Antibody Responses of Heifers Vaccinated with Reduced Doses of *Brucella abortus* Strain 19

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Summary

Serum samples were obtained over a six month period from heifers that were either vaccinated with *Brucella abortus* Strain 19 at one of two reduced doses (10^9 or 10^{10} CFU) or not vaccinated. Reactor status was determined by 5 standard tests as well as the hemolysis in gel test (HIGT) and enzyme-linked immunosorbant assay (ELISA). With the standard tests, 50% of the low dose vaccinates and 68% of the higher dose vaccinates were serologically positive 1 month after vaccination. Six months after vaccination, only 8% of the low dose vaccinates and 27% of the higher dose vaccinates were serologically positive by at least one standard test. HIGT failed to detect any cattle as serologic reactors. All cattle were seronegative by ELISA by 5 months after vaccination.

Introduction

Brucellosis continues to be a problem to the cattle industry despite the availability of an efficaceous vaccine, *Brucella abortus* Strain 19 (20). Vaccination of heifers with Strain 19 effectively increases resistance to brucellosis and thereby helps control the disease. The antibody response to Strain 19 is usually transient (4,5,9,18). However, detection of antibody to *B. abortus* is the current means of detecting infected cattle in the field and occasionally vaccine induced antibody titers persist resulting in vaccinated cattle being designated falsely as reactors (20). The detection of false reactors has been one of several aspects of the vaccination program that have caused some cattlemen to loose confidence in Strain 19 as an effective part of brucellosis control. Recently, several studies have demonstrated that reduction of the standard dose of Strain 19 from 9×10^{10} colony forming units (CFU) to approximately 1×10^9 CFU or lower does not reduce protection against experimental challenge exposure to virulent *B. abortus* but may reduce some of the undesirable side effects such as persistance of vaccine titers (1,4,5,6,20).

There are numerous serologic tests available for the detection of antibody to *B. abortus* (3,7,8,9,10,11,12,13,14, 15,16,17,19). These tests vary in sensitivity and specificity and none are 100% reliable in detecting infected cattle nor can they differentiate between antibody titers induced by Strain 19 or field strains. Within the past few years, several new tests have been studied which may eventually become incorporated into the brucellosis control program. Of these, the hemolysis in gel test (HIGT) and enzyme-linked immunosorbant assay (ELISA) show promise (3,8,9,11,13,14,17).

The purpose of this paper is to report the serologic responses over a 6 month period of heifers vaccinated with Strain 19 at one of two reduced doses (10^9 and 10^{10} CFU). Serologic data was obtained from 5 standard tests as well as HIGT and ELISA.

Materials and Methods

Cattle - Cattle used in this experiment were 69 crossbred heifers 10 to 12 months of age. The cattle were obtained from two certified brucellosis free beef herds, transported to the Livestock Health Research Center in Hugo, Oklahoma, maintained together on a bermuda grass pasture, and fed a protein supplement.

Experimental design - The CFU were determined on the lot of lypohilized Strain 19 vaccine used in this study during the week prior to vaccination. For vaccination, the Strain 19 vaccine was reconstituted in phosphate buffered saline (PBS) such that 2 ml contained 10^9 or 10^{10} CFU. Cattle were randomly selected and divided into 3 groups: Group A (24 heifers) received 10^9 CFU of Strain 19 subcutaneously, group B (22 heifers) received 10^{10} CFU of Strain 19, group C (23 heifers) remained as nonvaccinated controls.

At monthly intervals, serum samples for serologic testing were obtained from each heifer.

Serology - The standard serologic tests utilized were the Brucellosis Card test (CARD), standard tube agglutination test (STT), Rivanol precipitation-plate agglutination test (RIV), plate test (PLATE), and a microtiter complement fixation test (CF) (19). The criteria of Deyoe et al. (5) were used to evaluate reactor status: CARD - any degree of readily visible agglutination, STT - + 100 or higher, PLATE - + 100 or higher, RIV - +25 or higher, CF - 3+ at 1:10 or higher.

The HIGT was performed as previously described (13).[a] Briefly, bovine J-negative blood was stored five to seven days in Alsever's solution. The erythrocytes (bRBC) were then washed three times in pH 7.2 to remove all serum. A 10% bRBC suspension was made in PBS. An equal volume of PBS (control) or PBS-antigen was added to the cells. A Strain 1119-3 phenol/water extract was prepared at a 15 mg/ml concentration (pre-alkalinization lyophilized weight) and used at a final concentration of 0.13 mg/ml in PBS. The KDO assay activity of this amount of antigen was 2.25 ng.

