

**The serological response of adult cattle after vaccination with  
*Brucella abortus* strain 19 and RB51\***

Resposta sorológica de bovinos adultos após vacinação com as amostras 19 e RB51 de  
*Brucella abortus*

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**SUMMARY**

Adult cattle vaccinated once or twice with  $2 \times 10^9$  viable *B. abortus* strain RB51 bacteria did not seroconvert in the rose bengal, serum agglutination and mercaptoethanol tests. Animals vaccinated while pregnant did not abort and no *B. abortus* was isolated from their vaginal mucus and milk.

UNITERMS: Brucellosis; Antibody; Bovine.

## INTRODUCTION

Brucellosis is an important zoonotic disease caused by bacteria of the Genus *Brucella*. In its principal animal hosts, it is characterized by abortion and impaired fertility<sup>1</sup>.

*Brucella abortus* is the main causative agent of brucellosis in cattle. The disease can be prevented by the use of the attenuated strain 19 vaccine, which induces resistance to infection and abortion lasting several years in a significant number of vaccinated animals<sup>10</sup>.

The vaccine may elicit persistent serological reactions, especially when animals are vaccinated as adults. These persistent serological reactions are mainly against the antigenic O-chain of the lipopolysaccharide present in smooth *Brucellae*<sup>8</sup>.

Recently, *B. abortus* strain RB51 has been approved as the official vaccine against bovine brucellosis in the USA (Federal Register (156):41730-41733,1996). The *B. abortus* strain RB51 vaccine is a stable, rough mutant, derived from virulent strain 2308 by serial passages of this strain on media containing rifampicin<sup>14</sup>. The vaccine induces protection against challenge with smooth virulent *B. abortus* in cattle<sup>7</sup> and against *B. abortus* and other *Brucellae* in mice<sup>9,15</sup>. Protection levels induced by the vaccine in cattle appear to be similar to levels achieved with strain 19, but strain RB51 vaccine does not induce the serological responses which interfere in the serodiagnosis of the disease, particularly when conventional serological tests are used<sup>7</sup>.

Currently, it is recommended that only calves from 4-10 months of age be vaccinated with  $1-3.4 \times 10^{10}$  organisms once. The recommended dose for adults is ten fold less and the vaccination of pregnant cattle is not recommended. Although in mice, multiple vaccinations do not lead to the production of anti-O antibodies and vaccination of pregnant mice does not lead to abortions, the effects of multiple vaccination as well as the effect of vaccination on pregnant cattle has not been studied in detail<sup>3</sup>.

The objectives of the present study were: 1) to determine if adult cattle vaccinated once or twice with RB51 maintain negative anti-O serology when compared to S19 adult vaccinated animals, 2) to determine the effect of vaccination and revaccination with RB51 on pregnancy, and 3) to determine if animals revaccinated with RB51 during pregnancy shed the vaccine strain after parturition in milk and vaginal discharges.

## MATERIAL AND METHOD

Three groups of 25 adult females Aberdeen Angus, 2-5 years old were used. One group received  $2 \times 10^9$  viable strain RB51 bacteria in 2 ml subcutaneously (SC). A second group received  $3 \times 10^9$  viable strain S19 bacteria (SC) and a third group served as controls receiving saline only. All animals in the strain RB51 group were revaccinated subcutaneously 6 months after the first inoculation with the same dose. Most animals were pregnant before revaccination. All animals were bled before vaccination and at 30, 90, 180, 210, 270 and 360 days after vaccination. The sera were tested with the following conventional tests: rose bengal (RB), serum agglutination test (SAT) and 2 mercaptoethanol (ME), according to Alton *et al.*<sup>2</sup> Two indirect ELISA (I-ELISA) tests were also performed. One ELISA used the lipopolysaccharide of *B. abortus* 1119-3 (SLPS) as antigen according to Nielsen *et al.*<sup>11</sup>. Briefly, a 1 µg/ml dilution of smooth lipopolysaccharide was prepared in 0.05M carbonate buffer (pH 9.6) and 100 µl was added to wells of 96 polystyrene plates (NUNC 2-69620) and incubated covered overnight at 4°C. After three wash cycles (Handiwasher-BDSL, UK) with 0.01 M phosphate buffered saline (pH 7.4) containing 0.05% Tween 20 (PBST), 100 µl of

