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To cite this version:

D. Levieux. BOVINE IMMUNOGLOBULINS AND BRUCELLOSIS. 3. Activity of IgG1, IgG2 and IgM versus different commercial batches of rose bengal antigen. Annales de Recherches Vétérinaires, INRA Editions, 1978, 9 (3), pp.489-493. hal-00901029

HAL Id: hal-00901029
https://hal.archives-ouvertes.fr/hal-00901029

Submitted on 1 Jan 1978

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BOVINE IMMUNOGLOBULINS AND BRUCELLOSIS
3. Activity of IgG1, IgG2 and IgM versus different commercial batches of rose bengal antigen

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Résumé
IMMUNOGLOBULINES BOVINES ET BRUCELLOSE. 3. ACTIVITE DES IgGl, IgG2 ET IgM CONTRE DIFFERENTS LOTS D’ANTIGENES DU COMMERCE AU ROSE BENGALE. — Le diagnostic sérologique de la Brucellose bovine par le test à l’antigène au Rose Bengale a été controversé quant aux doses d’immunoglobulines actives et aux résultats obtenus sur le terrain.

Pour tenter d’expliquer ces discordances, l’activité de différentes préparations commercialisées d’antigène au Rose Bengale a été comparée vis-à-vis d’immunoglobulines purifiées IgG1, IgG2 ou IgM à activité anti-Brucella ou de sérums à anticorps essentiellement IgG ou IgM. Deux antigènes qui donnent le même titre à des IgG1 diffèrent d’un facteur 8 dans le titrage des IgM. Certains antigènes tintent les IgG2, d’autres peu ou pas du tout.

Après injection expérimentale par Brucella abortus de génisses à 6' mois de gestation, la moitié des animaux est positive dès le 12' jour pour un antigène et seulement au 30' jour pour un autre.

Des génisses vaccinées à 6-8 mois au B19, qui réagissent encore au test Rose Bengale 14 mois plus tard, sont quatre fois plus nombreuses avec l’un des antigènes qu’avec un autre.

Introduction

A wide range of serological tests is available for the serological diagnosis of brucellosis (reviewed by Morgan, 1967). While the serum agglutination test has been extensively used for control and eradication pro-

grammes, the test has limitations, especially in the incubative and chronic stages of the disease, and in differentiating antibodies resulting from infection and vaccination; the complement fixation test is now widely used as an aid to proper diagnosis in these circumstances.

More recently, a modification of the acid-plate-agglutination test (Rose and Roepke, 1957), using a suspension of Brucella abortus cells stained with Rose Bengal dye and
buffered at pH 3.65, was introduced by Dr. D. Pietz of the United States Department of Agriculture, as the basis of a card test for bovine brucellosis. In field trials, this test was found to be a more accurate indicator of infection than the serum agglutination test (Nicoletti, 1967), and it has been accepted with enthusiasm in some countries either as a definitive test (Schiff, 1967), or as a screening test (Morgan et al., 1969); in other countries, its efficiency has been questioned (O'Reilly and Cunningham, 1971; Alton et al., 1975).

The antibody active in the Rose Bengal test belongs to the IgG1 class, while the IgG2 are inactivated at pH 3.65 (Diaz and Levieux, 1972). IgM are inactive for Corbel (1972), but active for Levieux (1974 b). In the work of Beh (1973), IgG1, IgG2 and IgM are active.

These discrepancies can be due:
1. To the use of insufficiently purified immunoglobulins.
2. To different properties of the commercial preparations of the Rose Bengal antigen.

The purpose of the present article is to evaluate this second hypothesis.

Materials and methods

SERA

Serum 365
This serum was obtained from a heifer (8 months) 14 days after subcutaneous vaccination with 2ml of freeze-dried living *Brucella abortus* strain 19. Antibodies are found essentially in the IgM class (Levieux, 1974 b).

Serum B1
Pool of 20 sera showing high titer antibodies after vaccination with *Brucella abortus* B19 and experimental infection with *Brucella abortus* biotype 1 strain 544 (Plommet et al., 1970). Antibodies are found essentially in the IgG1 class (Levieux, 1974 b).

ISOLATION OF BOVINE IMMUNOGLOBULINS

Each serum was fractionated by combination of column chromatography on DEAE cellulose and Sephadex G 200 as described previously (Levieux, 1974 a).

Freeze-dried immunoglobulin preparations were solubilized in phosphate buffered saline pH 7.2 to their respective concentrations in the sera, and serially diluted in the same buffer.

SEROLOGICAL TESTS

Rose Bengal plate test

25 μl of test substance was added to 25 μl of Rose Bengal antigen on a haemagglutination tray and mixed on a rotary agglutination for 4 minutes. Agglutination was assessed on a scale 1 + to 4 +.

All the antigens used for this test were commercial batches: one English (Weybridge), two Israeli, six French.

Serum agglutination and complement fixation tests

The procedures used for these tests have been described or referred to previously (Levieux, 1974 b).

Results

ACTIVITY OF PURIFIED IMMUNOGLOBULINS VERSUS DIFFERENT BATCHES OF ROSE BENGAL ANTIGEN (Fig. 1)

The titer obtained with the different antigens for the IgG1 preparation is very similar. The antigen 7 is a little more sensitive, and the antigens 8 and 9 are less sensitive by one dilution.

The IgG2 preparation is not titrated by 5 antigens, and partially titrated by the other. The antigen 2 is very sensitive for the IgM preparation while the antigen 1 is particularly insensitive. The other antigens have a similar activity.