The antigen treated and control cells were incubated one hour in a 37°C water bath with occasional agitation. The sensitized and control cells were then washed three times in PBS and resuspended a final time in veronal buffered saline (VBS)-azide to a concentration of 3% bRBC. The cells were distributed 3 ml per 13 x 100 mm test tube and heated to 42° in a water bath. Meanwhile, a 2% agarose solution was prepared using a low gelling temperature agarose[b] in VBS-azide and maintained at 42-45°C until used. When the bRBC and agarose were equilibrated to 42°C, 4 ml of 2% agarose and 1 ml of undiluted guinea pig complement[c] were added to each tube of bRBC, mixed well and poured into 95 mm x 45 mm polystyrene immunodiffusion plates.[d] Calibrated plates were used for the antigen-treated cells, uncalibrated plates were used with the control bRBC. The final bRBC concentration in the plates was 1.13%. The plates were allowed to gel for 10-15 minutes at room temperature before 17 (4mm) wells were cut in the agar using a standard radial immunodiffusion template.[d] Twenty microliters of serum were added to each well with a total of 15 test samples and a positive and negative reference serum on each plate. Each set of sera was added to an antigen-bRBC plate and to a control-bRBC plate. The plates were incubated overnight at 4°C, then 1-2 hours at 37°C. The diameter of zones of hemolysis were measured using the plate calibrations. Control plates were used to insure lysis was antigen specific. Samples with similar zones of lysis on antigen and control plates were interpreted as being the result of non-specific hemolysins.

The ELISA was performed as previously described (14). The test was run in 96 well polystyrene plates,[e] the results were read on an automated ELISA reader.[f] The test was run on serum dilutions of 1:200, 1:500, 1:1000, 1:1500, 1:10,000. Higher dilutions (up to $1:10^8$) were made on higher titered sera as necessary. Positive and negative control sera were run on each plate. Optical density (O.D.) readings of test sera (T) twice the average of the negative controls (N) were interpreted as positive (T/N 2). Titers were recorded as the reciprocal of the dilution. A titer of 1:1000 or greater was considered positive.

Analysis - The number of reactor cattle in groups A and B were compared by Chi-square analysis (2).

Results

The results of the standard serologic tests are listed in Table 1. Within one month after vaccination 12 (50%) of 24 group A heifers and 15 (68%) of 22 group B heifers were positive by at least one test. These differences were not significant ($p>0.05$). In both groups, there was a decline in the number of positive cattle such that 6 months after vaccination 2 (8%) of group A heifers and 6 (27%) of group B heifers were positive by at least one test. These differences were not significant ($p>0.05$). Postive reactions were not detected in any group C heifers.

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[a] *Stemshorn, B. W., Ruckerbauer, G. M. Brucellosis hemolysis-in-gel test protocol ADRI. Nepean, Canada.*

[b] *Litex agarose, Accurate Chemical and Scientific Corp., Westbury, N.Y.*

[c] *Pel Freeze frozen guinea pig serum, Rogers, Arkansas*

[d] *Miles Laboratories, Elkhart, Indiana*

[e] *Immulon Substrate Plates; Dynatech, Alexandria, Va.*

[f] *Mr 580 MicroelisaR Auto Reader, Dynatech, Alexandria, Va.*

Table 1 - Number of Serologic Reactors as Detected by Standard Tests at Monthly Intervals After Vaccination

	No. of Cattle	Month after vaccination						
		0	1	2	3	4	5	6
Group A (10^9)	24							
Card		0	11	2	0	1	2	2
Plate		0	5	0	0	0	0	0
STT		0	6	3	0	1	0	0
RIV		0	11	5	0	0	0	0
CF		0	6	1	0	0	0	0
No. of calves positive by at least 1 test		0	12	5	0	2	2	2
Group B (10^{10})	22							
Card		0	15	4	4	3	2	3
Plate		0	12	4	1	1	1	2
STT		0	14	8	2	2	3	5
RIV		0	15	6	2	1	0	0
CF		0	9	2	2	0	0	0
No. of calves positive by at least 1 test		0	15	10	8	7	6	6
Group C (Control)	23							
Card		0	0	0	0	0	0	0
Plate		0	0	0	0	0	0	0
STT		0	0	0	0	0	0	0
RIV		0	0	0	0	0	0	0
CF		0	0	0	0	0	0	0
No. of calves positive by at least 1 test		0	0	0	0	0	0	0

None of the cattle among the groups were positive by the HIGT (Table 2). Incomplete and very faint hemolysis were seen with sera from both groups A and B. Those reactions were more common with sera from group B. Five heifers (4 in group A and 1 in group B) were detected positive by the ELISA one month after vaccination (Table 3). Of these, 4 were positive on all standard tests and 1 was negative on all standard tests. None of these had any reaction in the HIGT.

