Evaluation of the double agar gel immunodiffusion test with serum agglutination in plate, tube, and 2-mercaptoethanol for cows vaccinated with five different reduced-dosages of *B. abortus*-Strain-19

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ABSTRACT. The control of bovine brucellosis requires the differential serological diagnosis of the non-vaccinated animals and animals vaccinated with *B. abortus*-S-19. This is almost impossible using the classic serological diagnostic tests, such as seroagglutination in plate (SAP), seroagglutination in tube (SAT), and 2-mercaptoethanol (2Me). In this study, serological titers, induced by 5 reduced-dosages, up to three times, corresponding to 6 x 10^5, 10^6, 10^7, 10^8, and 10^9 CFU/ml of S-19 were seen during a 120-day period and analyzed in 5 groups of cows numbering 7, 6, 13, 12, and 11 cows, respectively. The titers showed variations up to 400 IU using SAP, up to 800 IU using SAT and 2Me. No precipitation of antigen *poly-O* of *B. abortus* 1119-3 was observed using double agar gel immunodiffusion (AGID). Considering the need for further investigation, this allows us to consider AGID a useful diagnostic test to distinguish between naturally infected seropositive animals and vaccinated cows with different reduced-dosages of *B. abortus*-S-19.
Key words: *Brucella abortus*, Brucellosis, Immunodiffusion.

RESUMEN. El control de la brucelosis bovina requiere la realización de un diagnóstico serológico diferencial de los animales no vacunados y de aquellos vacunados con *B. abortus* S-19. Esto es casi imposible de ser hecho utilizándose las pruebas diagnósticas clásicas, tales como suero aglutinación en placa (SAP), suero aglutinación en tubo (SAT) y 2-Mercaptoetanos (2Me). En el presente estudio, los títulos serológicos inducidos por 5 dosificaciones reducidas, hasta tres veces, correspondientes a 6x10^5, 10^6, 10^7, 10^8 y 10^9 de UFC/ml de S-19 han sido observados durante un período de 120 días y analizados en 5 grupos de vacas con 7, 6, 13, 12 y 11 animales, respectivamente. Los títulos presentan variaciones de hasta 400UI (Unidades Internacionales) utilizándose el SAP y hasta 800UI utilizándose el SAT y el 2 Me. No fue observada precipitación del antígeno *poly-O* de *B. abortus* 119-3 utilizándose la inmunodifusión doble de agar gel (AGID). Teniendo en cuenta la necesidad de investigaciones adicionales, este episodio nos permite considerar el AGID como una prueba diagnóstica util para identificar animales soropositivos naturalmente infectados por animales vacunados con diferentes dosificaciones reducidas de *B. abortus* S-19.
words: *Brucella abortus*, Brucellosis, Immunodiffusion.

INTRODUCTION

Bovine brucellosis is a disease that causes high economic toll and is hazardous to human health worldwide. Accurate diagnosis must include laboratory tests that allow the direct or indirect demonstration of *Brucella*. Classical serological tests are routinely used for the diagnosis of different diseases. These tests deactivate IgM, which is responsible for non-specific reactions. These tests are highly sensitive, but have low specificity and are ineffective in discriminating vaccine antibodies from those produced by infection. *B. abortus* vaccine S-19 has been successfully used in preventive vaccination of heifers, as well as in control programs for infected herds, where vaccination of

adult cows is effective.[12, 2] Correa and Correa[7] recommended observing whether the vaccinated females immune system reacted well to S-19 vaccine using post-vaccine seroagglutination tests. However, S-19 stimulates the formation of agglutinating antibodies, which might interfere with the correct interpretation of serological tests, preventing the discrimination between vaccinated and infected cows.[3]

Reduced-dosages of S-19 have been recommended to avoid persistence of residual post-vaccine titers. These control the disease and may also eliminate it with time.[13]

Díaz et al,[8] Lord et al[9] and Nielsen[14] reported that the double agar-gel immunodiffusion test (AGID) performed with the B-polysaccharide antigen of *B. melitensis* differ-

entiates between antibodies resulting from infection and those resulting from vaccination with S-19. Bruce and Jones[4] in 1958 were the first to report that there might be serological differences between these two categories of antibodies.

The objective of this study was to compare the double agar-gel immunodiffusion test (AGID) with seroagglutination in plate (SAP), tube (SAT), and 2-mercaptoethanol (2Me) in non-vaccinated cows then vaccinated with 5 different reduced-dosages of *B. abortus* S-19.

