

A SEROLOGICAL INVESTIGATION ON ADULT CATTLE VACCINATED WITH *BRUCELLA ABORTUS* STRAIN 19

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ABSTRACT

WORTHINGTON, R. W., MÜLDERS MARIA, S. G., McFARLANE, I. S. & BECKER, DAPHNE. A serological investigation on adult cattle vaccinated with *Brucella abortus* strain 19. *Onderstepoort J. vet. Res.* 40(1), 7-12 (1973)

Serum antibody titres were followed for a period of two years in 99 beef and 29 dairy cows which had been vaccinated as adults with *B. abortus* strain 19. Agglutination titres remained above diagnostic levels throughout but complement fixation, mercaptoethanol agglutination and rivanol agglutination titres generally returned to negative levels within 6 months. Coombs tests and agglutination at 56° proved to be of limited value for recognising antibody titres caused by vaccination.

INTRODUCTION

In the control of brucellosis in cattle Strain 19 (S19) vaccine is frequently used but it has the disadvantage that it causes many non-infected animals to react positively to the serum agglutination test thus affecting the diagnostic value of the test. In order to overcome this difficulty vaccination is generally restricted to heifer calves 4 to 8 months old. Vaccinated calves become negative to the agglutination test relatively quickly whereas vaccinated adult cattle retain their agglutination titres much longer. The importance of restricting vaccination to young calves has therefore often been stressed (Kuttler, 1954; Morgan, 1964, 1970; Fichandler, 1967; Manthei, 1968). Vaccination of young calves has also been shown to be as effective as vaccination of adult cattle and there is no evidence that re-vaccination serves any useful purpose (McDiarmid, 1957; Gregory, 1958; Morgan, 1964, 1970; Manthei, 1968).

In South Africa a calfhood vaccination scheme has been started and the vaccination of adult cattle is forbidden except under special circumstances where permission has been granted by a State Veterinarian. Vaccination cannot be relied upon to completely eliminate brucellosis. According to Manthei (as quoted by Morgan, 1964) vaccination of calves reduces the overall incidence of infection by 80% and the number of infected herds by 20%.

Eradication of the disease is therefore dependent on the elimination of all infected animals, a step which will have to be started after calfhood vaccination has been in progress for a number of years. Because South African farmers have been using S19 vaccine in adult cows for many years and emergency vaccination of adults will still have to be used during outbreaks of abortion, titres resulting from vaccination will cloud the interpretation of sero-diagnostic tests for a long time. Two methods of combating this problem are presently available - the use of non-agglutinogenic vaccines in place of S19 vaccine in adult cattle and the use of serological tests capable of distinguishing between natural infection and antibody titres due to vaccination. The former method cannot be discussed in this article and the interested reader is referred to the work of McDiarmid (1962), Roerink (1966, 1967a, 1967b, 1969), Cunningham (1966, 1967, 1968) and Morgan & McDiarmid (1968).

Traditionally the agglutination test has been the main test used for the diagnosis of brucellosis because it is easy to perform in great numbers and has been well

standardized (Stableforth, 1954). The complement fixation (CF) test is, however, much more useful for distinguishing titres due to vaccination from those of natural infection. Hill (1963a, 1963b), MacKinnon (1963), Burki (1963), Van Waveren (1965) and many others have confirmed the original observations of the Russian workers (see Morgan, 1964) that CF titres soon become negative in vaccinated cattle whereas they remain high in infected animals. The CF test has the additional advantage that CF titres are often positive in chronically infected animals which react negatively to the agglutination test.

