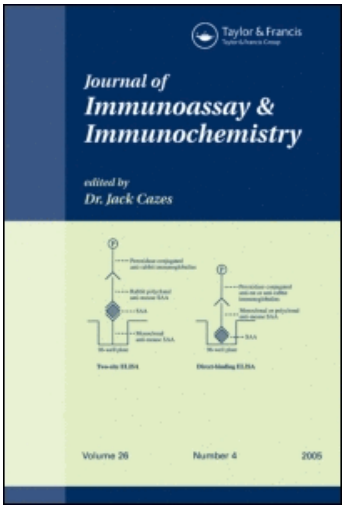

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

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Online publication date: 21 October 2002

To cite this Article Aguirre, N. P. , Vanzini, V. R. , de Echaide, S. Torioni , Valentini, B. S. , De Lucca, G. , Aufranc, C. , Canal, A. , Vigliocco, A. and Nielsen, K.(2002) 'ANTIBODY DYNAMICS IN HOLSTEIN FRIESIAN HEIFERS VACCINATED WITH *BRUCELLA ABORTUS* STRAIN 19, USING SEVEN SEROLOGICAL TESTS', Journal of Immunoassay and Immunochemistry, 23: 4, 471 — 478
To link to this Article: DOI: 10.1081/IAS-120015478
URL: http://dx.doi.org/10.1081/IAS-120015478

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JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY
Vol. 23, No. 4, pp. 471–478, 2002

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DOI: 10.1081/IAS-120015478
1532-1819 (Print); 1532-4230 (Online)

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ABSTRACT

The serological response induced by *Brucella abortus* strain 19 was evaluated in 52 Holstein females from a brucellosis-free herd using seven serological tests. Each calf was vaccinated at an age of 4 and 8 months old with 3×10^{10} CFU *B. abortus* S19 and the antibody response was determined as the proportion of positive results to each test. The antibody dynamics, measured with the buffered plate antigen (BPA) test and the rapid automated presumptive (RAP) test, were similar. The proportion of positive reactions in these tests reached 100% one week after vaccination and remained at this level for seven weeks, after which the proportion of positive samples slowly declined to 8% (BPA) and 2% (RAP) at week 50. The response in the indirect enzyme immunoassay (i-ELISA) was similar, but shorter than that observed with the BPA/RAP. The antibody dynamic, measured using the seroagglutination test (SAT) in parallel with the 2-mercaptoethanol (2-Me) test and the complement fixation test (CFT) were similar to the RAP/BPA, but of slightly shorter duration. The competitive ELISA (c-ELISA) was positive in all animals for 3 weeks, followed by a rapid decline. The fluorescence polarization assay (FPA) reached a maximum of 68.5% positive animals at week 4 and then declined. Based on these data, the c-ELISA and FPA discriminated residual antibody activity due to vaccination more efficiently than the other tests.

Key Words: *Brucella abortus* S19; ELISA; Fluorescence polarization assay; Rapid automated presumptive test; CFT; BPA

INTRODUCTION

The control and eradication of bovine brucellosis includes the elimination of infected cattle from herds and the vaccination of female calves between 3 and 8 months of age. In Argentina, the official vaccine is *Brucella abortus* strain 19 and the vaccination is mandatory.[1] It is known that this vaccine induces *O*-polysaccharide (*O*-PS) specific antibodies that interfere with the standard serological tests which employ smooth lipopolysaccharide (S-LPS) as the antigen. To overcome this problem, a competitive ELISA (c-ELISA) and the fluorescence polarization assay (FPA) to differentiate vaccinal anti-

bodies from those induced by natural infection were developed.[2,3] Both tests were evaluated in Argentina,[4–7] but no data on female dairy calves are available. The c-ELISA and the FPA are included on the list of tests for international trade by the Office International des Epizooties (OIE) as prescribed, and alternative tests, respectively.[8]

This paper describes the antibody dynamics in Holstein Friesian female calves vaccinated with *B. abortus* strain 19 using the official (in Argentina) and the OIE sanctioned tests to provide basic information for future test selection.

EXPERIMENTAL

Origin of Samples

Fifty two 4–8 month old Holstein female calves were selected from a herd free from brucellosis for at least seven years. Each calf was vaccinated with 3×10^{10} CFU *B. abortus* S19 (Bay-Vac®, Bayer, Argentina). The sampling schedule was designed to detect the peak and decline of antibody levels over time. Serum samples (S) were obtained as follows: S1 on the day of vaccination; S2 to S5 weekly from day 7 to 28 post-vaccination (PV), S6 and S7, 14 days apart, S8 and S9, 30 days apart, S10 and S11, 60 days apart and S12 one year PV. In addition, two samples were obtained, one month before and 15 days after calf delivery. On the last sampling, a milk sample for indirect ELISA (i-ELISA) was also obtained.

