Immune Responses and Efficacy After Administration of a Commercial *Brucella abortus* Strain RB51 Vaccine to Cattle*

Steven C. Olsen, DVM, PhD

United States Department of Agriculture
Bacterial Diseases of Livestock Research Unit
Agricultural Research Service
National Animal Disease Center
2300 Dayton Avenue
Ames, IA 50010

**ABSTRACT**

*Brucella abortus* strain RB51 (SRB51) is a newly approved live vaccine to protect cattle against brucellosis. The purpose of this study was to evaluate the immunologic responses of cattle to a commercially available SRB51 vaccine and to characterize the efficacy of the vaccine to protect against abortion or infection after midgestational challenge with virulent *B. abortus* strain 2308 (S2308). All cattle were pasture bred, and pregnancy was confirmed by rectal palpation. Pregnant cattle were intraconjunctivally challenged with $1 \times 10^7$ colony-forming units (CFUs) of S2308 at 180 days gestation. Serologic responses were monitored in all heifers after vaccination using the standard tube agglutination test and a dot-blot assay using killed SRB51 as antigen.

In one study, 3-month-old Hereford heifers were subcutaneously inoculated with $10^9$ CFU or $10^{10}$ CFU of a commercially available SRB51 vaccine or 2 mL of 0.15M sodium chloride (saline). In this study, four of eight nonvaccinates, two of four $10^9$ CFU SRB51 vaccinates, and two of 14 $10^{10}$ CFU SRB51 vaccinates aborted after midgestational challenge with virulent S2308. The challenge strain was recovered at necropsy from maternal or fetal tissue from six of nine nonvaccinates, four of four $10^9$ CFU SRB51 vaccinates, and seven of 14 heifers vaccinated with $10^{10}$ CFU of SRB51.

In a separate study, 6-month-old Hereford heifers were subcutaneously inoculated with $10^{10}$ CFU of SRB51 or saline. Peripheral blood mononuclear cells proliferative responses to $\gamma$-irradiated SRB51 were monitored. In this study, three of seven nonvaccinates and one of 18 $10^{10}$ CFU SRB51 vaccinates aborted. The S2308 challenge strain was recovered from maternal or fetal tissue of five of seven nonvaccinates and seven of 18 SRB51 vaccinates.

In both studies, cattle vaccinated with $10^{10}$ CFU of SRB51 had greater ($P < .05$) antibody responses to SRB51 at 4 and 8 weeks after vaccination than nonvaccinates. SRB51-vaccinated cattle had greater lymphocyte proliferative responses to killed SRB51 at 10, 12, 14, and 16 weeks when compared with nonvaccinates.

The data presented in this study indicate that $10^{10}$ CFU of the commercial SRB51 vac-
cine was highly efficacious \((P < .02)\) in preventing \textit{Brucella}-induced abortion or fetal infection. Considering that fetal infection and abortion are the predominant mechanisms for transmission of brucellosis, our data suggest that the commercially available SRB51 vaccine will be efficacious in preventing abortion and controlling brucellosis in cattle under field conditions. However, incidence of maternal infection with \textit{Brucella} at necropsy did not differ \((P > .05)\) among 10\(^{10}\) CFU SRB51 vaccinates and nonvaccinates.

\section{INTRODUCTION}

In February 1996 a new brucellosis vaccine, \textit{Brucella abortus} strain RB51 (SRB51), was conditionally licensed by the USDA Animal and Plant Health Inspection Service (APHIS) for use in cattle in the United States. Conditional approval of a commercial vaccine is granted under expedited conditions in order to meet an emergency condition, limited market, local situation, or other special circumstances. The conditional approval is only granted when purity and safety of the product have been demonstrated and available data are sufficient to give a reasonable expectation of efficacy. In this instance, the decision on efficacy was based on published data obtained in cattle experiments at the National Animal Disease Center with the ARS/1 strain of SRB51, the master seed source for the commercial product.\(^1\)\textsuperscript{1}\textsuperscript{4}\textsuperscript{6} However, for APHIS to grant full licensure for the commercial vaccine, additional scientific data were required that demonstrated the efficacy of the commercial vaccine.

The SRB51 strain is a rough mutant of \textit{B. abortus}\textsuperscript{7} that does not induce antibody responses that react with conventional brucellosis surveillance tests.\textsuperscript{3}\textsuperscript{8} When compared with the \textit{B. abortus} strain 19 vaccine, SRB51 offers similar efficacy in cattle without confounding the ability to detect individuals infected with field strains of \textit{B. abortus}.\textsuperscript{2}\textsuperscript{3}

Because the SRB51 vaccine is being widely used in the United States and other countries and it is recognized as an official calfhood vaccine against brucellosis, it was important to evaluate the commercial product so that full licensure of the vaccine might be obtained. The purpose of the studies reported here was to evaluate the safety and efficacy of a commercially available vaccine made from SRB51.

