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SEROLOGICAL TITRES FOLLOWING VACCINATION OF SHEEP AND GOATS WITH *BRUCELLA MELITENSIS* REV I VACCINE

R. W. WORTHINGTON[1], MARIA S. G. MÜLDERS[1], I. S. McFARLANE[1] and DAPHNE BECKER[2]

ABSTRACT

WORTHINGTON, R. W., MÜLDERS, MARIA S. G., McFARLANE, I. S. and BECKER, DAPHNE. Serological titres following vaccination of sheep and goats with *B. melitensis* Rev 1 vaccine. *Onderstepoort J. vet. Res.* 40(1), 1–6 (1973)

Rev 1 vaccination of sheep induced complement fixation titres to both *Brucella abortus* and *Brucella ovis* antigens, the complement fixing antibody titres were, however, higher with the *B. abortus* antigen. These titres generally fell to negative levels (below 1/20) within 5 to 6 months of vaccination. In goats Rev 1 vaccination induced complement fixation titres against *B. abortus* antigen which fell to negative levels within 5 to 6 months. Agglutination at 37°C or 56°C, agglutination in 5% NaCl, and Coombs tests are less useful in vaccinated animals as titres remain at positive levels for much longer periods after vaccination.

INTRODUCTION

In South Africa the incidence of *Brucella ovis* infection of rams is high (Worthington, Van Tonder & Mülders, 1972) and infection of sheep and goats with *Brucella abortus, Brucella melitensis* and *Brucella suis* also occurs although these infections are less common (Van Drimmelen, 1962, 1966). Rev 1 vaccine is the only *Brucella* vaccine used for control of brucellosis in sheep and goats in South Africa. It has been shown to be effective in controlling *B. ovis* in rams (Van Heerden, 1962, 1964), and is the vaccine of choice for controlling other forms of brucellosis in sheep and goats (Joint FAO/WHO Expert Committee on Brucellosis, 1971)*. In South Africa only rams are vaccinated to control *B. ovis* infection and vaccination of ewes for the control of the other forms of brucellosis is not widely practised. For this reason only 400 000 doses of vaccine are issued annually by the Veterinary Research Institute, Onderstepoort, although the South African sheep population is over 35 million and the goat population about 5,5 million.

In South Africa the complement fixation test (CF test), using *B. ovis* and *B. abortus* antigens in sheep and *B. abortus* antigen in goats, is the main test used for the diagnosis of brucellosis. Other serological tests including Coombs, 5% salt agglutination, and the standard agglutination test at 37°C (Joint FAO/WHO Expert Committee on Brucellosis, 1971)* are sometimes used as additional diagnostic tests. The question of how the results of serological tests should be interpreted following Rev 1 vaccination therefore periodically arises. The Joint FAO/WHO Expert Committee on Brucellosis (1971) has given clear directives concerning the use of Rev 1 vaccination in goats. They state that "goats that have been vaccinated with Rev 1 vaccine or H38 adjuvant vaccine may have suspicious or positive agglutination reactions for several years after vaccination" and "the complement fixation test is very useful for routine tests on animals that have been vaccinated with Rev 1 vaccine, since the complement fixation reactions have generally returned to negative levels by 6 months after vaccination". In the case of sheep the position has not been so clearly defined and in particular the effect of Rev 1 vaccination on the *B. ovis* titres is not known.

The object of this investigation was to test the effect of Rev 1 vaccination of adult sheep and goats on the persistence and specificity of serum antibodies.

METHODS

Experimental animals

One hundred adult ewes of various breeds (Merino, German Merino, Letelle and cross-breds) and 44 adult Boerbok goats were vaccinated with Rev 1 vaccine (dose $2{,}5 \times 10^4$ viable organisms). All animals were bled at the time of vaccination and at monthly intervals thereafter for a period of 2 years. Sera from both sheep and goats were subjected to the following tests: CF, agglutination at 37°C and 56°C, Coombs test and 5% salt agglutination test with *B. abortus* antigen. Sheep sera were also submitted to the CF test with *B. ovis* antigen.