Serological Tests

The buffered plate antigen (BPA) test was previously described by Angus and Barton.[9] It was performed according to the procedures recommended by the Servicio Nacional de Sanidad Animal y Calidad Agroalimentaria (SENASA) of Argentina.[10] Results were expressed as positive or negative.

The rapid automated presumptive (RAP) test[11] was performed in 96-well microplates (Falcon 3910, Becton Dickinson, Oxnard, CA, USA) and then read in a Bio-Tek® Ceres 900 (Bio Tek Instruments Inc., Winooski, USA) autoreader with a 690 nm filter. Briefly, 30 μL of test serum was added to each well. Negative and a positive control sera were included in triplicate. To each well, 20 μL of *Brucella* card test antigen was added. The plate was inserted into the instrument carrier and, after 15 min of shaking, the transmission profile of each well was measured as initial reading. The plate was placed on a rotator (Autolex® 2342, Viral Antigens Inc., Menphis, USA) for 10 min and then allowed to settle for 20 min. The software monitored the incubation time and prompted the final scan of the plate.

Each initial reading was subtracted from the final reading, generating a transmission change which represents the degree of agglutination. The results were expressed as percent of agglutination (%AGG). Samples with ≥5% AGG were considered positive.

The seroagglutination test (SAT) and the 2-mercaptoethanol (2-Me) test were performed in parallel, as described by Alton et al.[12] The interpretation of the results was made following the procedures recommended by SENASA.[10] Sera with titers ≥1 : 200 to SAT and/or ≥1 : 25 to 2-Me were considered positive.

The complement fixation test (CFT) was performed following Hill's method of 50% hemolysis,[12] using the second International Standard Anti-*B. abortus* Serum (ISAbS) as reference. Sera with ≥30 International Units were considered positive.[8]

The ELISA used was described by Nielsen et al.[2,13] The i-ELISA for serum and milk samples was performed using a *B. abortus* smooth lipopolysaccharide (SLPS) antigen, a mouse monoclonal anti-bovine IgG_1 (M23) conjugated with horseradish peroxidase (HRPO) as described by Vanzini et al.[14] The cut off points were set at 40 and 53 percent positivity (PP) for milk and serum samples, respectively. The c-ELISA also used the SLPS as antigen, a mouse monoclonal antibody (M84) for competition and a goat anti-mouse IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, USA) conjugated with HRPO for detection.[6] The cut off point was fixed at ≥40 percent of inhibition (%I).

The FPA was performed as described by Nielsen et al.[3] Briefly, 20 μL of serum was added to 2 mL of 0.1 M phosphate buffer pH 7.0, containing 0.01% NaN_3, 0.15 M NaCl and 0.5% lithium dodecyl sulphate. The diluted serum was incubated at room temperature for 2 min and, after equilibration, a baseline reading was obtained in a fluorescence polarization analyzer. Antigen, *B. abortus O*-polysaccharide conjugated with fluorescein isothiocyanate, appropriately diluted, was added, mixed, and incubated for 2 min. A final reading was performed in the fluorescence polarization analyzer (FPM-1, Jolley Consulting and Research Inc., Graslake, USA). The difference between the initial and final reading was expressed in millipolarization (mP) units. The cut-off point for FPA was fixed at ≥95 mP. The biological reagents for i-ELISA, c-ELISA, and FPA were provided by the Canadian Food Inspection Agency (CFIA).

Statistical Analysis

The difference between proportions was assessed by the chi-square test using Medcalc® software.

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RESULTS

The antibody dynamics of BPA and RAP were similar. The proportion of positive samples reached 100% one week PV and remained at this level for seven weeks. There were no statistical differences, except in S9 ($p = 0.003$) at 17 weeks PV.

The antibody response measured by the SAT/2-Me and CF tests were similar to the BPA/RAP tests, but declined earlier.

The response to i-ELISA was similar to, but shorter than, those observed in the 2-Me and CFT.

The FPA showed the lowest proportion of positive reactions, reaching 68.5% of positive samples at week 4 PV and then declining. It is interesting to note that the onset of the antibody response measured with the FPA is later and slower than when using the other tests. There were statistical ($p = 0.0001$) differences when comparing with BPA, RAP, and i-ELISA (Fig. 1). Five heifers (9.6%) were always negative, 11 heifers (21.2%) were positive in one sampling, 7 heifers (13.5%) were positive in two samplings, and 7 heifers (13.5%) were positive in three samplings.

More than 94% of samples became positive to c-ELISA, CFT, and 2-Me from week 2 PV. The CFT and 2-Me remained at higher levels up to week eight and then began to decline. In contrast, the proportion of reactors in the c-ELISA remained at this level only two weeks (Fig. 2).