test sera (in duplicate) and control sera (in quadruplicate) diluted 1:200 in PBST was added to the wells for 1 hour at 37°C. Four controls were included: a strong positive serum, a weak positive serum, a negative serum and a buffer control. After three more wash cycles, 100 µl of a mouse monoclonal anti bovine IgG<sub>1</sub> conjugated to horseradish peroxidase (HRPO) properly diluted in PBST was added to each well and the plates incubated as described above. After three final wash cycles, 100 µl of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 1mM 2,2-azinobis (3-ethylbenzthiazoline sulfonic acid) (ABTS) dissolved in 0.05M sodium citrate/citric acid (pH 4.5) was added to all wells. The plates were incubated for 10 minutes at 37°C with continuous shaking for color development. To stop the reaction, 100 µl of sodium dodecyl sulphate (SDS) was added to all wells. Optical density readings were obtained using a spectrophotometer (Titertek Multiskan Plus, Flow Lab. McLean,VA) at 405 nm. The results were expressed as percent of positivity of the positive control serum (PP). Any serum with a PP of 35% was considered as positive.

The other ELISA used acetone killed strain RB51 whole cells as the antigen according to Colby<sup>4</sup>. Briefly, lyophilized RB51 cells were reconstituted in sterile distilled water to 5% transmittance at 525 nm to form an antigen stock solution, which was stored at 4°C. Before use, this stock solution was centrifuged, the supernatant discarded and the original volume diluted 1:20 v/v in bicarbonate buffer (pH 9.6). Appropriate wells of 96-well polystyrene plates were coated with 200 µl of the antigen and incubated overnight at 4°C. After four cycles in PBST, 200 µl of each serum samples diluted 1:50 in PBST was appropriated dispensed and the plates incubated at 37°C for 30 minutes. After four wash cycles, each well was allowed to stand at 37°C with 200 µl of a mouse monoclonal anti-bovine IgG<sub>1</sub> conjugated to HRPO and properly diluted. After four final wash cycles, 200 µl of developing solution (H<sub>2</sub>O<sub>2</sub> -ABTS) was dispensed and the reaction was stopped as described before. Four controls were included in duplicates: a strong positive serum, a negative serum, wells with no antigen and wells with no serum, the last two in order to prevent nonspecific binding. The optical densities and the results were obtained and expressed as described before.

Vaginal swabs and milk were collected once and immediately after parturition from 15 cows of the strain RB51 vaccinated group and the samples were bacteriologically analysed for the presence of the vaccine strain using selective media according to Alton *et al.*<sup>2</sup>

## RESULTS AND DISCUSSION

The serological results of vaccinated animals in the RB, SAT, ME and I-ELISA tests are shown in [Tab. 1](#). Control animals remained serologically negative throughout the study. The animals vaccinated with S19 showed a classical antibody curve<sup>10</sup> with most animals being serologically negatives by 270 days post vaccination. The strain RB51 vaccinated animals remained negatives throughout the experiment in the traditional RB, SAT and ME tests even after revaccination. The I-ELISA with SLPS showed positive results with a small percentage of sera from the RB51 group after the first vaccination with a greater number of positive results 30 days after revaccination. This reactivity is probably due to specific antibodies directed against surface antigens common to both *Brucella* strains.

**Table 1**

Number and percentage of positive animals in four serological tests at different times after vaccination with reduced doses of strain 19 and RB51 vaccines. FEPAGRO, São Gabriel – RS, 1996.

Days After Vaccination	R B				S A T				2 ME				I - ELISA			
	S19		RB 51		S19		RB 51		S19		RB 51		S19		RB 51	
	+ T	%	+ T	%	+ T	%	+ T	%	+ T	%	+ T	%	+ T	%	+ T	%
0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0
30 (1m)	25/25	100	0/25	0	24/25	96	0/25	0	21/25	84	0/25	0	25/25	100	1/25	4
90 (3m)	5/25	20	0/25	0	8/25	32	0/25	0	3/25	12	0/25	0	14/25	56	1/25	4
180 (6m)	2/25	8	0/25	0	3/25	12	0/25	0	0/25	0	0/25	0	3/25	12	2/25	8
210 (7m)	1/25	4	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	2/25	8	6/25	24
270 (9m)	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	1/25	4	1/25	4
360 (1Y)	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0	0/25	0

RB-Rose Bengal;

