incubated for 7 days at 37°C under 5% CO2. After 7 days of incubation, cell cultures were pulsed with 1 μCi of [3H]thymidine per well for 18 h. Cells were harvested onto glass filter mats and counted for radioactivity in a liquid scintillation counter.

**Cytokine transcription after initial or booster vaccination.** At 4-week intervals between 4 and 24 weeks after the initial vaccination in study 1, PBMC were isolated from RB51 vaccinates and controls (5 bison/treatment/time). In a similar manner, PBMC were isolated from booster vaccinates and controls at 4, 8, and 12 weeks after the booster vaccination (5 bison/treatment/time) in study 1. Fifty microliters of purified PBMC, containing 5×10^6 cells, was added to each of four separate flat-bottom wells of 96-well microtiter plates that contained 100 μl of only RPMI 1640 medium (control) or 1640 medium containing gamma-irradiated RB51 (10^9 bacteria per well). After 18 h of incubation at 37°C and 5% CO2, cells were pelleted by centrifugation, resuspended in 100 μl of RNA later (Ambion, Austin, TX), and refrigerated at 4°C until processed for purification of RNA.

RNA was purified from cells using a commercial kit (QIA shredded spin columns and RNAseasy; Qiagen, Valencia, CA) in accordance with the manufacturer’s recommendations. RNA was converted to cDNA by adding 1 μg of oligo(dT) primer (Invitrogen) and RNase-free water and denaturing at 70°C for 10 min, followed by use of a commercial kit (SuperScript III reverse transcriptase; Invitrogen) in accordance with the manufacturer’s directions. The cDNA was purified using RNA H (New England BioLabs, Ipswich, MA) and a commercial kit (quick-clean enzyme removal resin; Clontech, Mountain View, CA) in accordance with the manufacturer’s directions. Total cDNA was determined on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA) in accordance with the manufacturer’s directions. Using a commercial real-time PCR kit (QuantiTech Sybr green PCR kit; Qiagen), 5 to 25 μg of cDNA was analyzed for quantitative cytokine transcription in triplicate on a real-time PCR machine (RotoGene 3000; Corbett Research, Australia) in a 25-μl reaction volume. Cycling conditions were as follows: 95°C for 15 min and 40 cycles of denaturation at 94°C for 15 s, 30 s of annealing at 60°C, and elongation for 30 s at 72°C. Melting curves were obtained at the end of each run to confirm that a single PCR product had the predicted melting temperature (Tm). Internal normalization of cytokine gene expression was carried out using beta-actin as a reference gene. Primers were generated with PrimerQuestSM (Integrated DNA Technologies, Iowa City, IA) using bovine cytokine sequences obtained using the NCBI GenBank database (Table 1). Integrity of the primers was also determined by sequencing of amplicons and alignment with the target gene sequence in the NCBI GenBank database.

