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Serologic Responses in Diagnostic Tests for Brucellosis in Cattle Vaccinated with *Brucella abortus* 19 or RB51

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Serologic responses in the particle concentration fluorescence immunoassay and the card, complement fixation, and tube agglutination tests were measured for 10 weeks after vaccination of cattle with either *Brucella abortus* 19 or the lipopolysaccharide O-antigen-deficient mutant, strain RB51. The responses of strain 19-vaccinated cattle were positive, whereas those of strain RB51-vaccinated cattle were negative, in all of the tests. These results indicate that cattle vaccinated with strain RB51 fail to produce antibodies that can be detected by conventional serologic tests that are used to diagnose bovine brucellosis.

Brucella abortus causes abortions and reduced fertility in cattle (4) and chronic zoonotic infections in humans, including undulant fever, endocarditis, arthritis, and osteomyelitis (11). Conventional serologic tests for diagnosing brucellosis in cattle include the card, complement fixation (CF), and standard tube agglutination (STA) tests and the particle concentration fluorescence immunoassay (PCFIA) (1, 5, 8). These and other serologic tests measure antibody responses to the lipopolysaccharide (LPS) O antigens of naturally occurring field strains of *B. abortus* (10). Prevention of brucellosis in cattle is currently achieved by vaccinating heifers with strain 19 (S19) of *B. abortus*, which induces the production of antibodies to the O antigens of LPS (6). Regulations by the federal government stipulate that the S19 vaccine is to be given only to 4- to 12-month-old heifers because, if vaccinated at this age, heifers rarely develop persistent antibody titers to the LPS O antigens that confuse serologic tests. Cattle that are vaccinated with S19 when they are older than 12 months of age have an increased risk of developing persistent antibody titers to the LPS O antigens. These S19-induced antibody responses to the O antigens confuse the interpretation of diagnostic tests in cattle because positive test results may indicate natural infections with field strains of *B. abortus* or vaccination with S19 (5, 10).

Strain RB51 (SRB51) of *B. abortus* is a laboratory-derived rough mutant of the standard virulent strain of *B. abortus* (S2308); this mutant lacks most of the LPS O side chain that is found in S19 and in naturally occurring field strains of virulent *B. abortus* (7). The reduced O-side-chain content in SRB51 prevents this bacterium from inducing in SRB51-vaccinated cattle antibodies that give positive results in the STA test (2, 3, 7). Thus, positive STA test results in SRB51-vaccinated cattle would indicate the occurrence of natural infections with *B. abortus*. In contrast, positive STA test results in S19-vaccinated cattle might result from either vaccination or natural infections. Therefore, it is usually necessary to conduct the card and CF tests and the PCFIA in conjunction with the STA test to differentiate between S19-vaccinated and naturally infected cattle (5, 10). It is not known whether cattle vaccinated with SRB51 will produce antibodies that are detected by the card and CF tests and the PCFIA, as occurs after vaccination with S19. In this study, antibody responses in these tests were measured after vaccination of cattle with SRB51, and the results were compared with those obtained after vaccination of cattle with S19.

Vaccination and serologic testing of cattle. A master seed stock of *B. abortus* SRB51 was obtained from Gerhardt Schurig (Virginia Polytechnic Institute and State University, Blacksburg). Cultures of SRB51 were grown for 48 h at 37°C on tryptose agar (BBL Microbiology Systems, Cockeysville, Md.) containing 5% bovine serum. The bacteria were harvested by aspiration with physiologic saline (0.15 M NaCl, pH 7.2), washed twice by centrifugation, and then resuspended in saline for use as a vaccine. The lyophilized *B. abortus* S19 vaccine (National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, USDA, Ames, Iowa) was reconstituted with saline according to the procedures recommended by the U.S. Department of Agriculture (USDA). Eight-month-old polled Hereford heifers were obtained from a nonvaccinated and brucellosis-free herd. Six animals were vaccinated with *B. abortus* S19, and 12 animals were vaccinated with *B. abortus* SRB51. All vaccines (1.0×10^{10} to 1.4×10^{10} total CFU) were subcutaneously injected in the axillary area.

Blood was obtained by jugular venipuncture before and at 2, 4, and 10 weeks after vaccination. Blood was allowed to clot for 12 h at 4°C before centrifugation. Serum was divided into aliquots and stored at −70°C. Thawed serum was measured for antibodies to *B. abortus* with the card, CF, and STA tests as described previously (1). Antibodies to *B. abortus* were also measured with the PCFIA as described previously (8) by the manufacturer (IDEXX Corp., Portland, Maine). Results of the card test were scored positive when the samples produced visible agglutination. CF test results were scored positive when the serum gave a 2+ reaction at a 1:10 or higher dilution (1). Serum samples in the STA test were classified positive, negative, or suspect on the basis of the standard criteria established by the USDA (1). In the PCFIA, a sample fluorescence/negative fluorescence ratio of 0.300 or lower was scored positive, a ratio of 0.301 to 0.600 was scored suspect, and a ratio of 0.601 or higher was scored negative.