Serological methods

The CF tests were done as previously described (Worthington & Mülders, 1969). CF antibody titres of 1/4 were regarded as suspicious and 1/8 as positive when the South African National Standard anti-*B. abortus* serum gave a titre of between 1/64 and 1/128 and our Standard anti-*B. ovis* serum gave a titre of between 1/64 and 1/128 in parallel control tests. It is usual to express CF titres in terms of the dilution of serum used in the test. An alternative method of expressing the titres is in terms of the final dilution of the serum after all the other test reagents have been added. Although the latter method is generally less acceptable, as in different test modifications varying volumes of the various reagents are added, we have chosen to use this method in the present investigation because the titres 1/2, 1/4, 1/8 etc. are then referred to as 1/10, 1/20, 1/40 etc. and are directly comparable to the agglutination results. Agglutination at 37°C and 56°C and Coombs tests were done by standard methods as described elsewhere in this issue (Worthington, Mülders, McFarlane & Becker, 1973).

The 5% NaCl agglutination test was done in exactly the same way as an ordinary agglutination test except that all serum dilutions were made with 10% NaCl

*Joint FAO/WHO Expert Committee on Brucellosis, Fifth report, 1971. *FAO agric. Stud.*, No. 85, pp 50-60.

[1]Veterinary Research Institute, Onderstepoort.
[2]Section of Biometry, Dept. of Agricultural Services - Present address Dept. of Mathematics and Applied Mathematics, University of South Africa.

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solution. After addition of the antigen the NaCl concentration was therefore about 5%. Doubling dilutions of serum from 1/10 to 1/320 were tested in all cases.

Statistical methods

The geometric means of the reciprocals of the titres for each test for each month were calculated as elsewhere described (Worthington, *et al.*, 1973).

Results

Studies on sheep

The sheep used in this experiment were unfortunately only available for a relatively short period. One year after the start of the experiment 97 of the original 100 animals were still in the trial, by 18 months 82 remained and in the next month a drastic reduction to 38 took place. At the end of the two years there were only 34 animals still in the experiment.

TABLE 1 CF and agglutination titres of 100 sheep before vaccination

	<1/10	1/10	1/20
CF (*B. ovis* antigen)	88	11	1
CF (*B. abortus* antigen)	100	—	—
Agglutination	78	18	4

The CF and agglutination titres at the start of the experiment are given in Table 1.

The monthly geometric means of the reciprocals of the titres of the sheep for each serological test are illustrated in Fig. 1.

The average titres in the 37°C agglutination test and the NaCl agglutination test rose sharply after Rev 1 vaccination and then fell by the 4th month after vaccination to levels (about 1/20-1/30) which were maintained for the rest of the experiment. Eighteen months

TABLE 2 Comparison of serum agglutination tests incubated at 56°C and 37°C on sera from sheep vaccinated with Rev 1

Month after vaccination	No. sera tested	56°C > 37°C*	56°C = 37°C**	56°C < 37°C***
1	100	59	23	18
2	100	16	42	42
3	100	16	48	26
4	99	7	79	13
5	99	18	73	8
6	98	4	69	25
7	97	2	23	72
8	97	5	62	30
9	96	1	64	31
10	97	0	49	48
11	97	0	15	82
12	97	0	7	90
13	96	1	42	53
14	93	0	14	79
15	90	0	11	79
16	88	0	9	79
17	84	2	30	52
18	82	0	2	80
19	38	0	3	35
20	37	0	8	29
21	37	0	1	36
22	36	0	1	35
23	34	0	3	31
24	34	0	3	31

*No. of sera with end titres greater in the 56°C test than in the 37°C test
**No. of sera with end titres equal in the 56°C and the 37°C test
***No. of sera with lower end titres in the 56°C test than in the 37°C test