### Table 1: Forward and reverse primers for real-time PCR analysis

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Primer Forward</th>
<th>Primer Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-actin</td>
<td>CGCCATGGGATGATGATATTGC</td>
<td>AAGCCTGGGCGTTCACAT</td>
</tr>
<tr>
<td>IL-2</td>
<td>CCTCAACTGCTGCCACAGTGA</td>
<td>GTTTGCAACAGTGGAAGGTATA</td>
</tr>
<tr>
<td>IL-4</td>
<td>GCCACAGCTGCTGAACAAA</td>
<td>CCTGGTGGCAACGTGTTGA</td>
</tr>
<tr>
<td>IL-6</td>
<td>GGCTCCCATGATTTGGTATTTT</td>
<td>GCCAGTGGAGCCAGGTTTCCTG</td>
</tr>
<tr>
<td>IL-7</td>
<td>CAAAAGCCGCTAAGTAAACTGA</td>
<td>GGGCTTGGCTTCCCTCATA</td>
</tr>
<tr>
<td>IL-10</td>
<td>CTTGCGGAAAATGCTACGTTTTT</td>
<td>TCAGGGCCGGTGTTCCTCA</td>
</tr>
<tr>
<td>IL-12</td>
<td>CAAGCTCGCGGTGCCAA</td>
<td>GGACACAGATGCCACAT</td>
</tr>
<tr>
<td>IL-15</td>
<td>GGCTGCAATCTAGTCTCCTCA</td>
<td>CAATCTGCGCATTCGCCGTTC</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>TTGGAGACCTGTCAAAACGCTGATT</td>
<td>TTATGGCCTTGCGGTGCA</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TCTACCGAGGGGAGGTCTCCTCA</td>
<td>GTCCGGCAGGTGAGCCACAT</td>
</tr>
</tbody>
</table>
Safety of the booster vaccination in early gestation. Bison in study 1 were rectally palpated at between 60 and 120 days of gestation to determine pregnancy status at approximately 16 to 18 months after initial vaccination. Animals were observed at least twice daily during gestation. After parturition, calves were sedated by dart delivery (Pneudart, Williamsburg, PA) of xylazine (2 to 3 mg/kg of body weight; Alcon, Decatur, IL) and ketamine (2 to 3 mg/kg; Fort Dodge, IA) and euthanized by intravascular injection of sodium pentobarbital (Sleepaway; Fort Dodge, IA) within 96 h of parturition. Fetals samples obtained included spleen, lung, blood, bronchial lymph node, gastric contents, and rectal swabs. Swabs and fluid samples were inoculated directly on tryptose agar plates containing 5% bovine serum. Tissue samples were triturated in 0.15 M NaCl using a tissue grinder and plated on both tryptose agar containing 5% bovine serum and Kudzas and Morse medium. After incubation at 37°C and 5% CO2, for at least 5 days, B. abortus bacteria were identified on the basis of colony morphology, growth characteristics, and a Brucella-specific PCR procedure (1, 5, 8).

Statistical analysis. Serologic, net IFN-γ, and [3H]thymidine data were statistically analyzed as the logarithms of their values. Serologic data were compared over all sampling times using a two-way analysis of variance model, whereas differences between treatments in stimulation indices and net IFN-γ data at each sampling time were compared by a general linear model procedure (SAS Institute, Inc., Cary, NC). Means for individual treatments were separated by the use of a least-significant-difference procedure (P < 0.05).

For determination of takeoff and amplification values, the threshold on the real-time PCR machine was set at a constant value for all runs. Expression of cytokine genes was normalized in relation to the expression of the housekeeping gene (beta-actin), and expression differences between vaccines and controls were determined by analysis of takeoff and amplification values on a commercial software program (REST 2009; Qiagen) using previously described mathematical algorithms (13). Results are expressed as ratios of gene induction in PBMC of vaccinates to that of controls after normalization against a housekeeping gene.

RESULTS

Vaccine dosages. Standard plate counts indicated the mean vaccination dosages for the initial and booster RB51 inoculations in study 1 as 1.1 × 10^8 and 2.2 × 10^10 CFU, respectively. In study 2, vaccination dosages for hand and dart vaccination treatments were 2.0 × 10^10 and 1.8 × 10^10 CFU, respectively.

Serologic responses. Compared to nonvaccinates, hand-vaccinated bison in study 1 had greater (P < 0.05) mean responses to RB51 beginning at 4 weeks after the initial vaccination and continuing through 24 weeks (data not shown). Mean responses in vaccinates peaked at 4 weeks after vaccination and had declined from peak responses by 20 and 24 weeks after initial vaccination with RB51.

After booster vaccination in study 1, bison inoculated with a second dose of RB51 had greater (P < 0.05) humoral responses to RB51 at all sampling times than bison vaccinated once with RB51 or nonvaccinates (data not shown). It should be noted that the magnitude of changes in mean responses over time after the booster vaccination was similar to that of changes after the initial vaccination when sera from identical time points after the initial and booster vaccinations were run simultaneously on an ELISA plate (Fig. 1). Responses of nonvaccinates and single vaccinates did not differ (P > 0.05) in any samplings obtained after the booster vaccination (60 to 84 weeks after the initial vaccination).

Bison in the hand and dart RB51 vaccination treatments in study 2 had greater (P < 0.05) humoral responses to RB51 at 4, 8, and 12 weeks than nonvaccinates (Fig. 2). With the exception of the 4-week-postvaccination sampling, mean responses of hand- and dart-vaccinated bison did not differ (P > 0.05).

