Revaccination with a reduced dose of \textit{Brucella abortus} strain 19 vaccine of breeding cows in the Pampas region of Argentina


Summary: In a breeding herd previously infected with brucellosis, 203 cows of various crossbreeds were inoculated subcutaneously with a dose of \(3.1 \times 10^9\) colony-forming units (CFU) of \textit{Brucella abortus} strain 19. They had been vaccinated as calves with the standard dose of \(60 \times 10^9\) CFU of this vaccine strain, and had become negative to the slow tube agglutination test (SAT) at the moment of revaccination. Another 137 cows in the same herd were not revaccinated in order to serve as controls for the natural course of infection.

One year after revaccination 12.3\% were positive to SAT and 12.8\% to the mercaptoethanol test, while two years after revaccination these percentages were 13.3\% and 3.4\%. First-calf heifers had higher and more persistent titres than the cows, and this tendency was also seen in zebu crosses as compared with European and Criollo crosses. There was no difference between pregnant and non-pregnant animals in their postvaccinal response.

The pronounced serological response and persistence of postvaccinal titres were probably due to the previous vaccination, in which case the reduced dose used may not have been the most appropriate. The persistent postvaccinal titres in the SAT show that it is necessary to conduct other serological tests in order to identify which animals are infected at the time of vaccination with reduced dosage. The percentage of abortions due to \textit{B. abortus} during the trial was similar (1.5\%) among revaccinated and control animals. Only one case of abortion, from which the vaccine strain was recovered, occurred in females revaccinated up to the 6th month of gestation.

KEYWORDS: \textit{Brucella abortus} - Brucellosis - Cattle diseases - Cow - Dosage - Immunisation - Live vaccines.

INTRODUCTION

The vaccination of calves with \textit{Brucella abortus} strain 19 is one of the principal control measures against bovine brucellosis. In various countries the use of this vaccine has reduced the prevalence of the disease to levels sufficiently low for the initiation
of eradication campaigns (6, 24, 25). Resolution 73/1982 of the Argentine Animal Health Service has made this vaccination compulsory within the Argentine Republic (23). Of course, this measure alone is inadequate to control the disease in herds having a high prevalence, and in such circumstances the heavy exposure to infection with *B. abortus* from the environment can overcome the protection conferred by the vaccine, resulting in the spread of infection and the creation of a problem herd (9).

By means of vaccination and/or revaccination with reduced doses (RD) of strain 19 vaccine under the right conditions (2, 3, 6, 12), adequate protection can be provided, enhancing the resistance of adult females to infection (17, 18, 19, 20). The national legislation makes provision for this possibility in certain herds undergoing eradication (23). The main disadvantage of using RD is the persistence of postvaccinal antibody titres in the blood serum, which may interfere with routine diagnostic tests, so that additional serological tests have to be used.

Since there is little information in Argentina about the use of RD, the present report has been prepared. Its purpose is to compare the serological response of cows of different breeds, ages and physiological states after revaccination with a reduced dose (5%) of *B. abortus* strain 19.

**MATERIALS AND METHODS**

**History and composition of the herd**

The animals were from a previously infected breeding herd in the South-East of Buenos Aires Province. The prevalence of infection (reactors to the slow tube agglutination test, SAT) and the percentage of abortions attributable to *B. abortus* were (respectively) 8.6% and 5.9% in 1979, 7.4% and 6.4% in 1980, and 5.5% and 16.5% in 1981. Table I gives details of the composition of the revaccinated herd according to category and physiological state of the animals. In order to evaluate the natural course of infection in the herd, the evolution of titres was monitored in a group of 137 Aberdeen Angus and Hereford cows, which served as controls, not revaccinated, for the animals revaccinated with strain 19. In this way it was possible to compare the infective titres with the serological conversion in animals inoculated with a reduced dose.

