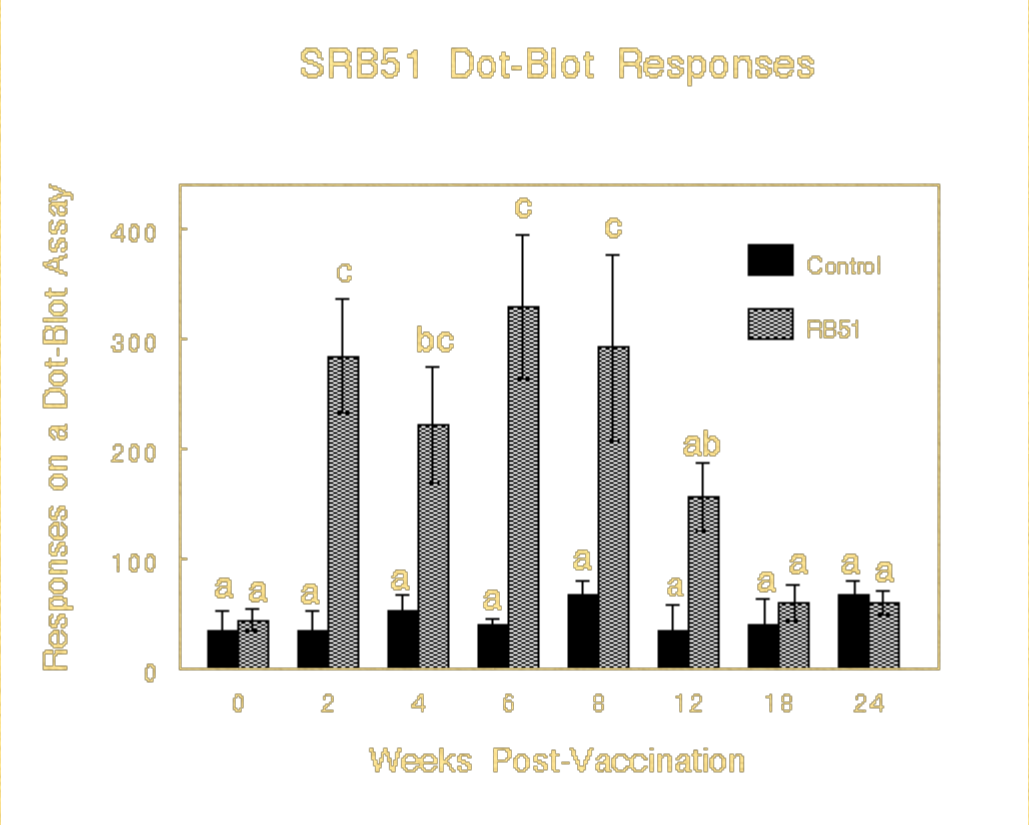
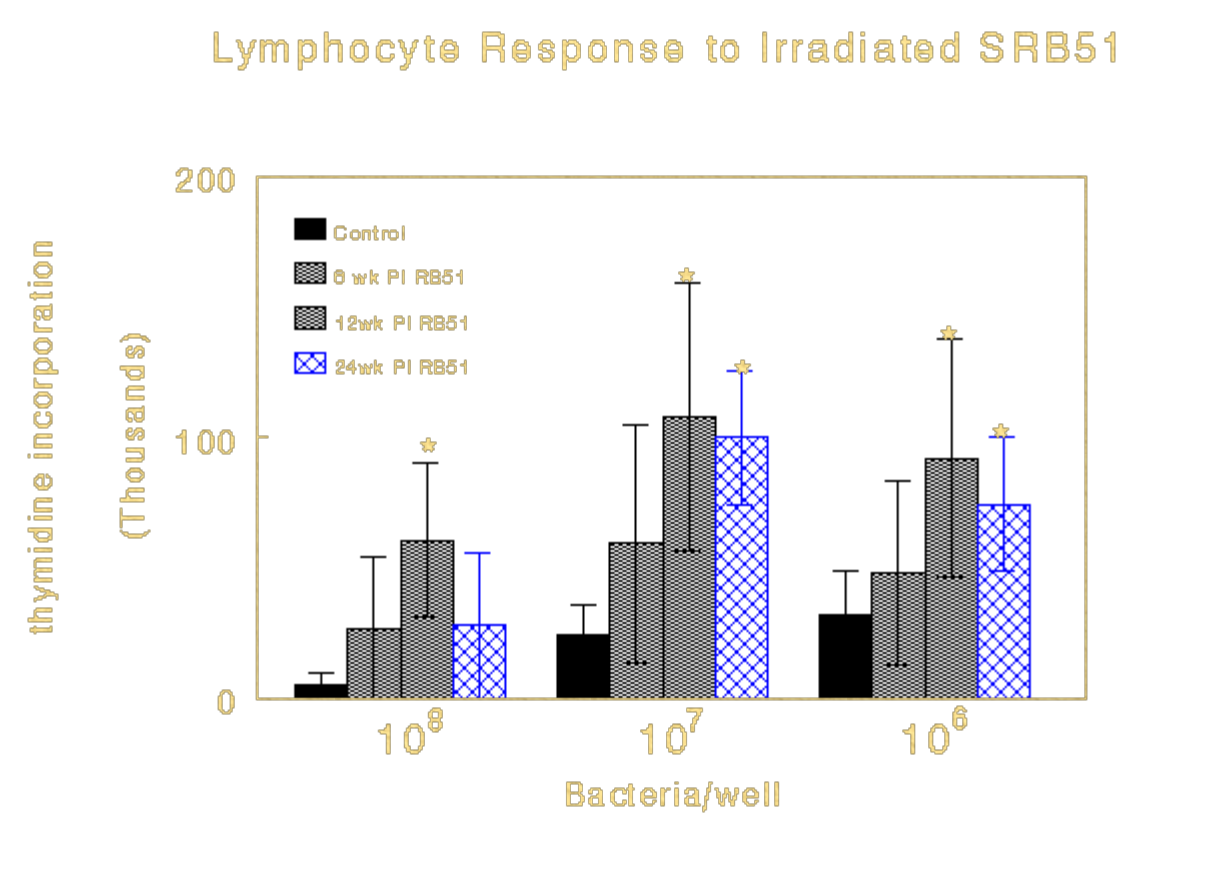
