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IMMUNE RESPONSES OF ELK TO VACCINATION WITH *BRUCELLA ABORTUS* STRAIN RB51

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ABSTRACT: In a study conducted from January to August 2000, elk (*Cervus elaphus*) were vaccinated with *Brucella abortus* strain RB51 (SRB51, $n=6$) or injected with 0.15 M NaCl solution ($n=3$) at approximately 6 mo of age. Beginning at 2 wk and continuing to 25 wk after vaccination, SRB51-vaccinated elk had greater antibody responses ($P<0.05$) to SRB51 when compared to nonvaccinated elk. Peripheral blood mononuclear cells (PBMC) from SRB51-vaccinated elk had greater ($P<0.05$) proliferative responses to SRB51 at 18 wk after vaccination when compared to responses of nonvaccinated elk. Strain RB51 was recovered from blood samples of all vaccinates at 2 wk, and three of six vaccinates at 4 wk after vaccination. The SRB51 vaccine strain was recovered from the superficial cervical lymph node of all vaccinates sampled at 6 wk after vaccination, but not from lymph node samples obtained from vaccinates at 12 or 18 wk after vaccination. At 34 wk after vaccination, SRB51 was recovered from the bronchial lymph node of one of five vaccinates but not from other tissues. Strain RB51 was not recovered at any time from samples obtained from nonvaccinated elk. This study suggests that following vaccination with SRB51, elk remain bacteremic for a prolonged period of time, rapidly develop high antibody titers, and are slower to develop detectable proliferative responses in PBMC when compared to responses of cattle or bison (*Bison bison*).

Key words: *Brucella, Cervus elaphus,* clearance, elk, RB51, serology, vaccine.

INTRODUCTION

A new official calfhood vaccine for cattle, *Brucella abortus* strain RB51 (SRB51), was conditionally approved in 1996 by the Animal and Plant Health Inspection Service, United States Department of Agriculture. The SRB51 strain, a laboratory derived, lipopolysaccharide O-side chain-deficient mutant (Schurig et al., 1991) of *B. abortus* strain 2308 (S2308), induces protection in cattle against challenge-exposure with virulent *B. abortus* strains (Cheville et al., 1993, 1996). Unlike its predecessor, *B. abortus* strain 19, cattle and bison vaccinated with SRB51 vaccine do not produce antibodies that react in conventional brucellosis serological tests (Stevens et al., 1994).

Although others have evaluated antibody responses and efficacy in elk vaccinated with the SRB51 vaccine (Cook et al., 2002; Kreeger et al., 2002), cell-mediated responses and clearance of SRB51 have not been characterized. Unlike data from cattle and other species, SRB51 was not found to be efficacious in protecting elk against brucellosis (Kreeger et al., 2002). Understanding immune responses of elk to SRB51 may be beneficial for development of an efficacious brucellosis vaccine. The purpose of the study reported here was to characterize immune responses after vaccination, evaluate clearance from lymphatic tissues, and compare histologic responses in SRB51-vaccinated and control elk.

MATERIALS AND METHODS

This study was conducted from January 2000 to August 2000 at the National Animal Disease Center (Ames, Iowa, USA; 42°52′N, 93°63′W). Nine female elk calves were obtained from a brucellosis-free area (Starkey Experimental Forest and Range, Oregon Department of Fish and Wildlife, La Grande, Oregon, USA). At approximately 6 mo of age, six elk were subcutaneously inoculated with a commercially available SRB51 vaccine (Colorado Serum Company, Denver, Colorado, USA) into both right and left cervical regions drained by the superficial cervical lymph node. The vaccine was prepared by dilution in 0.15 M NaCl (saline) to approximately 1×10^{10} colony-forming units (CFU)

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based upon standard plate counts on other vials with the same lot number. The concentration of viable bacteria within the inoculum was determined by standard plate counts to be 1.03×10^{10} CFU. An additional three elk were subcutaneously inoculated with 2 ml of saline.

At 0, 2, 4, 6, 8, 12, 18, 25, and 33 wk after vaccination, blood was obtained via jugular venipuncture for serologic analysis. Serum for serologic evaluation was obtained by centrifugation after allowing blood to clot for 12 hr at 4 C. 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).