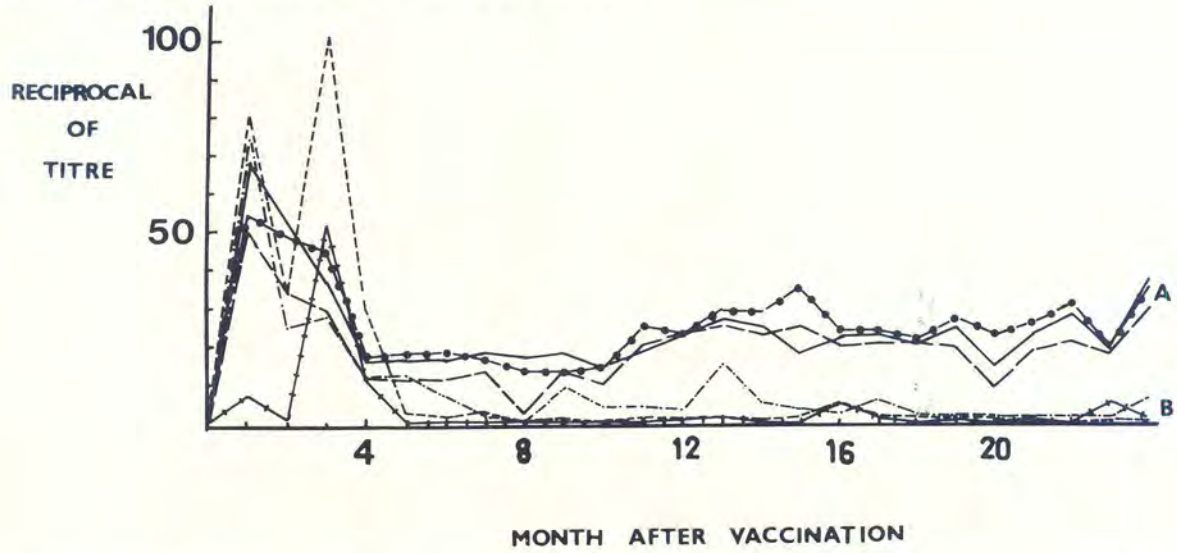

FIG. 1 Monthly geometric means of titres in - - - CF (*B. abortus* antigen), —+—+— CF (*B. ovis* antigen), — — — — 37°C Agglutination, ·—·—·—· 56°C Agglutination, ——— 5% NaCl agglutination and •—•—• Coombs tests in 100 sheep vaccinated as adults with Rev I.

after vaccination 64 of the 82 animals still in the experiment had titres of 1/20 or greater.

Comparison of agglutination tests done on the same sera at 56°C and 37°C showed that in the 1st month after vaccination the majority of sera had higher titres in the 56°C test than in the 37°C test. From 2 to 10 months after vaccination the majority of sera had the same titres in the two tests and from the 11th month after vaccination until the end of the experiment the titres were generally lower when incubated at 56°C. These results are summarized in Table 2.

In the Coombs test a fourfold increase in the agglutination titre after addition of the antiglobulin serum was regarded as indicating the significant amount of monovalent antibody. Of the 1 886 sera which were suitable for test (titres of 1/80 or less) 61 (3,2%) had fourfold Coombs titre increases.

The geometric mean of the CF titres rose sharply after vaccination but fell 2 months after vaccination and rose again to peak levels on the 3rd month. Thereafter the antibody titres fell rapidly and by the fifth month after vaccination most were again negative. During the initial antibody response *B. abortus* titres were higher than *B. ovis* titres. In the first 3 months *B. ovis* titres were higher than, equal to or less than *B. abortus* titres 9, 42 and 249 times respectively. As the serum antibody levels fell the difference between the end titres of sera tested with the two antigens became negligible. The CF titres at the test done 6 months after vaccination, which is the time at which differentiation between antibody titres resulting from vaccination and infection is usually attempted, are given in Table 3.

TABLE 3 CF titres of 98 sheep 6 months after vaccination with Rev 1

Antigen	<1/10	1/10	1/20	>1/20
B. ovis	87	11	—	—
B. abortus	78	16	1	3

All the CF titres for the period 6-24 months after vaccination are shown in Table 4.

Fewer serum antibody titres occurred in the period 6 to 24 months after vaccination in the CF test using *B. ovis* antigen than in the test with *B. abortus* antigen (Table 4) but less animals had CF titres to the *B.*

TABLE 4 CF titres of all tests done on sheep vaccinated with Rev 1, in the period 6 to 24 months after vaccination

Antigen	<1/10	1/10	1/20	>1/20
B. ovis	1 242	147	38	18
B. abortus	1 085	280	45	35

abortus antigen. In 61 animals the serum CF titres against *B. ovis* antigen remained below 1/20 from 6 months after vaccination until they were removed from the experiment and 36 had occasional titres of 1/20 or above. In the same period the CF titres remained below 1/20 to *B. abortus* antigen in 72 animals, 23 had occasional titres of 1/20 or over and three animals remained positive throughout. Two of the animals which remained positive for long periods were negative in all the tests before vaccination while one had a titre of 1/20 in the agglutination test although it was negative in both CF tests.

The effect of increased salt concentration on the occurrence of prozones could not be gauged as prozones did not occur in this investigation.

Studies on goats

We were unable to keep all the goats in the experiment for long periods and the numbers retained fell steadily. The reduction in numbers can be seen in Table 5 (Number of sera tested = number of animals still in the experiment). At the start of the investigation none of the goats had titres of more than 1/10 in the CF or agglutination tests. The monthly geometric means of the reciprocals of the titres for each serological test are shown in Fig. 2.