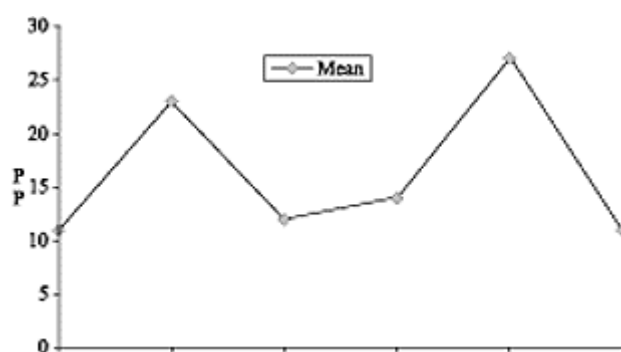
SAT-Serum Agglutination in tubes;

2ME-2 Mercaptoethanol;

I-ELISA - Indirect Elisa using SLPS antigen;

m = months; + = positive; T = total; Y – year.

The mean of the percentage of positivity (PP) values of I-ELISA with RB51 antigen of all adults vaccinated with RB51 is shown in [Fig. 1](#). A peak in the antibody response was observed 30 days after vaccination and again 30 days after revaccination.

**Figure 1**

Mean of PP values of the I- ELISA with RB51 antigen using sera from 25 adult cattle vaccinated with strain RB51 at day 0 and 180.

PP – percentage of positivity.

Although 100% of the strain 19 vaccinated animals were positive in the I-ELISA at 30 days post vaccination, only a small percentage (4-8%) of strain RB51 vaccinated animals were positive after the first vaccination. This percentage increased to 24%, 30 days post vaccination and declined to 0% at 6 months post vaccination. The smooth LPS used as antigen in the I-ELISA contains the O-chain antigen, LPS core sugars, lipid A and a variety of contaminating *Brucella* proteins tightly

bound to the smooth LPS<sup>3</sup>. The absence of serological reactions with the sera from RB51 vaccinated animals in the conventional tests indicates that these animals did not developed anti-O antibodies as expected with strain RB51 vaccination<sup>5,6,7</sup>. The responses observed in the I-ELISA are most likely directed against contaminating proteins or non O-chain components of the LPS, which are present in strain RB51<sup>3</sup>.

Since RB51 is essentially devoid of O-antigen, the RB51 ELISA indicated that cattle vaccinated with strain RB51 responded serologically to RB51 antigens distinct from the O-chain antigen<sup>13,14</sup>.

No abortions were observed in the strain RB51 vaccinated group, neither was RB51 cultured from milk or vaginal secretions. Cultures in the S19 vaccinated group were not done as the animals were vaccinated once and none were pregnant at the time of vaccination.

None of the pregnant animals aborted or demonstrated post partum shedding of the vaccine strain in milk or vaginal secretions, suggesting that a dose of  $2 \times 10^9$  RB51 bacteria injected into pregnant cattle may carry a very low risk of inducing abortions<sup>12</sup>.

## CONCLUSIONS

This study confirms previous observations that adult cattle, pregnant or not, do not seroconvert in the traditional brucellosis tests after vaccination with strain RB51. Importantly, revaccination does not change the conventional serological status indicating that multiple vaccinations with strain RB51 of adult normal or pregnant cattle can be carried out without affecting serological results. This characteristic is of practical importance since it permits revaccination of animals to increase immunity without serological consequences.

## RESUMO

Bovinos adultos vacinados uma ou duas vezes com  $2 \times 10^9$  bactérias viáveis da amostra RB51 de *Brucella abortus* não reagiram sorologicamente nas provas de rosa de bengala, soro-aglutinação em tubos e mercaptoetanol. Animais vacinados durante a prenhez não abortaram e não foi isolada *B. abortus* das secreções vaginais ou do leite.

UNITERMOS: Brucelose; Anticorpos; Bovinos.