Lymphocyte proliferation assays. After initial vaccination in study 1, PBMC from RB51-vaccinated bison had greater (P < 0.05) proliferative responses at 8, 16, 20, and 24 weeks after vaccination compared to responses of PBMC from nonvaccinates (Fig. 3).

After the booster vaccination at 60 weeks, PBMC from booster-vaccinated bison had greater proliferative responses at 16, 20, and 24 weeks than PBMC from nonvaccinates (Fig. 4). However, proliferative responses of PBMC from bison vaccinated only once with RB51 had responses greater (P < 0.05) than those of controls at all time points after booster vaccination and greater (P < 0.05) than responses of PBMC from booster vaccinates at 4, 8, and 12 weeks.

In study 2, PBMC from bison vaccinated by hand or dart with RB51 had greater (P < 0.05) proliferative responses at 16, 20, and 24 weeks compared to the responses of PBMC from control bison (Fig. 5). Proliferative responses of PBMC from hand and dart vaccination groups differed (P < 0.05) only at 16 weeks after inoculation.

Gamma interferon production. After initial vaccination in study 1, mean production of IFN-γ from PBMC of bison vaccinated with RB51 was greater (P < 0.05) at 4 and 20 weeks than IFN-γ production by PBMC from nonvaccinates (data not shown). In the booster vaccination phase of study 1, PBMC from booster-vaccinated bison had greater (P < 0.05) mean production of IFN-γ at 8, 20, and 24 weeks than those from nonvaccinated bison (Fig. 6). PBMC from bison vaccinated once with RB51 approximately 1 year earlier had greater (P < 0.05) mean production of IFN-γ at 16 and 20 weeks than those from nonvaccinated bison. With the exception of higher values in booster vaccinates at 24 weeks, the mean production of IFN-γ did not differ (P > 0.05) between the single-vaccine and booster vaccine treatments in samples obtained after the initiation of the booster vaccination phase of the study. It should also be noted that net production of IFN-γ was not detected in any treatment in samples obtained at 12 weeks after booster vaccination.
In study 2, PBMC from hand RB51 vaccinates had greater ($P < 0.05$) mean production of gamma interferon at 4, 8, 16, 20, and 24 weeks than PBMC from nonvaccinated bison (Fig. 7). In comparison, PBMC from bison vaccinated by dart had greater ($P < 0.05$) mean production of IFN-γ at 4, 8, 20, and 24 weeks than those from nonvaccinated bison. The mean production of IFN-γ from PBMC of hand RB51 vaccinates was greater ($P < 0.05$) than that of PBMC from dart vaccinates at 16 weeks, whereas dart vaccinates did not have greater responses than hand RB51 vaccinates at any time after vaccination.

**Relative expression of cytokine genes.** After initial vaccination in study 1, differences in expression of cytokine genes were not detected between RB51 vaccinates and controls until 12 weeks after vaccination. At 12 weeks after vaccination, PBMC from RB51 vaccinates had greater ($P < 0.05$) expression of interleukin-4 (IL-4), IL-7, IL-10, and IL-12 compared to that of PBMC from nonvaccinated controls (Table 2). At 16 weeks after initial vaccination, PBMC from RB51 vaccinates had upregulation of IL-2 and IFN-γ compared to expression by PBMC from control bison. Expression of IFN-γ by PBMC from RB51 vaccinates was also greater ($P < 0.05$) at 20 weeks than expression in PBMC from nonvaccinated bison.

In the second phase of study 1, PBMC from booster-vaccinated bison had greater ($P < 0.05$) expression of IFN-γ and IL-2 at 8 and 12 weeks compared to the gene expression by PBMC from control bison. Booster vaccinates also had greater ($P < 0.05$) expression of tumor necrosis factor (TNF) at 8 weeks compared to that in PBMC from nonvaccinates. The IL-4 gene at 4 weeks was the only gene downregulated in PBMC from RB51 vaccinates after booster vaccination compared to the expression in PBMC from control bison.

**Safety of the booster vaccination in early gestation.** No abortions were observed in booster-vaccinated bison during the subsequent pregnancy. Rectal palpation did not suggest that any cows that did not calve were pregnant at the time of booster vaccination. Four of the seven bison in the booster group calved between 36 and 42 weeks after the booster vaccination. In comparison, 5 of 7 bison in the control treatment and 6 of 7 in the single vaccination treatment calved between 35 and 38 weeks after inoculation of the booster vaccination group. *Brucella* was not cultured from any samples obtained at necropsy from the 4 calves evaluated in the booster vaccination group or from the 2 calves evaluated in the single RB51 vaccination treatment.