At 4, 6, 8, 12, 18, and 25 wk after vaccination, blood was obtained from the jugular vein of all elk 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, St. Louis, Missouri, USA). Cell culture experiments used RPMI 1640 medium (GIBCO Laboratories, Grand Island, New York, USA) containing L-glutamine, 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10% fetal bovine serum (Hyclone Laboratories, Logan, Utah, USA), 100 U of penicillin per ml, 100 mg of streptomycin per ml, and 5×10^{-5} M 2-mercaptoethanol. Peripheral blood mononuclear cells were diluted in RPMI 1640 medium 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} PBMC was added to each of three 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). In a similar manner, responses of PBMC from vaccinated and nonvaccinated elk to 1640 media containing concanavalin-A (ConA; 10, 1, and 0.1 μg/ml), phytohemagglutinin (PHA; 10, 1, and 0.1 ug/ml) and pokeweed (PKW; 20, 2, 0.2 μg/ml) mitogens (Sigma) were evaluated 19 wk after vaccination. Cell cultures were incubated for 7 days at 37 C under 5% CO_2. Microtiter plates were placed on a shaker (MicroShaker II, Dynatech Laboratories Inc., Alexandria, Virginia, USA) every 2 days during the incubations 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 counted for radioactivity in a liquid scintillation counter. Counts per minute (cpm) were converted to $\log_{10}$, or stimulation indices (cpm of wells containing antigen/cpm in absence of antigen), for statistical comparisons. Lymphocyte blastogenesis cpm data were analyzed using analysis of variance (SAS Institute Inc., Cary, North Carolina, USA). Means for each treatment were compared by use of a least significant difference procedure and are reported as least square means±SEM. Significant differences between treatments were reported when $P\leq0.05$.

To determine the persistence of SRB51 in blood and if vaccinates transmit SRB51 to nonvaccinates housed in close association, 15 ml of blood was obtained from all elk at 2 and 4 wk after inoculation and mixed 1:1 with tryptose broth (Difco Laboratories, Detroit, Michigan, USA) containing 1% sodium citrate. One ml from blood cultures of each elk were directly plated onto a selective media for SRB51 (RBM; Hornsby et al., 2000) which contains antibiotics, including rifampicin (Sigma). The antibiotics in RBM media minimize growth of contaminants without inhibiting growth of SRB51 and thereby enhance the ability to detect small numbers of SRB51 within samples. Remaining blood cultures were held at −5 C for 24 hr and then placed at 37 C and 5% CO_2. One ml volumes were removed from blood cultures at 7, 14, 21, and 28 days incubation and plated onto RBM media. Following incubation of plates at 37 C and 5% CO_2 for 72 hr, SRB51 was identified on the basis of colony morphology, growth characteristics (Alton et al., 1988), and resistance to rifampin (Schurig et al., 1991). Isolates were confirmed as SRB51 in a polymerase chain reaction (PCR) procedure using primers specific for SRB51 (Vemulapalli et al., 1999).

At 2 and 4 wk after inoculation, rectal, vaginal, nasal, and ocular swabs (American Scientific Products, McGaw Park, Illinois, USA) were obtained from all elk. Swabs were plated on RBM media. Isolates were confirmed as SRB51 as described above.

To determine the persistence of SRB51 in lymphoreticular tissues, SRB51-vaccinated ($n=3$) and nonvaccinated ($n=1$) elk were randomly selected at 6, 12, and 18 wk after vaccination for biopsy of the left or right superficial cervical lymph node. After aseptic surgical removal as previously described (Cheville et al., 1993; Olsen et al., 1997a) the lymph node was divided into proximal, middle, and distal portions. Areas adjacent to samples collected for bacteriology were placed into neutral, buffered, 10% formalin for histologic evaluation. Lymph node sections were weighed, triturated using a tissue grinder, serially diluted in saline, and placed on tryptose agar plates containing 5%

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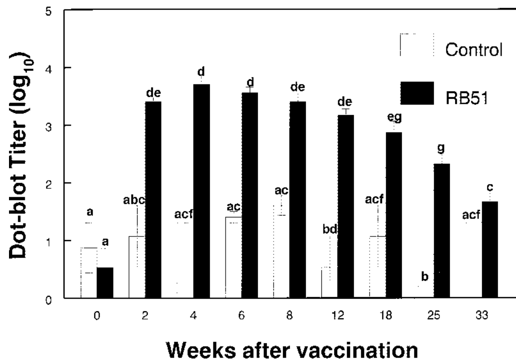

FIGURE 1. Serologic responses of SRB51-vaccinated (n=6) or control elk (n=3) to γ-irradiated SRB51 in a dot blot assay. Responses are presented as mean antibody titer±SEM. Means with different superscripts are significantly different (P<0.05).

bovine serum. Following incubation at 37 C and in 5% CO_2, bacterial cell counts were made from each dilution by standard plate counts. Strain RB51 was identified as described above.