A number of different classes of antibodies are known to exist and it is important to note that *Brucella* agglutinins may be either of the high molecular mass (19S) IgM type, or of the conventional (7S) IgG type (Morgan, 1967, 1970). In *Brucella* infection both IgM and IgG are produced but IgM levels fall much more rapidly and later IgG is the predominant type of antibody. In the case of animals vaccinated with S19 both types are produced but IgG antibody levels decline more rapidly than IgM. The different pattern of antibody production in vaccination and in infection makes available a number of methods of distinguishing antibody titres due to vaccination from those of infection. IgM antibodies are more easily inactivated by heat, low pH and thiol reagents such as mercaptoethanol (ME) than IgG antibodies. Sera which are positive in conventional agglutination tests but show negative or reduced titres in the presence of ME or at high temperature or low pH are therefore more likely to be due to vaccination (Morgan, 1967, 1970). Rivanol is a reagent which precipitates most serum proteins including IgM but does not precipitate IgG. If the supernatant fluid from a serum precipitated by Rivanol retains its agglutinins it is assumed that they are predominantly IgG in nature and are therefore due to natural *Brucella* infection (Morgan, 1967, 1970).

The Coombs anti-globulin test demonstrates the presence of monovalent antibodies which would not be shown up by any of the other modifications of the agglutination test (Hill, 1963a; Morgan, 1964, 1967) It will thus identify chronic cases which are producing only monovalent antibodies and which would not be recognized by the above-mentioned tests (with the exception of the CF test). The test has also been used to distinguish between antibodies from vaccination and natural infection since it had been found that in infected

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animals the Coombs titre is generally higher than the agglutination titre whereas in the vaccinated animal this is not the case (Hill, 1963a). The presence of antibodies in the udder generally indicates udder infection which seldom occurs with vaccination. The milk ring test (Van Drimmelen, 1950, 1951; Ferguson & Robertson, 1960; Hill, 1963a), and the whey agglutination test (Biberstein, Cameron & Meyer, 1961) are sometimes used to distinguish vaccinated from naturally infected animals.

The problem of brucellosis diagnosis has been a great challenge to the ingenuity of serologists and probably a greater number of techniques have been developed for the diagnosis of brucellosis than for any other animal disease. Because of the vast literature on the subject no attempt has been made in the above discussion to review these methods and the reader is referred to the reviews of Hill (1963a), Morgan (1964, 1967, 1970) and Manthei (1964, 1968).

In the present investigation we have studied the use of a number of serological tests in adult vaccinated cattle so as to assess their usefulness and to determine the duration of titres in cattle vaccinated with S19 as adults.

METHODS

Experimental animals

S19 vaccine (dose 100×10^9 viable organisms) was used to vaccinate 99 pregnant Afrikaner and Afrikaner cross cows maintained under ranching conditions and 29 dairy cows. The daily milk production and rectal temperature of the dairy cows were recorded for a period of three weeks following vaccination. Records were kept of the stage of pregnancy at vaccination and of the subsequent calving dates. All animals were bled at the time of vaccination and at monthly intervals thereafter for a period of two years. Agglutination at 37°C and 56°C, CF, ME agglutination (ME test), Rivanol agglutination (Rivanol test) and Coombs tests were performed on the sera.

Serological methods

The agglutination test antigen was standardized against the South African national anti-*B. abortus* serum which contains 800 IU of agglutinin per vial. The antigen density used in the tests was adjusted so that 50% agglutination occurred in a serum antigen mixture (equal parts) containing a final concentration of 1.6 IU per ml. The agglutination test was performed by adding 1 ml of antigen to 1 ml of serially diluted serum. Agglutination tests were set up in duplicate and incubated at 37°C and 56°C for 24 hours. All tubes not showing complete agglutination at 37°C were submitted to the Coombs test. This test was performed by resuspending the antigen cells, which had been sedimented by centrifugation, in anti-bovine gamma globulin at a suitable concentration, and incubating for a further 24 hours. Rivanol and ME tests were done according to methods of W. J. B. Morgan, Central Veterinary Laboratory, Weybridge England (Personal Communication 1968). In the Rivanol test 1.5 ml of 0.4% Rivanol was added to 0.5 ml of serum and allowed to stand for 20 min. A small amount of activated charcoal was then added to each tube and after centrifugation the clear supernatant fluid was used in an agglutination test as described above. The ME test was carried out by adding 0.8 ml of saline and 1 ml of 0.2 M mercapto-ethanol solution to 0.2 ml of serum and incubating at 37°C for 1 hour. Serial dilutions were made from this tube and agglutination tests performed as described above. In the ME

test doubling dilutions of serum were tested from 1/20 to 1/320; in the other tests serum dilutions tested were 1/10 to 1/320.