\section{MATERIALS AND METHODS}

\textit{Brucella abortus} Culture

A master seed stock of SRB51 was obtained from Dr. Gerhardt Schurig (Virginia Tech, Blacksburg, VA). After one passage on tryptose agar the seed stock was designated ARS/1. For experimental use in serologic or lymphocyte proliferation assays, SRB51 (ARS/1) bacteria were grown on tryptose agar (Difco Laboratories, Detroit, MI) for 48 hours at 37\(^\circ\)C. For the dot-blot assay, SRB51 suspensions (1.3 \(\times\) 10\(^{12}\) colony-forming units [CFU]/mL) were inactivated by \(\gamma\)-irradiation (1.4 \(\times\) 10\(^6\) rads). After irradiation, suspensions were washed in 0.15M sodium chloride (saline) and stored in 1 mL aliquots at \(-70\)^\(\circ\)C.

For the challenge portion of the experiment, \textit{B. abortus} strain 2308 (S2308) (Brucella culture collection, National Animal Disease Center, Ames, IA) was grown on tryptose agar for 48 hours at 37\(^\circ\)C. The bacteria were harvested from the agar by aspiration using saline. Suspensions of S2308 were adjusted by use of a spectrophotometer (Bausch and Lomb, Rochester, NY) and concentrations of viable bacteria were determined by plate counts.

For vaccination of cattle, a commercially prepared product (Colorado Serum Company, Denver, CO) derived from ARS/1 was used according to the product outline. The vaccine was diluted in saline to approximately 10\(^9\) or 10\(^{10}\) CFU based on standard plate counts on other vials with the same lot number. After dilution,
the concentration of viable bacteria within the inoculum was determined by standard plate counts.

**Animals and Inoculation**

In two separate studies, 30 10-week-old and 28 5-month-old Hereford heifers, respectively, were purchased from brucellosis-free herds and randomly assigned to treatments. In the first study, after acclimation for 2 weeks, 15 of the 30 heifers were vaccinated subcutaneously (SQ) with $1.22 \times 10^{10}$ CFU of SRB51, six heifers were SQ inoculated with $1.04 \times 10^9$ CFU of SRB51, and nine heifers were inoculated SQ with 2 mL of saline at 12 weeks of age. After acclimation for 4 weeks in the second study, 20 of the 28 heifers were SQ innoculated with $1.09 \times 10^{10}$ CFU of SRB51 and eight heifers were SQ inoculated with saline at 6 months of age. All vaccinations were administered in the left cervical region drained by the superficial cervical (prescapular) lymph nodes.

**Serologic Evaluation**

Blood samples were collected by jugular venipuncture before vaccination and at 4, 8, and 12 weeks after vaccination. Blood was allowed to clot for 12 hours at 4°C and centrifuged. Serum was divided into 1-mL aliquots, frozen, and stored at −70°C. Serologic titers to *Brucella* were determined by a standard tube agglutination test (STAT). Serologic titers to SRB51 were determined using previously described antibody dot-blot assay in which γ-irradiated SRB51 is used as antigen. Samples were coded such that serologic evaluations were conducted blindly.

**Preparation of Peripheral Blood Mononuclear Cells and Lymph Node Cells for Lymphocyte Proliferation Assays**

Five SRB51-vaccinated and five saline-inoculated heifers were randomly selected at the initiation of the 6-months-of-age vaccination study to evaluate proliferative responses of peripheral blood mononuclear cells to SRB51. At 10, 12, 14, and 16 weeks after vaccination, blood was obtained from the jugular vein of selected heifers and placed into an acid-citrate dextrose solution. Peripheral blood mononuclear cells were enriched by density centrifugation using a Ficoll-sodium diatrizoate gradient.

Fifty μL of each cell suspension containing $5 \times 10^5$ peripheral blood mononuclear cells were 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 ($10^7$ to $10^9$ bacteria per well). Cell cultures were incubated for 7 days at 37°C under 5% CO$_2$. Microtiter plates were placed on a MicroShaker II (Dynatech Laboratories, Inc, Alexandria, VA) every 2 days during the incubations and mixed at an instrument setting of 3.5 for 1 minute. After 7 days incubation, cell cultures were pulsed with 1.0 μCi of [3H] thymidine per well for 18 hours. Cells were harvested onto glass filter mats and counted for radioactivity in a liquid scintillation counter (1450 Microbeta scintillation counter, Wallac, Inc, Gaithersburg, MD). Cell proliferation results were converted to logarithm of the counts per minute and stimulation indices (counts per minute [cpm] of wells containing antigen/cpm in the absence of antigen) for statistical comparisons.