All the females used had been vaccinated as calves with the standard dose of strain 19 (60 x 10⁹ colony-forming units [CFU]), and they had become serologically negative to SAT at the start of the trial. Artificial insemination was carried out during a period of three months each year, using semen from bulls under supervision. The percentage pregnant at the time of revaccination was 69% in the revaccinated group and 71% in the controls, and none was more than 6 months pregnant. In the second year the percentages pregnant were 75% and 76%, respectively. Any female which aborted during the trial was removed from the herd.

**Dose of vaccine**

Once the insemination period of three months had been completed, 203 crossbred females aged 27 months or more were inoculated subcutaneously with a reduced dose (RD) of 3.1 x 10⁹ CFU of *B. abortus* strain 19, contained in 5 ml of normal saline.
TABLE I
Composition of the herd revaccinated with a reduced dose of B. abortus strain 19, according to crosses and categories

<table>
<thead>
<tr>
<th>Crosses</th>
<th>European (a)</th>
<th>Criollo (b)</th>
<th>Zebu (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
<td>VC (d)</td>
<td>VQ (e)</td>
<td>VC</td>
</tr>
<tr>
<td>Number</td>
<td>74</td>
<td>8</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>VC + VQ</td>
<td></td>
<td>82</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>203</td>
</tr>
</tbody>
</table>

(a) European : Aberdeen Angus crosses with Hereford (39 cattle), Limousin (22), Argentinian Dutch (15) and Simmental (6)
(b) Criollo : Criollo crosses (49 cattle) and crosses with Aberdeen Angus (33)
(c) Zebu : Nelore × Aberdeen Angus crosses
(d) VC : Cows which have calved twice or more
(e) VQ : First-calf heifers, 27 months old or more

solution. This RD was prepared from a commercially available vaccine (Brucella abortus Bang®, Bayer Laboratory, batch B 21) having an initial concentration of $12.3 \times 10^9$ CFU/ml. After dilution the final dose was about 5% of the standard dose.

Samples and serological tests

Blood samples were obtained from all animals at the time of revaccination and 1, 3, 4, 7, 12, 20 and 24 months afterwards. The SAT (8) and ME (13) tests were performed according to the techniques recommended by, and with antigens supplied by the Pan American Zoonosis Center. Interpretation of titres by the SAT was: doubtful up to 100 international units (IU), inconclusive up to 200 IU and positive 200 IU or more (17, 19, 25). The ME test was considered to be positive at titres of 25 IU or above (14, 19).

Bacteriology

A post-mortem examination was conducted on aborted fetuses and samples of organs were sown on tryptose serum agar incubated aerobically under 10% carbon dioxide at 37°C for 7 days (4). Strains of B. abortus isolated were typed by the Pan American Zoonosis Center.

Statistical analysis

The serological responses of the two groups were compared by means of Fisher’s precise test. The chi-squared test (22) was used when more than two groups were analysed. In each case the level of significance $P < 0.05$ was chosen.

RESULTS

Slow tube agglutination test

The serological responses of revaccinated animals and the controls are depicted in Table II and Fig. 1. There were significant differences between the two groups up to 7
1066

months after revaccination. The highest titre observed in revaccinated animals was 800 IU, and only 7 animals (3.4%) reached this titre during the first month after revaccination.

TABLE II

Animals having a significant serological response in slow tube agglutination and mercaptoethanol tests in the group revaccinated with a reduced dose of strain 19 vaccine and in the control group

<table>
<thead>
<tr>
<th>MPR(a)</th>
<th>Group/Test</th>
<th>SAT(b)</th>
<th>ME(c)</th>
<th>SAT</th>
<th>ME</th>
<th>SAT</th>
<th>ME</th>
<th>SAT</th>
<th>ME</th>
<th>SAT</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduced dose (203)</td>
<td>163 (80.3)</td>
<td>194 (95.5)</td>
<td>96 (47.3)</td>
<td>107 (52.7)</td>
<td>46 (22.4)</td>
<td>53 (26.1)</td>
<td>25 (12.3)</td>
<td>26 (12.9)</td>
<td>27 (13.3)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td></td>
<td>Controls (137)</td>
<td>5 (3.6)</td>
<td>8 (5.8)</td>
<td>7 (5.1)</td>
<td>10 (7.3)</td>
<td>12 (8.7)</td>
<td>14 (10.2)</td>
<td>14 (10.2)</td>
<td>10 (7.3)</td>
<td>15 (10.9)</td>
<td>12 (8.7)</td>
</tr>
</tbody>
</table>