The CF tests were done as previously described (Worthington & Mülders, 1969). In this test the South African National anti-*B. abortus* serum has a titre of between 1/64 and 1/128 and in vaccinated animals a titre of 1/4 is regarded as suspicious and 1/8 as positive. It is normal to refer to CF antibody titres in terms of the serum dilution used in the test and not in terms of the final dilution of the serum when all the other reagents are added. In this case we have, however, used the latter method as the results are then directly comparable to the agglutination test for statistical purpose, i.e. serum dilutions of 1/2, 1/4, 1/8 etc. are multiplied by the further dilution factor of 5 and become 1/10, 1/20, 1/40 etc.

Statistical methods

The reciprocals of the end titres of every serological test done on each animal were punched on computer cards and statistical analyses performed by standard methods (Snedecor, 1956). The arithmetic means and variances, and the geometric means and variances of the reciprocals of the end titres were calculated for each month. Correlation coefficients were calculated between all possible pairs of tests for each month of the experiment.

RESULTS

Although the herd was believed to be free of brucellosis when the experiment was begun it was found that one cow had a CF titre of 1/160 and an agglutination titre of 1/320 (512 IU). The cow subsequently aborted and remained strongly positive to all the serological tests throughout the duration of the experiment. Results of tests done on this cow were excluded from the final analysis. Of the remaining 127 animals one had a titre of 1/80 (128 IU) six of 1/40 (64 IU) and 26 of 1/20 (32 IU) to the agglutination test and all except one which had a titre of 1/20 were negative to the CF test. These animals were retained in the experiment as they were considered to be non-specific reactors or reactors due to previous vaccination and not naturally infected cows. Vaccination was not practised in the herd but some of the older animals introduced into the herd could possibly have been vaccinated before introduction. Careful scrutiny of the records of the herd over many years revealed that the herd had an exceptionally high calving rate and that abortions were rare.

Twenty-two cows were in milk at the time of vaccination and two calved within six days of vaccination. No measurable change in milk production was seen in the 21 days following vaccination in 23 of the 24 milking cows. The remaining animal lost her milk entirely on the eighth day but returned to normal on the following day. A slight rise in temperature was seen in thirteen cows which persisted from one to six days with maximum temperatures between 38.8 and 39.9°C. All 99 of the beef cows and 18 of the 29 dairy cows were in calf at the time of vaccination. The period of gestation varied from 65 days to 173 days in the beef cows and from 75 days to 280 days in the dairy cattle. Three of the beef cows, including the one infected cow already mentioned, aborted and one calf was born dead at full term. One abortion and one death at full term occurred in the dairy herd. As the foetuses were not made available for examination it could not be determined if the abortions were due to S19.

The geometric means were regarded as the true average because the reciprocals of the titres are a geometric series. The geometric means of the titres of the various tests are illustrated graphically for each month for a period of two years in Fig. 1 and 2. It can

be seen that the results were essentially similar in the beef cattle and the dairy cattle.

Vaccination of adult cattle clearly induced agglutination titres which remained well above the diagnostic level for a period of at least two years (Fig. 1). At the