**Experimental Brucella Challenge**

Animals were raised to adulthood and pasture bred between 14 and 18 months of age. Breeding dates were determined by observation, and pregnancy was confirmed by rectal palpation between 60 and 90 days gestation. After transfer to a biolevel 3 containment facility, pregnant animals were intraconjunctivally challenged at 180 days gestation with 1 ×
10⁷ CFU S2308 suspended in 100 µL of saline (50 µL/eye). Conjunctival swabs were obtained from all cattle at 2 and 5 days after experimental challenge exposure to verify persistence of the challenge strain of *B. abortus*.

Abortion was defined as premature expulsion from the uterus of the embryo or a nonviable fetus. Immediately after abortion or 1 week before estimated parturition, cows were euthanized with intravenous administration of sodium pentobarbitol (Sleepaway, Fort Dodge Labs, Ft. Dodge, IA). Because many contaminating bacteria impair identification of *Brucella*, nonaborting cattle were euthanized before parturition to prevent contamination of fetal or uterine tissue samples.

The most common maternal tissues for isolation of *Brucella* are reproductive, mammary, and lymphoreticular tissues. In the study reported here, maternal samples obtained at necropsy included bronchial, hepatic, internal iliac, mandibular, parotid, prescapular, retropharyngeal, and supramammary lymph nodes; blood; milk from all four quarters; mammary gland tissue from all four quarters; placenta or caruncle; spleen; liver; and vaginal swab. Fetal 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 0.15M NaCl using a tissue grinder and plated on tryptose agar containing 5% bovine serum. After incubation at 37°C and 5% CO₂, *B. abortus* bacteria were identified on the basis of colony morphology and growth characteristics.⁹ Cattle were considered to be infected if a single colony of *B. abortus* was recovered from any sample obtained at necropsy.

**ANALYSIS**

Serologic data were converted to the logarithm of the titer for analysis. Serologic responses of calves in both studies were 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, NC). Means for individual treatments were separated by use of a least significant difference procedure (*P* < .05). Fisher's exact test was used to compare the incidence of abortion or S2308 infection after S2308 challenge.

**RESULTS**

No clinical illness or adverse reactions were noted in any heifer at any time after vaccination with 10⁹ or 10¹⁰ CFU of SRB51.

**Serologic Evaluation**

Cattle vaccinated with SRB51 in both studies remained negative on the standard tube agglutination test at all times after inoculation. Dot-blot titers of SRB51-vaccinated and unvaccinated calves did not differ in either study before vaccination. Calves vaccinated at 12 weeks of age with 10¹⁰ CFU of SRB51 had greater (*P* < .05) antibody titers on the dot-blot test at 4 and 8 weeks but not 12 weeks after vaccination when compared to nonvaccinates (Figure 1). Dot-blot titers of calves inoculated with 10⁹ CFU of SRB51 at 12 weeks of age did not differ at any sampling time from titers of nonvaccinates.

In the second study, heifers vaccinated at 6 months of age with 10¹⁰ CFU of SRB51 had greater (*P* < .05) dot-blot titers at 4, 8, 12, and 16 weeks after vaccination when compared with titers of nonvaccinates (data not shown).

**Lymphocyte Proliferation Assays**

Heifers vaccinated with 10¹⁰ CFU of SRB51 at 6 months of age had greater (*P* < .05) proliferative responses and stimulation indices to γ-irradiated SRB51 bacteria at 10, 12, 14, and
16 weeks after vaccination as compared to responses of nonvaccinates (Figure 2).

Experimental Challenge and Bacteriologic Evaluation

**SRB51 Vaccination at Twelve Weeks of Age**

Rectal palpation indicated that 14 of 15 $10^9$ CFU SRB51 vaccinates, four of six $10^8$ CFU SRB51 vaccinates, and nine of nine nonvaccinates were pregnant. After intraconjunctival challenge of pregnant heifers with S2308, the challenge strain was recovered from conjunctival swabs at both 2 and 5 days after challenge in all but four heifers. In two heifers vaccinated with $10^9$ CFU of SRB51 and one nonvaccinated heifer, S2308 was recovered only at 2 days, whereas in the remaining heifers vaccinated with $10^8$ CFU of SRB51 swabs were culture positive for S2308 only at 5 days after challenge.