Significant results (P < 0.05): (a) MPR = months post revaccination
  * = between groups
  • = between tests
(b) SAT = slow tube agglutination test on serum, 100 IU or more
(c) ME = mercaptoethanol test, 25 IU or more
( ) figures within brackets are percentages

FIG. 1

Serological response in cows revaccinated with a reduced dose of strain 19 vaccine and in unvaccinated controls to the SAT (expressed as % of reactors at titres of 100 IU or more)

MPR = months post revaccination
Mercaptoethanol test

Table II shows the results of this test in revaccinated and control cattle. The highest titre reached in the revaccinated group was 400 IU, in 11 animals (5.4%) one month after revaccination. There was a significant difference after 24 months, with the highest proportion of reactors in the control group. At the end of the trial, 3.4% of cows in the revaccinated group had titres between 25 and 100 IU. The complement fixation test was performed on blood from 4 of these reactors, giving titres of 1:20 or more in all of them.

Comparison of results for SAT and ME

The ME test revealed significant differences from the SAT at 1 and 24 months (Table II). Analysis of the serological response to SAT and ME of pregnant and non-pregnant revaccinated cows reveals a greater initial response (at 1 month) to SAT in pregnant than in non-pregnant animals (82.2% against 69%). At the same sampling there were also significant differences between results of the two tests. Thus 82.2% of pregnant animals were positive to SAT and 97.1% to ME, while 69% of non-pregnant cows were positive to SAT and 90.9% to ME.

Although the initial response to revaccination was similar in cows and heifers, the latter showed a significant persistence of postvaccinal titres in both tests up to the 18th month (Table III). At the end of the trial the percentages of SAT reactors were similar, while the heifers submitted to ME still had significant residual titres (Table III, Fig. 2). In zebu crosses, the response of cows and heifers showed a similar tendency, expressed at 24 months by 42% of the heifers and 5% of the cows positive to SAT, and 10.5% of the heifers and none of the cows positive to ME.

### TABLE III

<table>
<thead>
<tr>
<th>Category/Test</th>
<th>MPR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows (162)</td>
<td>SAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ME&lt;sup&gt;c&lt;/sup&gt;</td>
<td>SAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ME</td>
<td>SAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ME</td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>154</td>
<td>71</td>
<td>81</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>(77.7)</td>
<td>(95.6)</td>
<td>(43.8)</td>
<td>(50)</td>
<td>(19.7)</td>
<td>(18.3)</td>
</tr>
<tr>
<td>Heifers (41)</td>
<td>SAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ME&lt;sup&gt;c&lt;/sup&gt;</td>
<td>SAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ME</td>
<td>SAT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ME</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>40</td>
<td>25</td>
<td>26</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>(90.2)</td>
<td>(97.6)</td>
<td>(60.9)</td>
<td>(63.4)</td>
<td>(34.1)</td>
<td>(21.9)</td>
</tr>
</tbody>
</table>

Significance of results (P < 0.05): (a) MPR = months post revaccination

* = between categories 

• = between tests

(a) SAT = slow tube agglutination test, 100 IU or above
(b) ME = mercaptoethanol test, 25 IU or above
(c) figures within brackets are percentages

Behaviour of the different breeds and crosses

The response was most pronounced in zebu crosses, which showed a tendency to maintain higher postvaccinal titres than European and Criollo crosses (Table IV, Fig. 3).
Serological response of cows and heifers revaccinated with a reduced dose of strain 19 vaccine to the mercaptoethanol test (expressed as % of reactors at titres of 25 IU or above)