MATERIALS AND METHODS

Animals. The groups consisted of dairy cows from farms located in the Botucatu area, São Paulo State, Brazil. Forty-nine cows, non-reactive to the serological tests used in this study, were divided into 5 groups. The following groups were formed so as to avoid interference with the daily activities of the farms.

Groups	Inoculum with reduced dosage of S-19
GROUP I: 7 cows	6×10^5 CFU S-19/ml
GROUP II: 6 cows	6×10^6 CFU S-19/ml
GROUP III : 13 cows	6×10^7 CFU S-19/ml
GROUP IV: 12 cows	6×10^8 CFU S-19/ml
GROUP V: 11 cows	6×10^9 CFU S-19/ml

Using a commercial suspension of *B. abortus* S-19, containing 6×10^{10} bacteria/ml as a matrix, dilutions were prepared in PBS corresponding to 6×10^5, 6×10^6, 6×10^7, 6×10^8, 6×10^9 CFU S-19/ml. The tested cows received 2 ml subcutaneous of their respective dilution and one month after vaccination, each cow was tested at 1:100 titer using SAT. The cows that did not present the titer were reimmunized up to 3 times. They were also submitted to monthly tests AGID, SAP, SAT, and 2Me over a period of 120 days. The SAP, SAT, and 2Me were performed following the procedures recommended by the World Health Organization (WHO)[1].

Double agar gel immunodiffusion (AGID). Agar noble diluted in Sorensen phosphate buffer, pH 7.2, and antigen containing polysaccharide extracted from *B. abortus* (*poly-O*-1119-3) were used. These were located in the central well of a rosette 3.4 mm in diameter, while the animals' sera were deposited in the surrounding wells.[8] The control tests were performed using negative and positive sera of the diagnostic kit (Fig. 5).

RESULTS AND DISCUSSION

In this study, the serological results induced by the five reduced-dosages of S-19 showed that, regardless of the vaccine concentration used, SAP ranged between 25 IU and 400 IU, and SAT and 2Me ranged between 25 IU and 800 IU (Fig. 1). Further details about the titer variation of SAP, SAT, and 2Me using titer mean values are shown in Figures 2-4. Within these vaccine serological variations, there was no precipitation of the antigen *poly-O* of *B. abortus* 1119-3 in AGID, which corroborates the observations.[10,11,6] These authors reported that vaccine antibodies did not precipitate the antigen *poly-O* and the serological reaction was attributable to the *B-polysaccharide*, which was due to the presence of the *O-polysaccharide* of *Brucella (poly-O)*.