At 5 weeks after challenge, two SRB51 vaccinates were lost because of a steam line pipe break at the biocontainment facility. At 7 weeks after challenge, one nonvaccinate had to be euthanized because of lameness. Four of the remaining eight nonvaccinated cattle aborted between 7 and 9 weeks after challenge. Two of the four heifers in the $10^9$ CFU SRB51 treatment aborted between 8 and 9 weeks after challenge. One heifer in the $10^8$ SRB51 treatment was found at necropsy to not be pregnant. Two of the remaining 11 heifers vaccinated with $10^{10}$ CFU of SRB51 aborted between 8 and 10 weeks after vaccination as compared to responses of nonvaccinates (Figure 2).
weeks after challenge (Table 1).

All nonvaccinates and SRB51 vaccinates demonstrated seroconversion on the standard tube agglutination test by 4 weeks after challenge. In most animals that did not abort, standard tube agglutination titers were negative at necropsy, whereas animals that aborted demonstrated high titers (>800) at necropsy. For regulatory purposes, cattle with standard tube agglutination titers of 1:100 or greater are classified as reactors.

The S2308 challenge strain was recovered at necropsy from maternal and/or fetal tissue of six of the nine nonvaccinated cattle including all animals that aborted. In heifers vaccinated with 10⁹ CFU of SRB51, the S2308 challenge strain was recovered from maternal tissue of all heifers and from fetal tissue obtained from the two abortions. In the 10¹⁰ CFU SRB51 group, the S2308 challenge strain was recovered from maternal and fetal tissue from both abortions and maternal tissue of five other heifers including the nonpregnant heifer. In all culture-positive SRB51 vaccinates that did not abort—two heifers in the 10⁹ CFU and five heifers in the 10¹⁰ CFU treatment—S2308 was recovered in low numbers only from mandibular or parotid lymph nodes. SRB51 was not recovered at necropsy from any sample.

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**TABLE 1. Recovery of *Brucella abortus* from Tissue Obtained at Necropsy After Midgestational Intraconjunctival Challenge of Nonvaccinated and *B. abortus* strain RB51 (SRB51)—Vaccinated Cattle with 1 × 10⁷ Colony-Forming Units (CFU) of *B. abortus* Strain 2308**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>Recovery of S2308</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Pregnant</td>
</tr>
<tr>
<td><strong>Study 1 (3 months of age)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>10⁹ CFU SRB51</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>10¹⁰ CFU SRB51</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td><strong>Study 2 (6 months of age)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
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<td>7</td>
</tr>
<tr>
<td>10¹⁰ CFU SRB51</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
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<td>16</td>
</tr>
<tr>
<td>10¹⁰ CFU SRB51</td>
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<td>31</td>
</tr>
<tr>
<td>10⁹ CFU SRB51</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

*Excludes one heifer euthanized because of lameness at 7 weeks after challenge.
†Excludes two heifers lost to a steam line pipe break at 5 weeks after challenge and one heifer found at necropsy to not have been pregnant.
‡Excludes one heifer found at necropsy to not have been pregnant.
§Excludes all nonpregnant heifers, euthanized heifers, heifers lost prematurely because of unrelated causes, and one SRB51 vaccinate that was culture negative for *Brucella* on eye swabs after challenge and did not seroconvert on the standard tube agglutination test.
||The overall incidence of abortion or fetal infection in 10¹⁰ CFU SRB51 vaccinates is reduced (*P* < .02) when compared to overall data for nonvaccinates.
SRB51 Vaccination at Six Months of Age

After pasture breeding, 17 of 20 SRB51 vaccinates and seven of eight nonvaccinates were determined to be pregnant by rectal palpation. After intraconjunctival challenge with S2308, the challenge strain could be recovered from conjunctival swabs at both 2 and 5 days in all but four heifers. In two SRB51 vaccinated heifers, S2308 was not recovered from conjunctival swabs at either time point. In two other SRB51 vaccinates, S2308 was recovered from conjunctival swabs only at 5 days after challenge.

With the exception of four heifers, all vaccinates and control heifers seroconverted on the standard tube agglutination test by 4 weeks after challenge. Three of the heifers that did not seroconvert [one nonvaccinate and two SRB51-vaccinates] had S2308-positive eye swabs at 5 days after challenge. The remaining heifer that did not seroconvert, an SRB51 vaccinate, had negative eye swabs for S2308 at both 2 and 5 days after challenge. All SRB51 vaccinates that did not abort had transient seroconversion on the standard tube agglutination test and were seronegative at necropsy.