TABLE IV

Cows of different crosses having a significant serological response in slow tube agglutination and mercaptoethanol tests following revaccination with a reduced dose of strain 19 vaccine

<table>
<thead>
<tr>
<th>MPR&lt;sup&gt;(a)&lt;/sup&gt;</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross/Test</td>
<td>SAT&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>ME&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>SAT</td>
<td>ME</td>
<td>SAT</td>
</tr>
<tr>
<td>European (82)</td>
<td>60 (73.1)</td>
<td>77 (93.9)</td>
<td>35 (42.6)</td>
<td>32 (39)</td>
<td>20 (24.4)</td>
</tr>
<tr>
<td>Zebu (39)</td>
<td>37 (94.8)</td>
<td>39 (100)</td>
<td>28 (77.1)</td>
<td>35 (89.7)</td>
<td>16 (41)</td>
</tr>
<tr>
<td>Criollo (82)</td>
<td>63 (76.8)</td>
<td>78 (95.1)</td>
<td>33 (40.2)</td>
<td>40 (48.9)</td>
<td>9 (10.9)</td>
</tr>
</tbody>
</table>

Significance of results (P < 0.05):  
- * = between crosses  
- ** = between tests  
- ( ) figures within brackets are percentages

(a) MPR = months post revaccination  
(b) SAT = slow tube agglutination test, 100 IU or above  
(c) ME = mercaptoethanol test, 25 IU or above
Serological response measured by the mercaptoethanol test in different crosses of cow breeds after revaccination with a reduced dose of strain 19 vaccine (expressed as % of reactors at titres of 25 IU or above)

E : European crosses
C : Criollo crosses
I : zebu crosses

Abortions

During the first year of the trial, the revaccinated group experienced three abortions (1.97%), from which B. abortus biotype 1 was isolated, and one in which the vaccine strain was isolated. There were two abortions in the control group (1.46%), from which B. abortus biotype 1 was isolated. In the second year only one cow of the revaccinated group aborted, and B. abortus biotype 1 was isolated.

DISCUSSION

Different ways of vaccinating young animals and adults with a reduced dose have been employed. Experience gained in infected herds has shown that the incidence of brucellosis can be reduced by 90% or more during one year, with some cases of residual antibody titres and slight vaccinal infection, regardless of the route and concentration of the vaccine (3, 18). The conjunctival route has been used for a dose of $4.5 \times 10^9$ CFU (20), or $6.1 \times 10^9$ followed by revaccination after 4-6 months (12). The intradermal route
has been used for a dose of $4 \times 10^9$ CFU (18). Recently the oral route has been tried with $500 \times 10^9$ CFU in the form of a paste (18). Some authors have used the subcutaneous route with a concentration of $5 \times 10^9$ CFU (10), while others have employed smaller doses: 4 or $2.8 \times 10^9$ (2, 3, 10) and even $0.3 \times 10^8$ CFU (15).

While most researchers have used RD for primary vaccination of calves and adult cattle, others have vaccinated calves with the standard dose of $60 \times 10^9$ CFU, with revaccination of heifers with RD by the conjunctival route at a concentration of $5.7 \times 10^9$ CFU (12). In our case the initial vaccination was also performed with the standard dose, while revaccination was done on adult animals, over 27 months of age, with a dose of $3.1 \times 10^9$ CFU subcutaneously. The serological response to SAT and ME of animals vaccinated in this way differed from the results obtained by Nicoletti et al. (18), employing the same tests and the same route and dose. The high percentage of reactors encountered in our trial may have been due to an anamnestic response to the antigenic stimulus of revaccination, in animals already vaccinated (19). In such circumstances the dose used was probably rather high, or the route of application may not have been the most suitable. According to Fensterbank et al. (12), revaccination by the conjunctival route should produce as good protection with less persistence of postvaccinal antibody titres. Considering that at the end of the trial some animals were positive to the complement fixation test, and that the control group had more animals positive to ME than the revaccinated group, it is possible that natural infection may have occurred in some animals with persistent antibody titres.