## REFERENCES

- 1- ACHA, P.N.; SZYFRES, B. **Zoonosis y enfermedades transmisibles comunes al hombre y a los animales**. 2.ed. Washington : Organización Panamericana de la Salud, 989p. 1989. (Publicación Científica, 503). [ [Links](#) ]
- 2- ALTON, J.J.; JONES, L.M.; ANGUS, R.D.; VERGER, J.M. **Techniques for the brucellosis laboratory**. Paris : INRA, 1988. 190p. [ [Links](#) ]
- 3- BUHRMAN, D.L. **The behaviour and effects of *Brucella abortus* rough strain RB51 in mice and cattle**. Blacksburg, 1989. 147p. Thesis. Virginia Polytechnic Institute and State University. Virginia. [ [Links](#) ]
- 4- COLBY, L.A. **The humoral immune response of Elk (*Cervis elaphus nelsoni*) and mice to vaccination with *Brucella abortus* strain RB51**. Blacksburg, 1997. 152p. Thesis. Virginia Polytechnic Institute and State University. Virginia. [ [Links](#) ]
- 5- CHEVILLE, N.F.; JENSEN, A.E.; HALLING, S.M.; TATUM, F.M.; MORFITT, D.C.; HENNANGER, S.G.; FRERICHS, W.M.; SCHURIG, G.G. Bacterial survival, lymph node changes, and immunological response of cattle vaccinated with standard and mutant strain of *Brucella abortus*. **American Journal of Veterinary Research**, v.53, n.10, p.1881-8, 1992. [ [Links](#) ]
- 6- CHEVILLE, N.F.; OLSEN, S.C.; JENSEN, A.E.; STEVENS, M.G.; PALMER, M.V.; FLORANCE, A.M. Effects of age at vaccination on efficacy of *Brucella abortus* strain RB51 to protect cattle against brucellosis. **American Journal of Veterinary Research**, v.57, n.8, p.1153-6, 1996. [ [Links](#) ]
- 7- CHEVILLE, N.F.; STEVENS, M.G.; JENSEN, S.C.; TATUM, F.M.; HALLING, S.M. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus*. **American Journal of Veterinary Research**, v.54, n.10, p.1591-7, 1993. [ [Links](#) ]
- 8- DIAZ, R.; JONES, L.M.; LEONS, D.; WILSON, J.B. Surface antigens of smooth brucellae. **Journal of Bacteriology**, v.96, n.6, p.893-901, 1968. [ [Links](#) ]
- 9- JIMENEZ DE BAGUES, M.P.; ELZER, P.H.; JONES, J.M.; BLASCO, J.M.; ENRIGHT, F.M.; SCHURIG, G.G.; WINTER, A.J. Vaccination with *Brucella abortus* rough mutant RB51 protects BALB/c mice against virulent strains of *B. abortus*, *melitensis* and *ovis*. **Infection and Immunity**, v.62, n.11, p.4990-6, 1994. [ [Links](#) ]
- 10- NICOLETTI, P. Vaccination. *In*: NIELSEN, K.; DUNCAN, J.R. **Animal Brucellosis**. Boston : CRC Press, 1990. p.283-300. [ [Links](#) ]
- 11- NIELSEN, K.H.; GALL, D.; KELLY, W.; *et al.* Immunoassay Development: Application to Enzyme Immunoassay for the Diagnosis of Brucellosis. **Agriculture and Agri-Food Canada**, Nepean, 1996, 118p. [ [Links](#) ]
- 12- PALMER, M.V.; CHEVILLE, N.F.; JENSEN, A.E. Experimental infection of pregnant cattle with the vaccine candidate *Brucella abortus* strain RB51: pathologic, bacteriologic, and serologic findings. **Veterinary Pathology**, v.33, n.6, p.682-91, 1996. [ [Links](#) ]

13- ROOP, R.M.; MOORE, P.D.; BAGCHI, T.; SCHIRIG, G.G. Rapid identification of smooth *Brucella* species with a monoclonal antibody. **Journal of Clinical Microbiology**, v.25, n.11, p.2090-3, 1987. [ [Links](#) ]

14- SCHURIG, G.G.; ROOP, R.M.; BAGCHI, T.; BOYLE, S.; BUHRMAN, D.; SRIRANGANATHAN, N. Biological properties of RB51: a stable rough strain of *Brucella abortus*. **Veterinary Microbiology**, v.28, n.2, p.171-88, 1991. [ [Links](#) ]

15- WINTER, A.J.; SCHURIG, G.G.; BOYLE, S.; SRIRANGANATHAN, N.; BEVINS, J.S.; ENRIGHT, F.M.; ELZER, P.H.; KOPEC, J.D. Protection of BALB/c mice against homologous and heterologous species of *Brucella* by rough strain vaccines derived from *B. melitensis* and *B. suis* biovar 4. **American Journal of Veterinary Research**, v.57, n.5, p.677-83, 1996. [ [Links](#) ]

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