Serum agglutination titres were maintained at levels which would seriously interfere with the use of the test for diagnostic purposes for at least 2 years. Average agglutination titres were generally lower from 4 to 8 months after vaccination than they were in the second year. The trend was exaggerated by the fact that more of the animals with low titres were removed from the experiment at an early stage than animals with higher titres. This trend could, however, also be observed in a number of individual animals remaining in the experiment. In the last month of the experiment six animals had titres of greater than 1/20, six of 1/20 and two of 1/10.

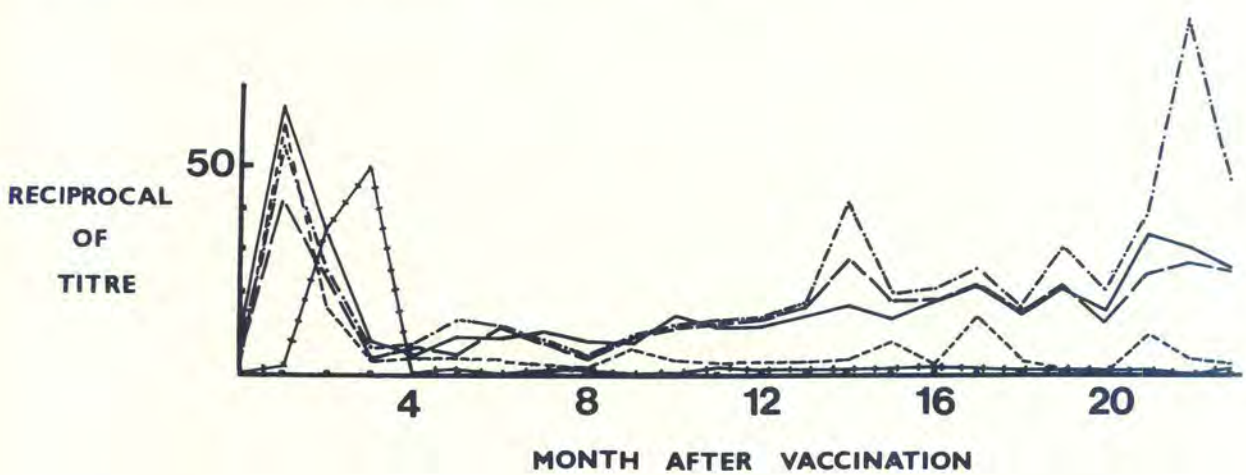

FIG. 2 Monthly geometric means of titres in —+—+— CF (*B. abortus* antigen), — — — 37°C Agglutination, - - - - 56°C Agglutination ——— 5% NaCl Agglutination and •—•—• Coombs test in 44 goats vaccinated as adults with Rev I.

TABLE 5 Comparison of serum agglutination tests incubated at 56°C and 37°C on sera from goats vaccinated with Rev 1

Month after vaccination	No. sera tested	56°C > 37°C*	56°C = 37°C**	56°C < 37°C***
1	44	23	19	2
2	44	4	20	20
3	41	2	32	7
4	39	8	17	14
5	35	1	21	13
6	35	0	16	19
7	35	0	11	24
8	35	1	24	10
9	33	1	23	9
10	33	0	10	23
11	33	0	9	24
12	32	2	8	22
13	30	0	7	23
14	27	0	2	25
15	26	0	14	12
16	19	0	2	17
17	18	0	12	6
18	18	0	2	16
19	15	0	1	14
20	15	0	3	12
21	15	0	5	10
22	15	0	0	15
23	14	0	1	13

*No. of sera with end titres greater in the 56°C test than in the 37°C test
**No. of sera with end titres equal in the 56°C and 37°C tests
***No. of sera with lower end titres in the 56°C test than in the 37°C test

As in the case of sheep there was a tendency for heat sensitivity of the agglutinins to increase with increasing time after vaccination. The monthly comparison of agglutination titres at 56°C and 37°C is given in Table 5.

The CF titres rose slowly after vaccination and peak titres were only reached on the 3rd month. Serum antibody levels then fell rapidly and generally remained at negative levels (CF titres less than 1/20) throughout the rest of the experiment. The serum of only one animal remained positive in the CF test for longer than six months after vaccination. This animal had serum antibody titres of more than 1/20 on 12 occasions and a titre of 1/20 once during the period 6-23 months after vaccination. In a total of 430 tests done on the sera of all the other animals during this period only seven titres of 1/20 were found.