Serologic responses in cattle vaccinated with S19 or SRB51. All cattle had negative results in the card, CF, and STA tests and in the PCFIA before being vaccinated with SRB51 or S19 (data not shown). All cattle vaccinated with SRB51 had

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TABLE 1. Serologic responses in cattle vaccinated with *B. abortus* SRB51 or S19[a]

Diagnostic test or assay	Wk after vaccination	No. of cattle with the indicated result after vaccination with:				
		SRB51 (n = 12)		S19 (n = 6)		
		Positive	Negative	Positive	Negative	Suspect
Card	2	0	12	6	0	
	4	0	12	6	0	
	10	0	12	4	2	
CF	2	0	12	6	0	
	4	0	12	6	0	
	10	0	12	6	0	
STA	2	0	12	6	0	
	4	0	12	4	0	2
	10	0	12	0	2	4
PCFIA[b]	2	0	12	4	0	2
	4	0	12	1	3	2
	10	0	12	1	3	2

[a] Cattle were vaccinated with SRB51 or S19, and serologic responses were measured with the card, CF, and STA tests and the PCFIA.

[b] Means ± standard deviations for sample fluorescence/negative fluorescence ratios in the PCFIA for SRB51-vaccinated cattle were 0.972 ± 0.036 (2 weeks), 1.011 ± 0.052 (4 weeks), and 0.868 ± 0.186 (10 weeks).

negative results in the card, CF, and STA tests and in the PCFIA at 2, 4, and 10 weeks after vaccination (Table 1). In contrast, during 2 to 10 weeks after vaccination with S19, cattle generally had positive or suspect responses in all of the tests (Table 1). These results indicate that cattle vaccinated with SRB51 do not produce antibodies that can be detected by conventional tests that are used to diagnose brucellosis in cattle. Previous studies showed that SRB51-vaccinated cattle do not produce agglutinating antibodies that are detected by the STA test (2, 3, 7), and these results were confirmed in the present study. The present study also determined that SRB51 fails to induce the production of antibodies that can be detected by the card and CF tests and the PCFIA, which are commonly used in conjunction with the STA test to diagnose bovine brucellosis. The inability of SRB51 to induce positive CF test results when given as a vaccine to cattle is important because this test is considered reliable for diagnosing brucellosis (1, 5). The lack of detectable responses in SRB51-vaccinated cattle in the PCFIA is also important because this automated procedure can rapidly analyze large numbers of samples in screening herds for brucellosis (8).

The inability of SRB51 to induce antibodies that are normally detected by the card, CF, and STA tests and the PCFIA probably resulted because this bacterium contains very small amounts of the LPS O antigens (7). The low content of the LPS O antigens in SRB51 is supported by a previous study that showed that SRB51 LPS reacts poorly with a monoclonal antibody to S2308 LPS O antigens and that no SRB51 LPS O antigens can be detected in silver-stained sodium dodecyl sulfate-polyacrylamide gels (7). Previous studies showed that SRB51 is cleared more rapidly than S19 from vaccinated cattle when the same bacterial concentrations as those that were used in the current study are used (2, 3). Therefore, rapid clearance of SRB51 could also explain the lack of antibody responses to the LPS O antigens in SRB51-vaccinated cattle in the current study. However, this possibility seems unlikely, because cattle that have been hyperimmunized with SRB51 in Freund's complete adjuvant also fail to produce antibodies to the LPS O antigens (7). In addition, cattle vaccinated with SRB51 at the same concentration as that used in the current study are protected from abortions following challenge with S2308 and have in vitro cell-mediated immune responses to S2308 that are similar to those that occur when cattle are vaccinated with S19 (3, 9). Therefore, despite the short persistence of SRB51, these studies demonstrate that SRB51 is antigenic because it induces cell-mediated immune responses and protective immunity to S2308. In the current study, it was shown that SRB51 does not induce antibodies that can be detected in standard serologic tests that are used to diagnose brucellosis in cattle. Collectively, our results and results from previous studies suggest that the SRB51 vaccine may be a candidate for use as a replacement for the S19 vaccine in preventing bovine brucellosis and in increasing the efficiency of serologic identification and removal of infected cattle from herds in the United States.

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