On the other hand, the control of bovine brucellosis requires the ability to differentiate between infected animals and those vaccinated with S-19. The antibodies pro-
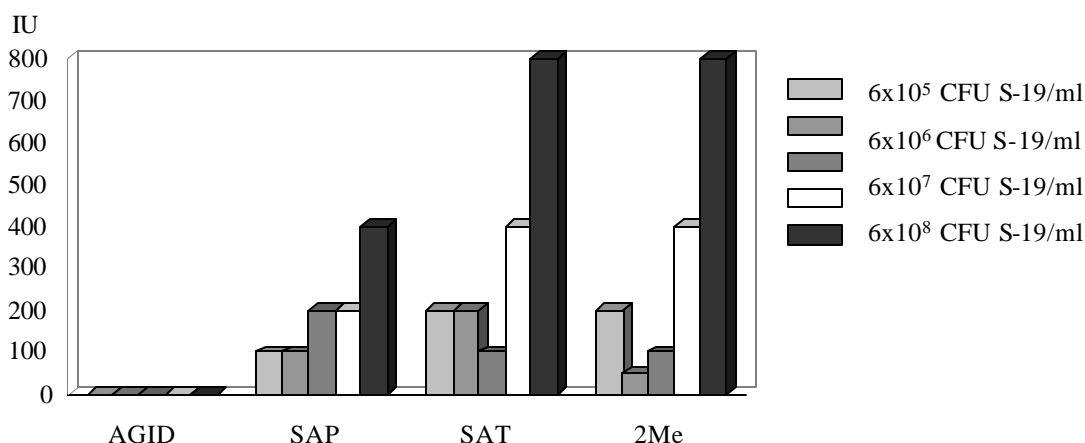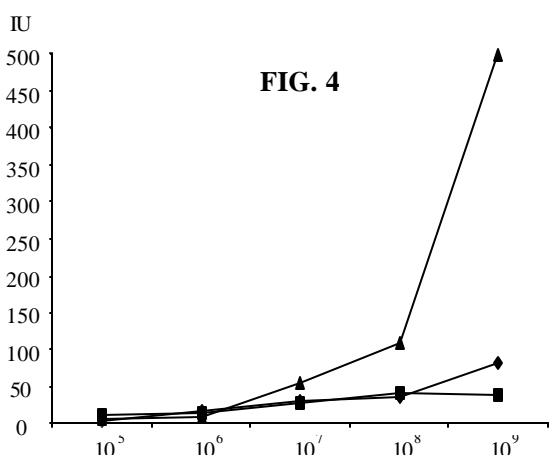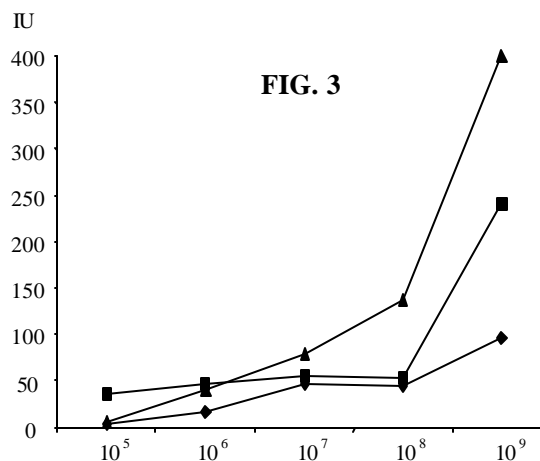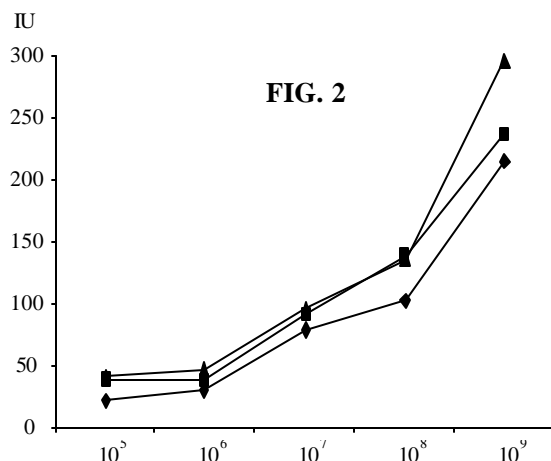

Fig 1. Distribution of the highest titers of AGID, SAP, SAT, and 2Me in cows vaccinated up to three times with five different reduced-dosages of *B. abortus* S-19.

FIG. 2

FIG. 3

FIG. 4

◆ 30, ■ 60, ▲ 90 days
6 x 10^5 CFU S-19/ml
6 x 10^6 CFU S-19/ml
6 x 10^7 CFU S-19/ml
6 x 10^8 CFU S-19/ml
6 x 10^9 CFU S-19/ml

Fig. 2-4. Distribution of mean values of SAP (Fig. 2), SAT (Fig. 3) and 2Me (Fig. 4) in vaccined up to three times cows with five different reduced dosages of *B. abortus* S-19.

duced by both categories of animals (IgG1, IgG2, IgM, and IgA) have only a proportional distribution between the two categories, it being impossible to distinguish the animals using classical serological tests, which vary in their ability to detect antibodies of a certain class of immunoglobulins.[3]

In addition, some murine monoclonal antibodies can precipitate the *poly-O* of *B. abortus* 1119-3 and *B. melitensis* 16M, probably because of their affinity for common structural entities within the *O-polysaccharides*, and also because the *poly-O* of *B. abortus* and *B. melitensis* are homopolymers.[10,5,11] This phenomenon makes possible the differentiation between vaccine antibodies and infection-produced antibodies using AGID.[8,9,14]