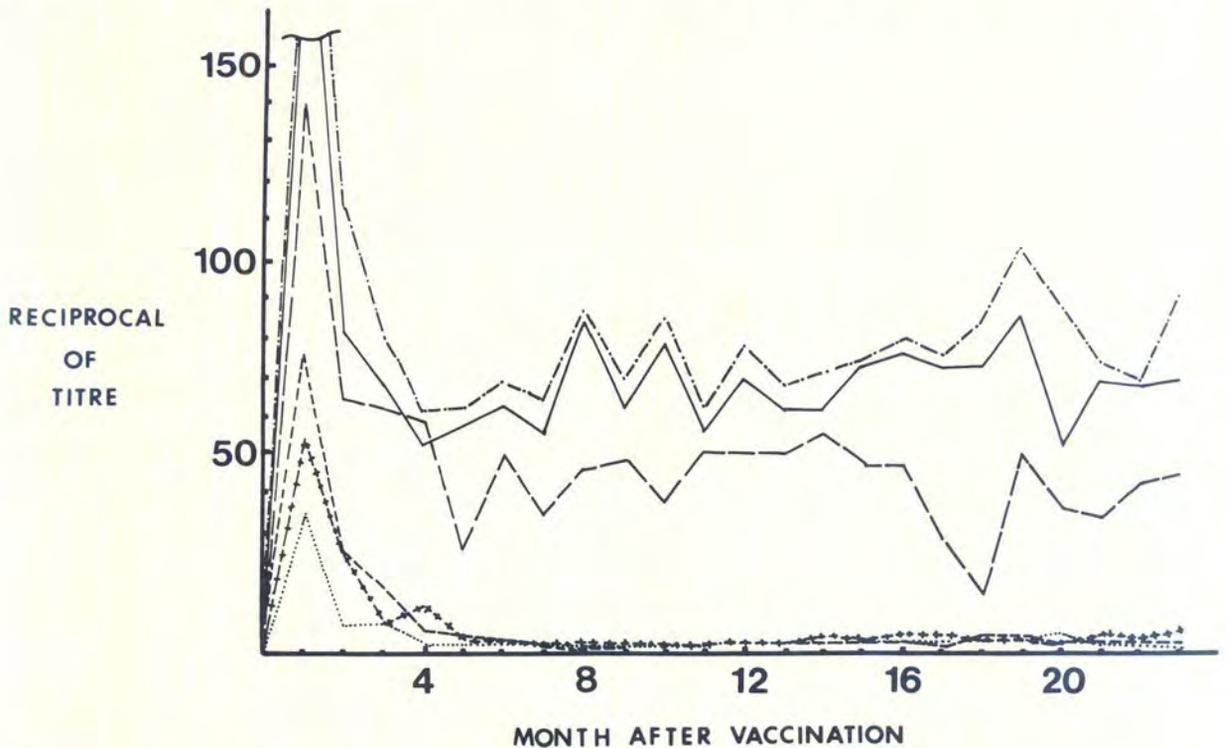


FIG. 1 Monthly geometric means of titres in ——— 37°C Agglutination, — — — 56°C Agglutination, — · — · — Coombs, · · · · · ME, + + + Rivanol and . . . CF tests in 98 beef cows vaccinated as adults with S19