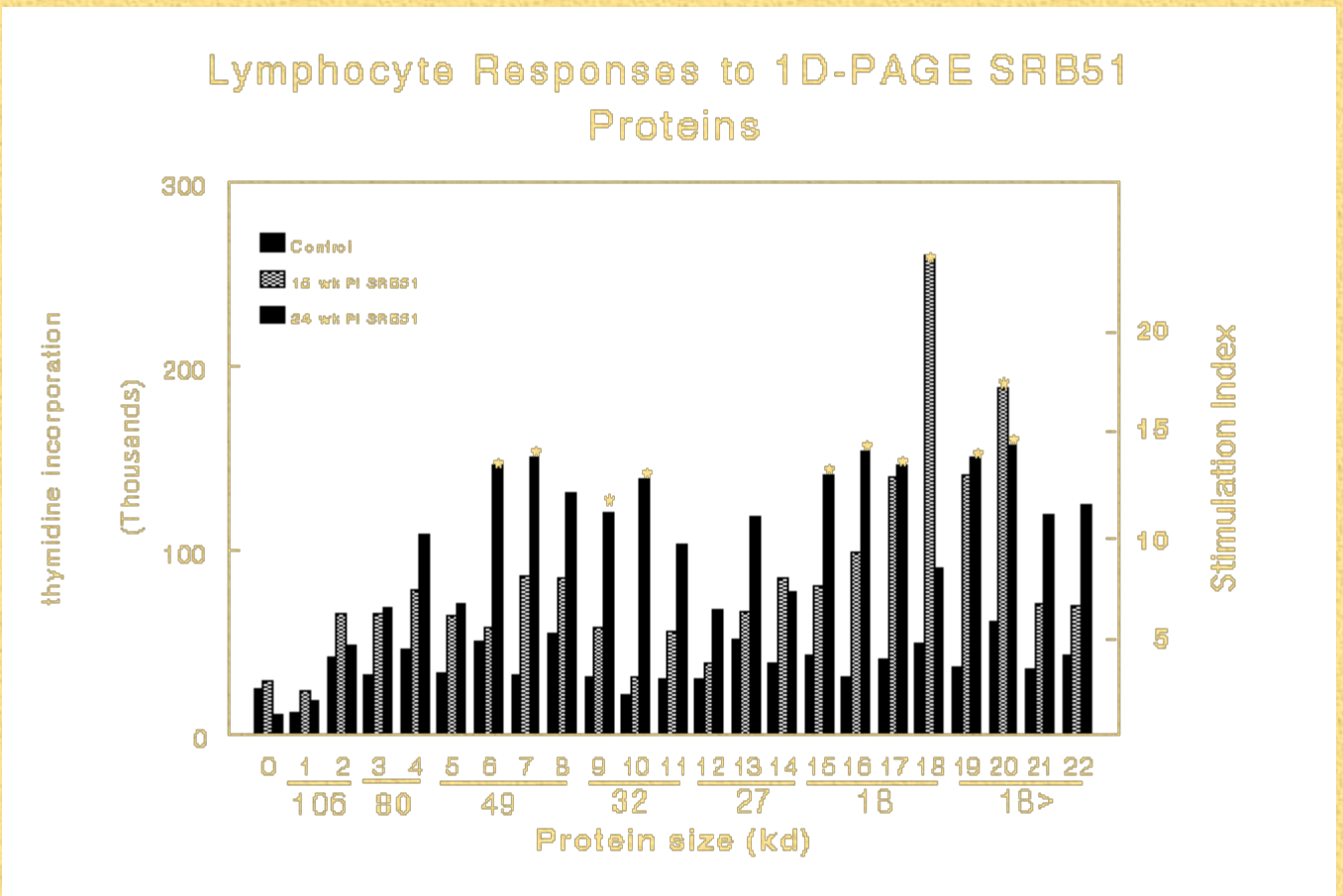

