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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Received 31 January 2000/Accepted 1 June 2000

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 $6 \times 10^5$, $10^6$, $10^7$, $10^8$, 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.

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. Correa and Correa recommended observing whether the vaccinated females immune system reacted well to S19 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. 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.

Díaz et al., Lord et al. and Nielsen reported that the double agar gel immunodiffusion test (AGID) performed with the B-polysaccharide antigen of B. melitensis differ-
entitates 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 seroagglutina-
tion in plate (SAP), tube (SAT), and 2-mercaptoethanol (2Me) in non-vaccinated cows then vaccinated with 5 dif-
ferent reduced-dosages of \textit{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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum with reduced dosage of S-19</th>
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<tr>
<td>GROUP I: 7 cows</td>
<td>6 x 10(^5) CFU S-19/ml</td>
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<tr>
<td>GROUP II: 6 cows</td>
<td>6 x 10(^6) CFU S-19/ml</td>
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<tr>
<td>GROUP III: 13 cows</td>
<td>6 x 10(^7) CFU S-19/ml</td>
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<tr>
<td>GROUP IV: 12 cows</td>
<td>6 x 10(^8) CFU S-19/ml</td>
</tr>
<tr>
<td>GROUP V: 11 cows</td>
<td>6 x 10(^9) CFU S-19/ml</td>
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Using a commercial suspension of \textit{B. abortus} S-19, containing 6 x 10\(^3\) bacteria/ml as a matrix, dilutions were prepared in PBS corresponding to 6 x 10\(^5\), 6 x 10\(^6\), 6 x 10\(^7\), 6 x 10\(^8\), 6 x 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 reimmu-
nized 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 Organiza-
tion (WHO)\(^1\).

**Double agar gel immunodiffusion (AGID).** Agar no-
ble diluted in Sorensen phosphate buffer, pH 7.2, and anti-
gen containing polysaccharide extracted from \textit{B. abortus}
\textit{poly-O-1119-3} were used. These were located in the cen-
tral well of a rosette 3.4 mm in diameter, while the ani-
mals’ 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 \textit{poly-O} of \textit{B.
abortus} 1119-3 in AGID, which corroborates the observa-
tions.\(^10,11,6\) These authors reported that vaccine antibodies
did not precipitate the antigen \textit{poly-O} and the serological
reaction was attributable to the \textit{B-polysaccharide}, which
was due to the presence of the \textit{O-polysaccharide} of
\textit{Brucella} (\textit{poly-O}).

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

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. This phenomenon makes possible the differentiation between vaccine antibodies and infection-produced antibodies using AGID.

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

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

The authors wish to thank -TECPAR-, Ms. Tania M. Martins, Ms. Adriana C. Pavan Vieira and Marcelo A. P. Cristofaro for technical assistance, Ms. Heloisa M. P. Toledo for English review, and to Ms. Márcia Chiozo for the final editing of the manuscript.

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