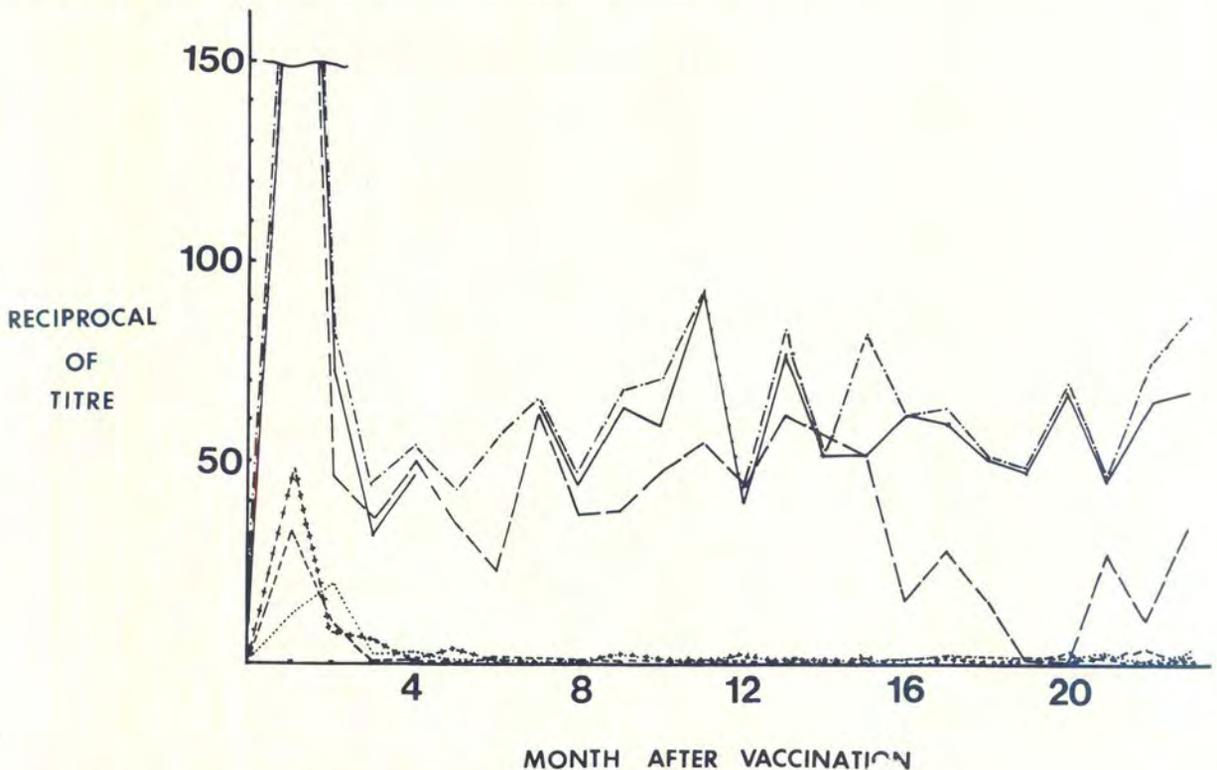


FIG. 2 Monthly geometric means of titres in ——— 37°C Agglutination, — — — 56°C Agglutination, — · — · — Coombs, · · · · · Me, + + + Rivanol and . . . CF tests in 29 dairy cows vaccinated as adults with S19

conclusion of the experiment the number of cattle had been reduced by deaths and culling to 105. At the test done 23 months after vaccination 96 had titres of 1/40 (64 IU) or more, 8 had titres of 1/20 (32 IU) and only one a titre of 1/10 (16 IU).

The results of agglutination tests incubated at 56°C and 37°C which were done on 2 650 sera were compared. In 151 sera the antibody titres were higher when incubated at 56°C, in 1 255 sera they were the same at both temperatures and in 1 244 higher when incubated at 37°C. Differences in titre in the same serum in these tests seldom varied by more than one step.

Sera which had titres of less than 1/320 in the agglutination test were tested by the Coombs test. Of 1 895 sera with agglutination titres of 1/80 or less only 16 showed an increase of titre of two steps or more after the addition of anti-bovine gamma globulin. Sera of vaccinated animals seldom showed any increase of titre if incubated at 56°C and seldom contained significant amounts of incomplete antibody.

The results of CF, ME and Rivanol tests closely paralleled each other (Fig. 1 and 2). Correlation coefficients between the reciprocals of the serum antibody titres in the CF and ME tests, CF and Rivanol tests and ME and Rivanol tests, in the beef cattle, were significant at the 1% level in every case except for the CF - ME and CF - Rivanol tests at 22 months after vaccination. In the dairy cows the numbers involved were smaller and because many of the cattle were negative in all three tests, after the initial antibody response, many results fell on the same point of the regression line and correlations were therefore not meaningful. During the initial antibody response correlation between the three tests was good.

The results of tests done on sera collected six months after vaccination from the 124 animals still left in the experiment at this stage are summarized in Table 1.

TABLE 1 Results of CF, ME and Rivanol tests done on sera from 124 adult cattle 6 months after vaccination with S19

Test	Titre		
	≤1/10	1/20	≥1/40
CF test	111	9	4
ME test	93	17	14
Riv test	107	10	7

Although the CF titres usually remained consistently negative after they had fallen to negative levels, positive or suspicious titres still occurred at irregular and unpredictable intervals in some animals. In the period from 6 to 23 months after vaccination 75.6% of the cattle remained consistently negative throughout, 7.9% occasionally had suspicious antibody titres (up to four times) or single positive titres and 16.5% had suspicious titres occurring five times or more, or positive titres on more than one occasion.