SRB51 Dot-Blot Responses
Responses on a Dot-Blot Assay
400
300
200
100
0
Control
RB51
a a
a c
a bc
a c
a c
a ab
a a
a a
0
2
4
6
8
12
18
24
Weeks Post-Vaccination


Lymphocyte Response to Irradiated SRB51
thymidine incorporation
(Thousands)
200
100
0
Control
6 wk PI RB51
12wk PI RB51
24wk PI RB51
10^8
10^7
10^6
Bacteria/well

Webmaster's note:
Figure 3 was missing on the original web server repository when this documented was converted to PDF.


Lymphocyte Responses to 1D-PAGE SRB51 Proteins
thymidine incorporation
(Thousands)
300
200
100
0
Control
16 wk PI SRB51
24 wk PI SRB51
20
15
10
5
Stimulation Index
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
106 80 49 32 27 18 18>
Protein size (kd)

G00042

Serologic responses, biosafety and clearance of *Brucella abortus* strain RB51 in 7 month old bison

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Abstract:
To determine the clearance of *Brucella abortus* strain RB51 (SRB51), 10 bison were subcutaneously vaccinated in the cervical region with 1.22 x 10^10 CFU SRB51 at 7 months of age. Four bison were SQ vaccinated with saline and housed with the SRB51-vaccinates. Serologic responses were monitored up to 24 weeks post-infection (PI) by use of a dot-blot assay. Clearance of SRB51 from the prescapular lymph node was evaluated in 4 vaccinates and 1 control at 6, 12, 18 and 24 weeks PI. To evaluate shedding of the SRB51 vaccine, nasal, conjunctival, vaginal and rectal swabs were obtained from all bison at 1, 2, 3, 4, 6, 8, 12, 18 and 24 weeks PI. Blood cultures were also evaluated in all bison at 2, 4, 6 and 8 weeks PI. Humoral responses were demonstrated in all SRB51-vaccinated bison but not in controls. Culture of biopsies from the prescapular lymph node indicated that SRB51 persisted in some animals until 18 weeks PI. The only culture-positive blood sample was in 1 SRB51-vaccinated bison at 2 weeks PI. SRB51 was not recovered at any time from vaginal, rectal, conjunctival, or nasal swabs obtained from SRB51-vaccinated or non-vaccinated bison. The results of this study suggest that SRB51 is safe as it was not shed from calfhood-vaccinated bison or spread to non-vaccinated bison. Our data also indicates that most bison clear SRB51 from the draining lymph node between 18 and 24 weeks PI.

Introduction:

With 37 states currently classified as free of cattle brucellosis, the goal of completion of the Brucellosis Eradication Program for cattle by 1998 may be achieved. However, the presence of *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; GYA) may threaten that goal. Transmission of brucellosis from elk or bison to cattle under experimental conditions has been demonstrated. Although the population will be significantly lower in the spring of 1997, it is estimated that average populations over the last 5 years in the GYA area were approximately 3,500 and 115,000 for bison and elk, respectively. Seroprevalence for brucellosis in these populations were approximately 50% for the total bison population, 37% for adult female elk on feedgrounds, and less than 2% in elk that overwinter off feedgrounds. The National Park Service has set a goal of elimination of brucellosis in the GYA by 2010.