At 34 wk after vaccination, elk were euthanized by intravenous injection of pentobarbital and tissues collected from five vaccinates and two nonvaccinates. Tissues collected at necropsy for bacteriologic and histologic evaluation included mammary gland, liver, lung, spleen, and uterus. Additional bacteriologic samples included bronchial, hepatic, parotid, popliteal, medial retropharyngeal, internal iliac, mandibular, mesenteric, and supramammary lymph nodes. Histologic evaluation was also conducted on kidney and cardiac samples from all elk. All tissues collected for histologic evaluation were fixed in neutral, buffered, 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

RESULTS

One SRB51 vaccinate was removed from the study due to a handling-associated injury 8 wk after vaccination. With the exception of this injury, no clinical illness was noted in any elk during the course of the study.

Elk vaccinated with SRB51 had greater (P<0.05) dot-blot titers than nonvaccinates at 2, 4, 6, 8, 12, 18, and 25 wk after vaccination (Fig. 1). Titers of SRB51-vaccinated elk did not differ from nonvaccinates at 33 wk after vaccination. All elk were negative on the STAT at all sampling times.

One colony of SRB51 was recovered from the nasal swab of a SRB51 vaccinated elk 2 wk after vaccination. Remaining swabs obtained from SRB51 vaccinated and nonvaccinated elk at 2 and 4 wk were negative for recovery of the vaccine strain. Strain RB51 was recovered from the blood of all six vaccinated elk at 2 wk, and three of six vaccinates at 4 wk after vaccination. The SRB51 vaccine strain was not recovered from the blood of any nonvaccinated elk.

Strain RB51 was recovered from the superficial cervical lymph node of all three vaccinated elk biopsied 6 wk after vaccination, but not from any biopsy sample obtained from SRB51 vaccinates 12 and 18 wk post-vaccination Mean SRB51 colonization of prescapular lymph node tissue 6 wk after vaccination was 6,852±4,190 CFU/g. Superficial cervical lymph node tissue of all nonvaccinates biopsied 6, 12, or 18 wk were culture negative for SRB51.

At necropsy, 34 wk after vaccination, SRB51 was recovered from the bronchial lymph node of one of five vaccinated elk, but not from any other tissue samples. The SRB51 vaccine strain was not recovered from any tissues obtained at necropsy from two nonvaccinated elk.

Peripheral blood mononuclear cell proliferative responses to γ-irradiated SRB51 bacteria did not differ (P>0.05) between SRB51-vaccinated and nonvaccinated elk at 4, 6, 8, and 12 wk after vaccination (Fig. 2). Peripheral blood mononuclear cells from SRB51-vaccinated elk had greater proliferative responses (P<0.05) to SRB51 bacteria at 18 wk after vaccination when compared to control elk. When incubated for 7 days with mitogens, PBMC demonstrated strong proliferative responses to 2 and 20 μg/ml concentrations of PKW, but poor proliferative responses to all concentrations of ConA or PHA mitogens (Fig. 3).

Histologic characteristics of superficial cervical lymph node sections did not vary

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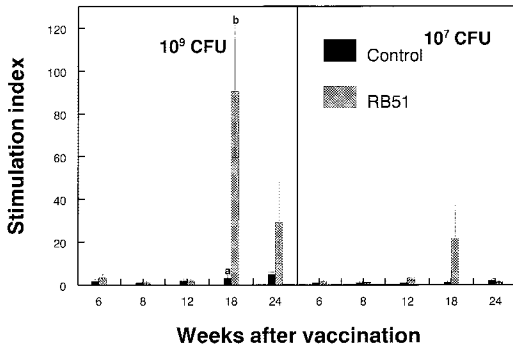

FIGURE 2. Proliferative responses of peripheral blood mononuclear cells from SRB51-vaccinated (n=6) or nonvaccinated elk (n=3) to 10^7 or 10^9 CFU of γ-irradiated SRB51 at various times after vaccination. Cells were incubated at 37 C in 5% CO_2 for 7 days and pulsed for 18 hr with [^{3}H]thymidine. Results are expressed as mean stimulation indexes. Means denoted with different superscripts are significantly different (P<0.05) from mean responses at other sampling times to that concentration of SRB51.