DISCUSSION

The herd used in this experiment was not a *Brucella*-free herd, although the incidence of infection was very low. One case of brucellosis was demonstrated among the 128 animals at the start of the experiment. The initial occurrence of some suspicious and positive titres to the agglutination test (all CF negative) indicated that some non-specific sensitization was also present or previous S19 vaccination had occurred in the herd. It

was subsequently found that a few of the animals had been introduced to the herd a number of years previously which may have been previously vaccinated. The breeding performance of the herd remained exceptionally good throughout the experiment.

Vaccination of pregnant and lactating cows did not interfere unduly with milk production or cause many abortions. Previous experience in the field and the view of veterinarians who have used this procedure tend to support this view. The procedure could therefore be used on pregnant or milking cows during an emergency (abortion storm) to try to arrest the spread of the disease. It must, however, be realized that in cases where cattle have previously been vaccinated during calthood re-vaccination is unlikely to be of much benefit (McDiarmid, 1957; Gregory, 1958; Morgan, 1964, 1970 and Manthei, 1968). Where cattle are unvaccinated or the vaccination methods are suspected of being inadequate (expired vaccine, exposure to hot sun, etc.) vaccination with S19 may be justified. In all cases where vaccination of adults is considered the cattle should be bled for the purpose of identifying naturally infected animals before vaccination is undertaken.

Vaccination of adult cattle induced persisting titres to the agglutination test and this test would clearly become unusable for diagnostic purposes for many years after vaccination of adult animals.

The Coombs test and the 56°C agglutination test showed little promise as tests which could be used to distinguish antibody titres due to vaccination from those of natural infection. Our results indicate that a serum which has a higher titre at 56°C than at 37°C and which has demonstrable incomplete antibody (Coombs titre) would not be typical of a vaccinated animal and would therefore indicate natural infection. In general the distinction was not clear-cut and in view of the great promise shown by the CF, ME and Rivanol tests the use of Coombs and 56°C agglutination tests for this purpose cannot be justified.

The CF, ME and Rivanol antibody titres fall rapidly after vaccination and within 5 months most animals were negative to these tests (Table 1). There was also a good correlation between the results with these three tests which seems to indicate that the technically simpler ME or Rivanol tests could be used instead of the CF test in future large scale investigations. It will, however, be necessary to investigate the use of these tests on large numbers of animals infected with virulent *Brucella* strains before seriously considering this possibility.

The CF test has been widely claimed to be of great use for distinguishing titres due to calthood vaccination from infection. In this investigation it was shown that the test can serve a similar purpose in cattle vaccinated as adults. In this experiment about 90% of the cattle could have been identified as vaccine reactors on the basis of CF tests done 6 months after vaccination. Repeated testing of the cattle over the 2 year period showed that in 25% of the cattle low fluctuating titres still occurred at unpredictable intervals. These tests were done on cattle which were vaccinated once only (with some possible exceptions as mentioned previously). The interpretation might be more difficult in cattle which have been revaccinated. At this point it is of interest to mention that vaccination of S19 vaccinated cattle with 45/20 adjuvant vaccine results in persisting CF titres (Morgan & McDiarmid, 1968). Despite the promising results obtained in this investigation with the CF test it is still clear that as far as possible vaccina-

tion should be restricted to heifer calves and no revaccination be allowed except under exceptional circumstances.

CONCLUSIONS

1. Vaccination of pregnant and lactating cows did not cause a drop in milk production and abortions were rare following this procedure
2. Most animals developed agglutination titres which persisted at levels above 64 IU for at least two years.
3. The CF, ME and Rivanol titres fell rapidly and were generally negative by six months after vaccination.

SUMMARY

Serological tests were carried out over a period of 2 years, on 99 beef and 29 dairy cattle vaccinated as adults with Strain 19. Vaccination had little adverse effect on lactating and pregnant cows. Agglutination titres remained at above diagnostic levels for at least 2 years. Complement fixation, mercaptoethanol and Rivanol titres generally returned to negative levels within 6 months of vaccination. Coombs tests and agglutination tests at 56°C showed limited promise as useful tests for distinguishing vaccination titres from titres due to infection.

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