Our results from the AGID test do not show precipitation bands at different antigen concentrations of S-19 vaccine. Considering the need for further investigations, this allows us to consider AGID a useful diagnostic test to distinguish naturally infected seropositive animals from vaccinated cows with different reduced-dosages of *B. abortus*-S-19.
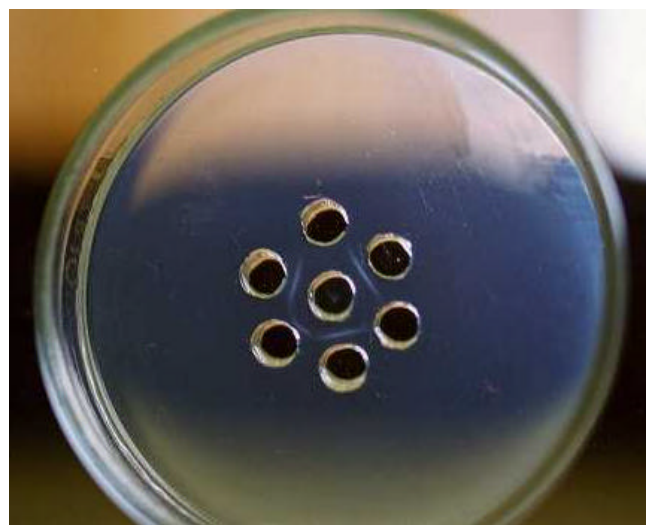

Fig. 5. Double agar gel immunodiffusion (AGID).

REFERENCES

1. Aalton, G. G, L. M. Jones. 1976. Las técnicas de laboratorio en la Brucelosis. Ginebra: FAO e OMS, 2nd ed., p.175.

2. Barton, C. E, J. R. Lomme. 1980. Reduce-dose whole herd vaccionation against Brucellosis: A review for recent experience. J. Am. Vet. Med. Assoc. 177:1218-20.

3. Beh, K. J. 1974. Quantitative Distribution of Brucella Antibody amongst Imunoglobulin Classes In Vaccinated and Infected Cattle. Res. Vet. Sci. 17:1-4.

4. Bruce, W, L. M. Jones. 1958. The use of gel diffusion precipitir plates in the study of brucella. Bull. Org. Mond. Santé. p.187-196.

5. Cherwonogrodzky, J. W, and M. B. Perry. 1986. Purification and characterization of *Brucella melitenses* in 86th Annu. Meet. Am. Soc. Microbiol., Abstr.B.186.

6. Cherwonogrodzky, J. W, and K. H. Nielsen. 1988. *Brucella abortus* 119-3 o-chain polysaccharinde to differentiate sera from *B. abortus* S-19-vaccinated and aeld-strain-infected cattle by agar gel immunodiffusion. J. Clin. Microbiol. 26:1120-1123.

7. Correa, W. M., and C. M. Correa. 1992. Bruceloses. Enferm. Infec. Mamíf. Dom. p.195-218.

8. Díaz, R., P. Garetea, L. M. Jones, I. Moriyon. 1979. Radio Immunodiffusion Test with a *Brucella* Polysaccharide Antigen for Differentiating Infected from Vaccinated Cattle. J. Clin. Microbiol. 17:37-41.

9. Lord, V. R, M. R. Rolo et al. 1989. Evaluation of humoral immunity to *Brucella sp* in cattle by use of an agar-gel immunodiffusion test containing a polysaccharide antigen. Am. J. Vet. Res. 50:1813-1816.

10. L´Vov, V. L, V. E. Mallkov, S. Shskov, E. Drandvskaya, B. S. Dmitriev. 1985. Somatic antigens of the genus *Brucella*. Structure of the O. specific polysaccharide chain of the lipopolysaccharide of *Brucella melitensis*. Bioorgan. Khim. 11:963.

11. Moreno, E, D. Borowiak, H. Mayer. 1987. *Brucella* lipopolysaccharides and polysaccharidarides. Ann. Inst. Pasteur. Microbiol. 138:102.

12. Nicoletti, P. A. 1976. Preliminary report on the efficacy of adult vaccination using strain 19 in sellected dairy herds in Florida. In: Proceeding. 80 Annu. Meet, US Anim.Health Assc., p.91-106.

13. Nicoletti, P, L. M. Jones, D. T. Berman. 1978. Adult vaccination whith standart and reduced doses of *Brucella abortus* strain 19 vaccine in a dairy heard infected with brucellosis. J. Am Med. Assoc. 173:1445-1449.

14. Nielsen, K. 1995. A brief review of diagnosis of bovine brucelosis by detection of antibody. Arch. Med. Vet. 27:9-17.