A brucellosis control or eradication program in the GYA most likely would include vaccination of bison with a safe and efficacious product. The current cattle vaccine, *B. abortus* strain 19 (S19), has not been found to be highly efficacious in bison under experimental conditions and appears to be more virulent as it causes a high incidence of abortion when given to pregnant bison. Strain 19 also induces serologic titers that interfere with the identification of Brucella-infected bison. We have evaluated a new vaccine in bison, *B. abortus* strain RB51 (SRB51), that has been recently approved as a calfhood vaccine for cattle. Following subcutaneous vaccination of bison with 10e10 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. Due to the very low expression of the lipopolysaccharide (LPS) O-side chain by SRB51, bison vaccinated with SRB51 remain seronegative on the card and standard tube agglutination tests at all times although antibody responses to SRB51 can be demonstrated using a dot-blot assay. The purposes of this study were to determine when SRB51 is cleared from the draining lymph nodes of vaccinated bison, characterize cell-mediated responses, and to assess the biosafety of this vaccine by determining if bison shed SRB51 following inoculation.

Materials and Methods:

A. Vaccination

Bison were vaccinated subcutaneously with 1.22e10 CFU of *Brucella abortus* strain RB51 at 7 months of age.

B. Clearance of the vaccine strain

Blood was obtained for culture at 2, 4, 6, 8, 12, 18 and 24 weeks post-innoculation.

The prescapular lymph node draining the site of vaccination was surgically biopsied from 3 SRB51 vaccinates and 1 nonvaccinate at 6, 12, 18 and 24 weeks. Lymph nodes were examined by culture and histological techniques.

C. Shedding of vaccine strain

Vaginal, rectal, conjunctival and nasal swabs were obtained at 1, 2, 3, 4, 6, 8, and 12 weeks post-innoculation for culture analysis.

D. Immune Responses

Blood was obtained at 2, 4, 6, 8, 12, 18 and 24 weeks post-innoculation for serologic evaluation using standard tube agglutination and SRB51 dot-blot tests.

At 6, 12, 18 and 24 weeks, PBMC and prescapular lymph node cells were incubataed with irradiated SRB51 bacteria and SRB51 proteins separated by one-dimensional polyacrylamide electrophoresis (1D-PAGE).

Results:

A. Clearance of the Vaccine Strain

Blood from 1 bison was culture positive for SRB51 at 2 weeks post-innoculation. All other blood samples were culture negative for SRB51.

SRB51 was recovered from the prescapular lymph node of 4/4, 3/4, 1/4, and 0/3 bison at 6, 12, 18 and 24 weeks post-innoculation, respectively (Table 1).

B. Shedding of the vaccine strain

SRB51 was not recovered from vaginal, rectal, conjunctival or nasal swabs of vaccinated or nonvaccinated bison at any sampling time.

C. Immune Responses

1. Antibody Responses

SRB51-vaccinated bison remained seronegative on the standard tube agglutination test at all times.

Bison vaccinated with SRB51 had greater antibody titers on the dot-blot test at 2, 4, 6, and 8 weeks post-innoculation when compared to nonvaccinated bison (Figure 1).

2. Cell-mediated Responses

PBMC and lymph node cells had proliferative responses to irradiated bacteria beginning at 12 weeks post-innoculation (Figure 2 & 3).

Lymph node cells had greater proliferative responses to 1D-PAGE-separated SRB51

proteins at 12 and 24 weeks when compared to responses of nonvaccinates over all sampling times (Figure 4).

Discussion and Conclusions:

1. Most bison clear *Brucella abortus* strain RB51 by 18 to 24 weeks post-innoculation.

2. Bison vaccinated with SRB51 do not shed the vaccine to nonvaccinated bison housed in close proximity or from mucosal surfaces into the environment.

3. Strain RB51 does not induce antibody responses which would interfere with serologic detection of *Brucella*-infected bison.

4. Cell-mediated reponses have been associated with protection against *Brucella abortus*. Strain RB51 induces cell-mediated responses in bison beginning at 12 weeks post-innoculation.

References:

Comments:

The authors would like to acknowledge the generous donation of the bison used in this project by the U.S. Fish and Wildlife Service.

Address questions and comments about this abstract to Steven C. Olsen (solsen@nadc.ars.usda.gov).

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Questions and Answers