In the Coombs test 22 sera out of 651 suitable sera tested showed fourfold increases in the agglutination titre after the addition of anti-goat gamma globulin. There was a sudden and unexplained increase in the number of positive Coombs titres in the 22nd month after vaccination when nine of the 15 animals tested had fourfold increments in the Coombs titre.

As in the case of the sheep the NaCl agglutination test was of no value as prozones did not occur.

DISCUSSION

The Joint FAO/WHO Expert Committee on Brucellosis (1971) has recommended that non-vaccinated sheep whose serum contains 40 IU of agglutinin be considered as reactors. We have considered sera causing 50% agglutination at a titre of 1/20 (32 IU) to be suspicious and sera containing 64 IU (titre 1/40) or more to be positive. The majority of sera in this experiment had titres of 1/20 or more for long periods. The agglutination test cannot therefore be used as a diagnostic test following Rev 1 vaccination in adult sheep. The position was similar in goats.

The CF titres (*B. abortus* antigen) rose rapidly after Rev 1 vaccination in both sheep and goats, but generally returned to negative levels (titres below 1/20) within five months of vaccination. In a few cases (3 sheep and 1 goat) CF titres (*B. abortus* antigen) remained positive for long periods. The reason for this is not known and detailed investigation of this type of case is indicated. In this regard the effect of revaccination is not known and although three of the four animals did not have any serum antibody titres at the time of vaccination the possibility of previous vaccination as young animals could not be excluded. Positive titres also developed in the CF test with *B. ovis* antigen following Rev 1 vaccination. In the initial antibody response these titres were clearly lower than the titres to *B. abortus* antigen indicating a closer serological relationship between Rev 1 (*B. melitensis*) and *B. abortus* than between Rev 1 and *B. ovis*. Titres fell to negative levels within five months. It should therefore generally be possible to distinguish between *B. ovis* infection and vaccination during the time of the initial antibody response, by testing with two antigens. As a general practice it would, however, be advisable not to do diagnostic serological tests within 6 months of vaccination. The effect of revaccination is not known and should be investigated.

In *B. ovis* infections the agglutination test and its modifications cannot be used for diagnostic tests because the rough *B. ovis* cultures are unsuitable as agglutinating antigens. The CF test is therefore the main diagnostic test used in South Africa for all forms of ovine brucellosis. In the case of *B. ovis* infections the CF test is probably very reliable. A number of authorities have successfully eliminated infection from flocks by culling animals with CF titres (Clapp, 1962; Ryan, 1964; Hughes & Clayton, 1968; Murray, 1969). In other forms of brucellosis the CF test is unfortunately not so reliable. According to the Joint FAO/WHO Expert Committee on Brucellosis (1971) the CF test will detect less than 70% of infected sheep "unless diagnostic criteria are adopted that classify as infected many animals known to be unexposed". The other serological tests are apparently not more reliable. Serological tests should therefore be used in sheep as flock tests and although clear positive titres are an indication of infection in individual sheep a negative test does not provide proof that an animal is not infected.

In our experiment the CF titres fell two months after vaccination and rose again at the third month (Fig. 1). This phenomenon remains unexplained. We do not believe that it was due to a technical error as the positive control sera gave their normal end point titre on these days.

Although the 56°C agglutination test showed some promise as a test for identifying vaccine reactors, there seems little justification for using the test when the superior results of the CF test are considered. The Coombs and salt agglutination tests did not show any particular advantage in this investigation but it is known that the Coombs test is a useful test for diagnosis of infection in sheep. (Joint F.A.O./WHO Expert Committee on Brucellosis, 1971).

The CF test should remain the basic test for diagnosis of brucellosis in sheep and goats where Rev 1 vaccination has been used. In non-vaccinated flocks the agglutination test may also be used for the diagnosis of *B. abortus* and *B. melitensis* infection.

Summary

The serological titres, as measured by agglutination at 56°C and 37°C, and in 5% NaCl, Coombs tests and complement fixation (CF) with *Brucella abortus* and *Brucella ovis* antigens, in Rev 1 vaccinated sheep and goats were followed for a period of two years.

Agglutination titres remained at close to diagnostic levels in the majority of sheep and goats throughout the experiment but CF titres fell to negative levels within 5 to 6 months. Rev 1 vaccine induced CF titres to both *B. abortus* adn *B. ovis* antigens, but the titres were higher with the *B. abortus* antigen. Other tests were shown to be less useful than the CF test for distinguishing vaccine titres from infection.

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