ACTIVITY OF SERA B1 AND 365 VERSUS DIFFERENT BATCHES OF ROSE BENGAL ANTIGEN (Fig. 2)

Some dilutions of serum B1 show incomplete reactions with antigens 3, 4 and 7, which are sensitive for IgG2 antibodies. However, antigens 2, 8, and 9 show normal reactions. Serum B1 contains many more antibodies of the IgG1 class than of the IgG2 class, and differences of competition of these two subclasses of antibodies for
the antigens may explain these discrepancies.

The antigens 8 and 9 are two-fold less sensitive than the others.

Serum 365 which contains antibodies of the IgM class shows the same pattern of reaction as the purified IgM: antigen 1 is only slightly sensitive, and antigen 2 is eight-fold more sensitive.

PRECOCITY OF ANTIBODY DETECTION AFTER EXPERIMENTAL INFECTION

Antigens 1 and 2 are used to study the appearance of antibody in the sera of 7 brucellosis-free heifers, experimentally infected at the 5th-6th month of pregnancy (Fig. 3).

Antigen 2, IgM sensitive, gives a positive reaction on 4 heifers for 12 days after infection. More than 30 days are needed to obtain the same results with the antigen 1.

Antigen 2 produces results similar to those obtained with the seroagglutination test, while those produced with antigen 1 are like the complement fixation test.

DETECTION OF POST VACCINAL ANTIBODIES

Antigens 1 and 2 were also used to study the antibody content of 12 brucellosis-free heifers 14 months after subcutaneous vaccination with strain B19 at the 6th-8th month of life.

Antigen 1 detected antibodies on 2 heifers, with little intensity while antigen 2 detected antibodies on 8 heifers.

This result is consistent with micro-chromatography analysis of these sera, which indicates that the persistant antibodies are only of the IgM class (to be published).

Discussion

The results published in this work show that the discrepancies are at least partly due to the antigens used. All these antigens are standardized for use in the serological detection of brucellosis on a national scale.

For a serum with class IgG1 antibodies (serum B1), which is characteristic of brucellosis, the antigens tested have a similar value, except for antigens 8 and 9 which are two-fold less sensitive. Thus, there is a risk that «positive» animals might be called
negative when these two antigens are used.

For a serum with class IgM antibodies (serum 365) which characterises either a very recent infection or antibodies remaining after vaccination, the antigens tested also have a similar value, except for antigen 2, which is eight-fold more sensitive than antigen 1. Thus, in a quantitative analysis, the same animal could be called «negative», with a titer of, for example, 20 international units, or «infected» with a titer of 160 international units, depending on the antigen used. The use of antigen 1 in the work of Corbel (1972) on serums with a fairly low titer of class IgM antibodies, explains why, for this author, the IgM were inactive in the Rose Bengal test.

Since the pH of these antigens is the same (3.65) the difference in activity is probably due to the industrial fabrication which varies slightly from one laboratory to another, although it is based on the recommendations of the USDA (United States Department of Agriculture).

This poses two problems: the choice of antigen, and its standardization.

The main value of the Rose Bengal test is that there is a good correlation between the results it produces, and those produced by the complement fixation test, which is generally considered as a very reliable test. As the immunoglobulins active in the complement fixation test are essentially of class IgG1, and to a lesser extent, class IgM, it is preferable to choose an antigen which titrates the IgG1. In this respect, the antigens tested are generally satisfactory: only antigens 8 and 9 lack sensitivity. The antigen used by O'Reilly and Cunningham (1971) has probably a low sensitivity to the IgG, for, according to the authors, it does not titrate complement fixing antibodies. IgM antibodies appear early post infection, but they can persist for a long time after vaccination; thus, an antigen sensitive to the IgM detects the infection in its early stages, but, on the other hand, after vaccination it is misleading in indicating positive. This type of antigen could be used in testing uncontaminated herds which have not been vaccinated.

The standardization of the Rose Bengal antigen ought to be done either with sera having a known immunoglobulin content, or with purified preparations of active immunoglobulins. In fact, if we compare, for example, the activity of antigens 1 and 2 for serum 365 (which is similar to the original International Standard Brucella Antiserum) antigen 1 is eight-fold less sensitive than antigen 2. On the other hand, for serum B1 (which represents the current International Standard Brucella Antiserum) these two antigens have the same sensitivity.

Thus, the standardization of any batch of antigens must be done for the IgG, as well as for the IgM. This is the only way we can test the real value of the Rose Bengal test for the serological detection of Brucellosis, and to avoid discrepancies between the results obtained in different laboratories.

Accepted for publication, April 5th 1978.

Acknowledgments

The author wishes to thank D’R. Gaumont and Mme D. Trap (Laboratoire Central de Recherches Vétérinaires, Maisons-Alfort, France) for kind hospitality in their Laboratory, and Dr Feinhaken (Kimron Veterinary Institute, Beit Dagan, Israel) for providing some of the Rose Bengal antigens.
The serological diagnosis of bovine Brucellosis by the Rose Bengal test has been disputed on account of the amount of active immunoglobulins and the results obtained in field trials. In order to try and explain these discrepancies, we have compared the activity of different commercial preparations of the Rose Bengal antigen for purified IgG1, IgG2 or IgM immunoglobulins with anti Brucella activity or for sera with essentially IgG or IgM antibodies. Two antigens which give the same titer for the IgG1 differ by a factor 8 in the titration of the IgM. Some antigens titer the IgG2, others, do not at all, or very little.

Heifers in the 6th month of gestation were experimentally infected with Brucella abortus and half the animals showed « positive » from the 12th day with one antigen, whereas with another, they did not register until the 30th day.

Heifers vaccinated at the age of 6-8 months with the B19 which still react to the Rose Bengal test 14 months later are 4 times more numerous with one of the antigens than with another.

References