**DISCUSSION**

The results of the current study suggest that bison booster vaccinates with RB51 do not demonstrate strong anamnestic responses, as measured by serologic data, PBMC proliferation, or production of IFN-γ by PBMC. Although serologic responses of booster vaccinates were higher than the responses after initial vaccination, they partially reflected a higher background response at the time of the second inoculation. Booster vaccinates also tended to demonstrate lower proliferative responses of PBMC to irradiated RB51 than paired bison that did not receive a second vacci-
nation. Although IFN-γ production tended to be higher in booster vaccinates, at most sampling times, responses of booster vaccinates did not differ \((P > 0.05)\) from responses of bison that received a single inoculation of RB51 more than 1 year previously.

The failure to detect strong anamnestic responses may be partially related to the long period of time required for bison to clear the live RB51 vaccine. In previous studies, clearance of RB51 from lymphoreticular tissues of vaccinated bison took approximately 24 weeks (15). One hypothesis for the immunologic observations in the current study is that a single RB51 vaccination can induce the equivalent of anamnestic responses due to the long period of time required for \textit{in vivo} clearance of the vaccine strain by bison. This hypothesis also suggests that immunologic responses are maximally induced by a single vaccination. Alternatively, the failure to detect \textit{Brucella}-responsive cells in the peripheral blood may be related to localization of antigen-specific cells in lymph nodes after booster vaccination, as this is the site for \textit{in vivo} colonization of RB51 bacteria after vaccination. Trapping of immune cells at the sites of \textit{in vivo} localization after booster vaccination may have resulted in a reduction in circulating immune-responsive cells within the bloodstream and, subsequently, reduced detection of cellular immune responses in cells isolated from the peripheral blood. However, additional data are required to determine why more-robust immunologic responses are not observed after booster vaccination.
Data from our study also suggest that immunologic responses of bison after dart vaccination with RB51 are similar to responses after parenteral inoculation with the vaccine. As remote delivery is a key component of the bison remote vaccination EIS, pneumatic dart delivery may be a viable route for remote vaccination at distances longer than those for previously described ballistic procedures (12).

As specific correlates of protective immunity against brucellosis have not been identified in domestic livestock, it remains unknown if booster or dart vaccinates will differ from single parenteral vaccinations in protection against exposure to virulent B. abortus strains. One potential immunologic predictor of protection is the production of IFN-γ, a cytokine associated with activation of macrophage effector function and bactericidal activity. As the RB51 vaccine has previously been shown to induce protection against experimental challenge (5, 10), the observation of increased IFN-γ expression after RB51 vaccination of cattle (14) supports a role for this cytokine in protective cellular immune responses. Although expression of IFN-γ is believed to be predictive of protection against other intracellular pathogens, others have reported that its expression in cattle is predictive of protection against Mycobacterium avium on a group, rather than individual, basis (4). In addition, the observation of increased IFN-γ responses to M. bovis antigens by PBMC from infected herds compared to responses by cattle in noninfected herds (3) suggests that the use of IFN-γ alone may not be fully predictive of protection status.

Expression of cytokines associated with TH-1 responses, including IFN-γ, is hypothesized to contribute to protection against intracellular pathogens. In the current study, increased in vitro expression of IFN-γ and upregulation of IFN-γ gene transcription were observed in RB51-vaccinated bison at multiple times after initial and booster vaccinations. Transcription of the TH-1 cytokine interleukin-2 was also upregulated in a pattern similar to the increases in expression of IFN-γ. As cytokines associated with TH-2 responses are generally believed to induce immunologic responses that are not protective against intracellular pathogens, the increased transcription of interleukin-10 at 12 and 16 weeks after initial vaccination and the general lack of a vaccination effect on IL-4 or IL-6 transcription were somewhat surprising. To some extent, our data correlate with a study evaluating M. bovis infection in cattle in which PBMC from cattle exhibiting more-severe lung lesions after experimental infection had higher expression of IFN-γ, tumor necrosis factor alpha (TNF-α), IL-10, and IL-4 than PBMC from cattle in the low-pathology group, which had lesions primarily in the lymph nodes associated with the head (17). Others have reported that upregulation of IL-10 and downregulation of IFN-γ expression increase intracellular survival and proliferation within bovine macrophages of another intracellular pathogen, Mycobacterium avium subsp. paratuberculosis (7). Cattle with more-severe lesions of M. avium subsp. paratuberculosis have increased expression of IFN-γ, IL-10, IL-13, IL-17, and TNF-α in mesenteric lymph nodes compared to that of infected cattle exhibiting less-severe histologic lesions (16).