Three of seven heifers in the control group aborted between 6 and 10 weeks after S2308 challenge. One SRB51-vaccinated heifer aborted 11 weeks after challenge. The S2308 challenge strain was recovered from maternal or fetal tissue obtained at necropsy from five of seven control heifers. In comparison, S2308 was recovered at necropsy from maternal tissue of seven of 18 SRB51 vaccinates, including one heifer that was not pregnant at necropsy. In four of the SRB51 vaccinates that did not abort, S2308 was recovered in low numbers only from mandibular or parotid lymph nodes. In two SRB51 vaccinates, tissue positive for S2308 at necropsy included the mammary gland, supramammary lymph node, and retropharyngeal lymph node, in addition to mandibular and/or parotid lymph nodes. Only fetal tissue from the SRB51 vaccinate that aborted was culture positive for the S2308 challenge strain. SRB51 was not recovered from any sample obtained at necropsy.

Efficacy of SRB51

For analysis of efficacy of SRB51, nonpregnant animals, noninfected animals, and animals lost prematurely to other causes in either study were excluded. When data from both studies were combined with these exclusions, statistical analysis indicated that calfhood vaccination with $10^{10}$ CFU of SRB51 significantly reduces ($P < .02$) the incidence of abortion or fetal infection with S2308 when compared with controls. However, incidence of recovery of S2308 from maternal tissue at necropsy did not differ ($P > .05$) among nonvaccinates and heifers that were vaccinated at 12 weeks or 6 months of age with $10^{10}$ CFU of SRB51.

DISCUSSION

Results of this study suggest that the commercial SRB51 vaccine is clinically safe and induces immune responses that protect against fetal infections or abortions caused by B. abortus. The limited data obtained in the studies reported here suggest that calfhood vaccination with $10^9$ CFU of SRB51 does not induce protection against brucellosis. Because calfhood vaccination with $10^{10}$ CFU of SRB51 did protect against experimental challenge with a virulent B. abortus strain, our data suggest that the current recommended calfhood dosage of SRB51 (1–3.4 $\times 10^{10}$) is appropriate. Because shedding of bacteria at the time of abortion or birth of a Brucella-infected fetus is the predominant method for transmission of brucellosis, our data suggest that the commercial SRB51 vaccine will be highly efficacious under field conditions.
Efficacy of brucellosis vaccines under field conditions has been found to be greater than efficacy noted under experimental conditions\textsuperscript{12} and most likely reflects differences under field conditions in exposure to brucellosis. Therefore, it is anticipated that protection induced by the commercial SRB51 vaccine under field conditions will be greater than protection from abortion and infection noted in the experimental studies reported here.

Data suggest that long-term protection of cattle against \textit{Brucella} infections is mediated through cell-mediated rather than humoral immune responses.\textsuperscript{13} Although blastogenic responses do provide evidence of stimulation of cell-mediated immunity, others have found no correlation between the magnitude of lymphocyte proliferative responses and protection against \textit{Brucella} infection.\textsuperscript{14,15}

Although data in the studies reported here did not demonstrate significant reductions in maternal infections, previous studies in our laboratory have demonstrated that calfhood vaccination with SRB51 significantly ($P<.05$) reduces recovery of S2308 at necropsy, 12 weeks after midgestational challenge, when compared with nonvaccinates (i.e., 20% and 60% S2308 recovery, respectively).\textsuperscript{2,3,4} Because previous studies in our laboratory have used SRB51 in log phase growth, one explanation for the difference may be that the commercial vaccine used in the current study was a lyophilized product. It should also be noted that the dosage used for vaccination in the current study was at the minimum recommended calfhood dosage, and the possibility cannot be excluded that higher dosages of the commercial product might enhance protective responses, although at this time there is no study planned to address this. In a previous study in our laboratory, cattle vaccinated at 7 months of age with $1.6 \times 10^{10}$ or $3.2 \times 10^{10}$ CFU of SRB51 in log phase did not differ in their protection against infection following experimental challenge.\textsuperscript{4}

Data from previous studies in our laboratory also have suggested that efficacy of SRB51 is greater when calves are vaccinated between 6 and 10 months of age. This trend is supported by data presented in this manuscript that suggest a slightly higher incidence of abortion and maternal infection in calves vaccinated at 12 weeks of age compared with calves vaccinated at 6 months of age.

\section*{CONCLUSION}

The commercially available SRB51 vaccine is efficacious in preventing abortions or fetal infections caused by virulent strains of \textit{Brucella abortus}.

\section*{ACKNOWLEDGMENTS}

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