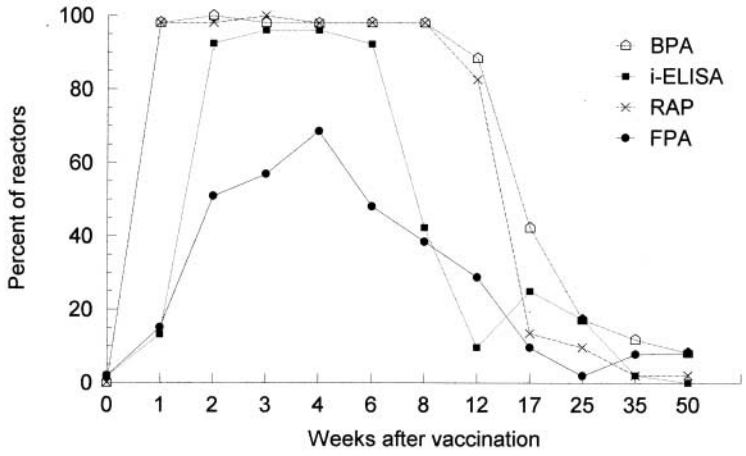

Figure 1. Proportion of positives serum samples to BPA, RAP, i-ELISA, and FPA of female calves vaccinated with *B. abortus* S19 at various weeks after vaccination (see text for abbreviations).

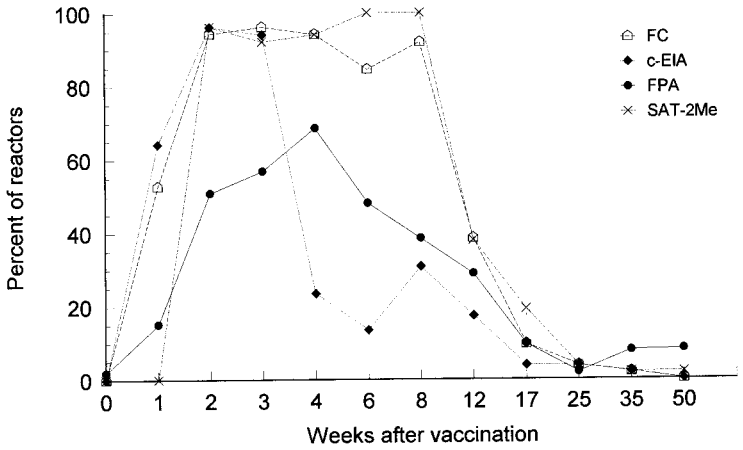

Figure 2. Proportion of positive serum samples to c-ELISA, 2-Me, and CFT of female calves vaccinated with *B. abortus* S19 at various weeks after vaccination. The FPA data were repeated in this figure for comparison (see text for abbreviations).

On week 17, more than 90% of the samples were negatives to the screening and confirmatory tests, except for BPA (67.7%), i-ELISA (75%), and 2-Me (80.8%).

Thirty-five heifers were sampled one month before and 15 days after delivery. The time elapsed between vaccination and delivery varied from 18 to 27 months. Only two (5.7%) were positive in the BPA before breeding (10 and 13 months PV) and negative in all other tests. After delivery, four samples (11.4%) were positive in the BPA and negative in all other tests; one of them had been positive one month before delivery. All milk samples were negative in the i-ELISA.

DISCUSSION AND CONCLUSIONS

Vaccination with *B. abortus* S19 in Argentina began in some provinces in 1960 as voluntary programs but, from 1981, they became mandatory. It is known that the persistence of vaccinal antibodies interfere with conventional diagnostics tests.[15] This gives rise to diagnostic problems when testing sexually mature animals. For this reason, tests including the c-ELISA and FPA, capable to differentiate vaccinal from infection antibodies, were developed and have been extensively evaluated in Argentina;[4–7] however, these assays were not included as official tests in the national program for control

and eradication of bovine brucellosis in Argentina. In this communication, it was clearly shown that the conventional tests detect significant antibody titers resulting from calves vaccinated according to the current Argentinean official control program for a prolonged period of time. Both the c-ELISA and the FPA have been shown to eliminate positive tests due to residual vaccinal antibody more efficiently and could, therefore, be considered for official status in the brucellosis eradication program. Both the c-ELISA and the FPA are recommended for international trade by the OIE.[8] While the c-ELISA declined slightly more rapidly than the FPA (the antibody dynamics of the FPA were different from the other tests, see figures), the FPA has certain advantages in that it is relatively inexpensive, very rapid and simple, and it can be performed in the field, offering accurate diagnosis without awaiting laboratory results, thereby allowing earlier elimination or segregation of infected animals.

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Received January 23, 2002
Accepted May 1, 2002
Manuscript 3063