In agreement with the findings of others, we observed a tendency for heifers to have high and persistent postvaccinal antibody titres more often than cows (6), and this tendency was more pronounced in zebu crosses than in European and Criollo crosses. Among the cows, the zebu crosses behaved serologically in a similar way to the heifers, with significant postvaccinal residual antibody titres. Although the validity of our findings might be questioned because of the small number of zebu females used, there is confirmation in other publications (15). Pending corroboration of these observations by tests with different concentrations of RD administered by various routes in adult zebu females, one should be cautious when using traditional diagnostic tests to evaluate the serological response of such crosses to revaccination with RD.

The humoral immunity which develops after vaccination is accompanied by an initial increase in immunoglobulin IgM, which reaches a peak between 12 and 21 days, and a later rise in IgG1, with a peak between 28 and 41 days (1, 7, 16). In the revaccinated group there was a significant difference between initial reactions to the SAT and ME tests, and this may have been due to the fact that the ME test detects principally IgG1 antibodies, and that the first testing probably coincided with the peak concentration of this immunoglobulin in the serum. Since the traditional serological methods are incapable of distinguishing residual postvaccinal antibodies from the antibodies resulting from infection, this interferes with the prompt identification of infected animals (20), giving rise to the need to use additional tests for distinguishing the two types of antibodies (13).

The SAT is regarded as inadequate for detecting infected animals, since *B. abortus* has been isolated from animals having low titres to this test (5). Nicoletti et al. (21) isolated *B. abortus* from 39% of a group of cattle which were negative to SAT. Later the same authors isolated *B. abortus* strain 19 from 14% of cattle which were negative to SAT but positive to other tests (17). Since SAT detects mainly IgM, its use for detecting infected animals, in which IgG1 dominates, is of doubtful value (16, 25). On the other hand it is necessary to take into account the fact that in certain cases the postvaccinal antibody
titres induced by strain 19 can be evaluated best by SAT (18), and that other tests may be necessary in order to avoid the unnecessary elimination of breeding stock. Of course, numerous other tests have been tried to overcome this problem, and the complement fixation test has proved to be the best (2, 3, 6, 10, 17, 19, 20, 21), although the Rivanol test has been used satisfactorily by many workers (3, 6, 10, 19, 20). The type of antibody detected by the latter is similar to that detected by the ME test, which confirms its suitability as a supplementary diagnostic test.

The occasional failure of an animal to develop a humoral immune response to *B. abortus* might be important in the spread of the disease. However, the diagnostic tests normally employed only measure humoral immunity, and are incapable of detecting females with chronic infection accompanied mainly by blocking antibodies (9, 13, 25). Cordes *et al.* (9) found that 34% of culturally positive animals were negative to complement fixation and Rose Bengal tests. Latently infected female cattle acted as a focus of infection for the herd, as in the case of calves born of infected dams, which may remain serologically negative, being detected only when they abort later in life (23). Because of this it is necessary to test problem herds throughout the year, and if necessary to conduct bacteriological tests on the milk (9).

Considering that we vaccinated animals up to six months pregnant, the percentage of abortions caused by the vaccine strain was low. This agrees with the findings of others (11, 17) and it confirms that RD vaccination is suitable for pregnant cows, serologically negative and exposed to the risk of infection. It must be remembered that a small percentage of cows (0.5-2.0%) will excrete strain 19 in their milk for up to 13 months after RD vaccination (1, 11, 17, 18).

Although there was a significant reduction in the number of abortions after 24 months, the disease was not eradicated. Elimination of reactors before RD revaccination, in a herd where the abortion rate was 16.5%, certainly contributed to a reduction in the degree of exposure of the herd to infection. The elimination of reactors is an effective means of controlling the disease, but this should not rule out an RD vaccination programme in herds having a high rate or imminent risk of infection.

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**REFERENCES**

(see p. 1060)