The observation that booster vaccinations did not induce abortion or fetal infection in the current study provides some indication of the safety of delivering a booster vaccination of RB51 to bison in early pregnancy. However, as the numbers of bison examined in the current study were small, additional data with greater numbers of animals are required to verify our observations. As vaccine dosage, method or route of vaccine delivery, and stage of gestation may influence the safety of a brucellosis vaccine in adult cows, additional studies need to evaluate these variables prior to widespread implementation of a vaccination program that includes adult females.

In conclusion, our study found relatively small differences in immunologic responses of bison after single RB51 vaccination delivered by parenteral or dart delivery or when comparing single and booster parenteral vaccinations. The lack of detection of anamnestic responses after booster vaccinations may be related to the in vivo pathogenesis of B. abortus in bison. Although robust differences were not found in immunologic responses, efficacy

TABLE 2 Relative expression of cytokine transcription by PBMC from RB51 vaccinates compared to that of nonvaccinated bison after initial or booster vaccination in study 1

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Initial 12</th>
<th>Initial 16</th>
<th>Initial 20</th>
<th>Booster 4</th>
<th>Booster 8</th>
<th>Booster 12</th>
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<tbody>
<tr>
<td>IFN-γ</td>
<td>1.07</td>
<td>6.30*</td>
<td>4.80*</td>
<td>0.94</td>
<td>4.05*</td>
<td>2.84*</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>5.29*</td>
<td>2.24*</td>
<td>2.57</td>
<td>0.51</td>
<td>3.64*</td>
<td>5.42*</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>2.94*</td>
<td>1.25</td>
<td>2.38</td>
<td>0.26*</td>
<td>1.39</td>
<td>1.75</td>
</tr>
<tr>
<td>Interleukin-7</td>
<td>5.14*</td>
<td>2.14</td>
<td>1.43</td>
<td>0.98</td>
<td>0.81</td>
<td>0.85</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>4.32*</td>
<td>1.76*</td>
<td>1.56</td>
<td>0.64</td>
<td>0.79</td>
<td>0.75</td>
</tr>
<tr>
<td>Interleukin-12</td>
<td>2.41*</td>
<td>1.41</td>
<td>1.62</td>
<td>1.02</td>
<td>1.53</td>
<td>1.39</td>
</tr>
<tr>
<td>TNF</td>
<td>2.22*</td>
<td>1.20</td>
<td>1.42</td>
<td>1.13</td>
<td>1.50*</td>
<td>1.18</td>
</tr>
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</table>

*PBMC were cultured in vitro with 10^6 CFU of gamma-irradiated B. abortus strain RB51 for 18 h. Data represent the ratios of gene induction in PBMC of vaccinates to that of controls after normalization against a housekeeping gene (beta-actin). Expression of IL-15 and IL-6 did not differ (P > 0.05) between vaccinates and nonvaccinated bison at any sampling time.

* †, upregulation (P < 0.05) of expression levels in vaccinates compared to expression in nonvaccinates; †, downregulation (P < 0.05) of expression levels in vaccinates compared to expression in nonvaccinates.
studies will need to be completed for an assessment of protection induced by the different vaccination procedures used in this study. However, our data do suggest that dart and booster vaccination strategies may be safely used in bison and can potentially be beneficial in addressing brucellosis in free-ranging bison in Yellowstone National Park.

ACKNOWLEDGMENTS

We thank Deb Buffington, Aileen Bryant, John Kent, Todd Pille, Darl Pringle, Dennis Weuve, and Larry Wright for their 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.

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REFERENCES

2. Reference deleted.