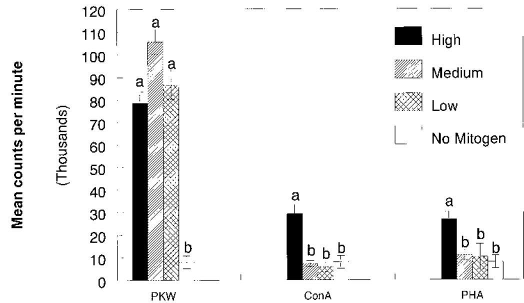

FIGURE 3. Proliferative responses of peripheral blood mononuclear cells from SRB51-vaccinated or nonvaccinated elk to high, medium, and low concentrations of concanavalin-A (ConA; 10, 1, and 0.1 ug/ml), phytohemagglutinin (PHA; 10, 1, 0.1 μg/ml), or pokeweed mitogen (PKW; 20, 2, and 0.2 μg/ml), respectively, or in the absence of mitogen. 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 counts per minute±SEM (n=9). Means denoted with different superscripts are significantly different (P<0.05) from mean responses to other concentrations of that mitogen.

between SRB51 vaccinated and nonvaccinated elk at 6 wk after vaccination. Beginning at 12 wk, and becoming more conspicuous at 18 wk after vaccination, lymph node sections from SRB51 vaccinated elk differed from nonvaccinates by the presence of two to 25 germinal centers of variable size in the superficial or deep cortex. At 18 wk after vaccination, SRB51 vaccinated elk also were characterized by diffuse to focally extensive infiltrates of neutrophils in medullary sinuses, the tunica intima and adventitia of arterioles, and the lymph node capsule. Mammary, liver, lung, spleen, kidney, cardiac, or uterine tissues obtained at necropsy from SRB51 vaccinated and nonvaccinated elk were histologically normal.

DISCUSSION

Data obtained in this, and other studies, suggests that elk have substantial differences in their response to vaccination with SRB51 when compared to cattle and bison (*Bison bison*). Our data, and those of others (Kreeger et al., 2002), suggest that elk rapidly develop strong antibody responses to SRB51. In our study, mean dot-blot antibody titers at 2 and 4 wk after calfhood vaccination of elk with 1×10^{10} CFU of SRB51 were 2,773 and 6,400, respectively. Others have reported mean dot-blot antibody titers >6,000 at 2 wk after vaccination of elk (n=16) with 1×10^{10} CFU of SRB51 (Kreeger et al., 2002). In comparison, unpublished data from studies in our laboratory using similar dosages of SRB51 have found mean dot-blot antibody titers at 2 and 4 wk after vaccination of 1,256 and 812, respectively, in bison (n=10), and 1,259 and 1,732, respectively, in cattle (n=31). Significant proliferative responses to SRB51 have been detected in PBMC from cattle and bison by 10 to 12 wk after SRB51 vaccination (Olsen et al., 1998; Olsen, 2000), whereas proliferative responses of PBMC to SRB51 could not be demonstrated in elk in the current study until 18 wk after vaccination. Because elk have predominately antibody and delayed proliferative responses to SRB51, we hypothesize that SRB51 induces a predominantly TH2 response in elk. If this hypothesis is correct, lack of proliferative responses could be explained by the fact that cytokines associated with TH2 responses inhibit cytokines associated with TH1 re-

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sponses (Fiorentino et al., 1989; O'Garra and Murphy, 1994). Additional studies evaluating cytokine transcription in elk mononuclear cells will be required to evaluate this hypothesis.

Elk also differ from other species in the length of SRB51 bacteremia. All vaccinated elk were bacteremic with SRB51 at 2 wk and half of the elk remained bacteremic at 4 wk after vaccination. In comparison, SRB51 was recovered from the blood of one of 10 bison at 2 wk after vaccination with 1.2×10^{10} CFU, but was not recovered from blood obtained at 4, 6, or 8 wk (Olsen et al., 1998). In cattle studies conducted in our laboratory, SRB51 was not recovered from blood obtained at 1, 2, or 3 wk after vaccination from 18 heifer calves inoculated with 8.5×10^{9} to 1.2×10^{10} CFU of SRB51 between the ages of 3 and 10 mo of age (unpubl. data).