Discussion

Persistance of antibody titers following Strain 19 vaccination appear to be related to such factors as age of cattle at vaccination, whether or not cattle become persistently infected with Strain 19, and the dose of the vaccine administered (4,5,6,12,18,20). It has been demonstrated that cattle vaccinated with the standard dose

Table 2 - Results of Hemolysis in gel Test for Brucella abortus

	No. of Cattle	Month after vaccination						
		0	1	2	3	4	5	6
Group A (10^9)	24							
Positive[a]		0	0	0	0	0	0	0
Incomplete[b]		0	2	1	1	2	2	0
Very Faint[c]		0	0	0	0	1	3	2
Group B (10^{10})	22							
Positive		0	0	0	0	0	0	0
Incomplete		0	3	3	4	3	7	1
Very Faint		0	0	0	0	2	6	2
Group C (Control)	23							
Positive		0	0	0	0	0	0	0
Incomplete		0	0	0	0	0	0	0
Very Faint		0	0	0	0	0	0	0

a - indicates zone of complete hemolysis
b - indicates incomplete hemolysis = negative
c - hemolysis very faint = negative

Table 3 - Numbers of Serologically Positive[a] Cattle as Detected by the ELISA for Brucella abortus at Monthly Intervals after Vaccination

	No. of Cattle	Month after vaccination						
		0	1	2	3	4	5	6
Group A (10^9)	24	0	4	0	0	1	0	0
Group B (10^{10})	22	0	1	0	0	0	0	0
Group C (Control)	23	0	0	0	0	0	0	0

a - positive indicates positive reaction at a serum dilution of 1:1000 or above

as adults had a longer persistance of antibody titers than did those vaccinated as calves (18). The decrease in positive reactors following vaccination with reduced dose vaccines seen in the present study is comparable to that published previously (5). In that study, a more rapid loss in positive serologic reactors as measured by standard tests was observed in heifers vaccinated with 10^9 CFU compared to those vaccinated with 10^{10} CFU. By 26 weeks after vaccination, only 1 of 17 heifers in the higher dose group was a serologic reactor and 0 or 17 of the low dose heifers were reactors. By comparison, as many as 8 or 17 heifers vaccinated with the standard dose (9×10^{10} CFU) were serologic reactors at that time. In contrast, the persistance of reactors in the 10^{10} dose group in the present study was more obvious and may reflect the lack of specificity or inconsistancy of the standard tests or some variation in actual CFU of Strain 19 administered in the different experiments.

The HIGT has been shown to be a sensitive assay for the detection of antibody to *B. abortus* lipopolysaccharide (LPS) antigens (13). The HIGT failed to demonstrate reactors in the present experiment. Very faint or incomplete hemolysis was found with sera from vaccinates in both groups. Complete hemolysis, however, can be demonstrated from sera from cattle known to be infected with field strain *B. abortus*. Therefore, it is probably important that less intense hemolytic reactions with HIGT be interpreted conservatively as they may indicate a vaccine induced immune response.

The ELISA as performed in the present study detected very few reactors. Several control sera were positive by ELISA at 1:1000 dilution initially but 3 to 5 additional tests on the same sera failed to again demonstrate such high titers. The ELISA is an extremely sensitive assay and may detect both specific and nonspecific reactions of immunoglobulins to *B. abortus* antigens (8,9). Specificity may at times be lost because of the extreme sensitivity of the assay. ELISA for *B. abortus* is presently used in several research laboratories and the antigens used vary from more crude sonicated LPS-rich preparations, as used in this study, to more purified soluble or LPS antigens (3,8,9,11,14,17). Further work is needed to determine the optimal antigen for use in the ELISA and to assure specificity of this assay.

In conclusion, vaccination of heifers with the present reduced standard dose of Strain 19 vaccines (10^9 CFU) resulted in only 2 of 24 heifers to be serologic reactors as determined by standard tests by 6 month after vaccination. HIGT and ELISA detected no reactors at that time. Further studies are needed to refine and better define these latter assays to determine if they can be effectively incorporated into control programs.

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