In bison, SRB51 was recovered from lymphatic tissues up to 26 wk after vaccination (Roffe et al., 1999). Although comparable data are not available for cattle, the persistence of SRB51 in lymphoid tissues 34 wk after vaccination in one elk in the current study exceeds the length of time that SRB51 persisted in bison. Inability to recover SRB51 from the superficial cervical lymph nodes of vaccinated elk at 12 and 18 wk after vaccination is surprising in light of its recovery from the bronchial lymph node of one elk at 34 wk. Strain RB51 was not recovered from the superficial cervical lymph node of this elk at 18 wk after vaccination. Although the authors cannot explain this disparity, the uneven localization of SRB51 in lymph nodes combined with the loss of portions of lymph nodes to histologic analysis, and the possibility that SRB51 was present in concentrations below detectable limits for culture methods, may be possible explanations for the inability to recover SRB51 from the superficial cervical lymph nodes of any vaccinates at 12 and 18 wk.

Recovery of SRB51 from a nasal swab is also unprecedented in other species and may reflect the persistent bacteremia in elk. However, failure of co-housed nonvaccinated elk to demonstrate significant seroconversion on the dot-blot assay, and the inability to recover SRB51 from blood of nonvaccinates at 2 or 4 wk, or from prescapular lymph nodes at 6, 12, or 18 wk, suggests that SRB51 was not transmitted from vaccinated to nonvaccinated elk.

Results of this study indicate that elk differ from cattle and bison in their response to SRB51 vaccine and suggest there may be differences among these species in their immunologic responses to infection with virulent field strains of *B. abortus.* Characterization of elk immune responses to *Brucella* vaccines may be required for development of an efficacious brucellosis vaccine and may also provide valuable insights into how elk respond immunologically to bacterial infections.

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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., M. G. STEVENS, A. E. JENSEN, F. M. TATUM, AND S. M. HALLING. 1993. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus.* American Journal of Veterinary Research 54: 1591–1597.

———, S. C. OLSEN, A. E. JENSEN, M. G. STEVENS, M. V. PALMER, AND A. M. FORANCE. 1996. Effects of age at vaccination of efficacy of *Brucella abortus* strain RB51 to protect cattle against brucellosis. American Journal of Veterinary Research 57: 1153–1156.

COOK, W. E., E. S. WILLIAMS, E. T. THORNE, T. J. KREEGER, G. STOUT, K. BARDSLEY, H. EDWARDS, G. SCHURIG, L. A. COLBY, F. ENRIGHT, AND P. H. ELZER. 2002. *Brucella abortus* strain RB51 vaccination in elk I. Efficacy of reduced dosage. Journal of Wildlife Diseases 38: 18–26.

FIORENTINO, D. F., A. ZLOTNICK, P. VIEIRA, T. R. MOSMANN, M. HOWARD, K. W. MOORE, AND A.

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O'GARRA. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. Journal of Immunology 146: 3444–3451.

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.

KREEGER, T. J., W. E. COOK, W. H. EDWARDS, P. H. ELZER, AND S. C. OLSEN. 2002. *Brucella abortus* strain RB51 vaccination in elk II. Failure of high dosage to prevent abortion. Journal of Wildlife Diseases 38: 27–31.

O'GARRA, J., AND K. MURPHY. 1994. Role of cytokines in determining T-lymphocyte function. Current Opinion in Immunology 6: 458–466.

OLSEN, S. C. 2000. Immune responses and efficacy after administration of a commercial *Brucella abortus* strain RB51 vaccine to cattle. Veterinary Therapeutics 1: 183–191.

———, 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. 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. Evaluation of serologic responses, lymphocyte proliferative responses, and clearance from lymphatic organs after vaccination of bison with *Brucella abortus* strain RB51. American Journal of Veterinary Research 59: 410–415.

ROFFE, T. J., S. C. OLSEN, T. GIDLEWSKI, A. E. JENSEN, M. V. PALMER, AND R. HUBER. 1999. Biosafety of parenteral *Brucella abortus* RB51 vaccine in bison calves. Journal of Wildlife Management 63: 950–955.

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.

STEVENS, M. G., S. G. HENNAGER, S. C. OLSEN, AND N. F. CHEVILLE. 1994. Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with *Brucella abortus* 19 or RB51. Journal of Clinical Microbiology 32: 1065–1066.

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.

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