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The serological response of cattle to vaccines against brucellosis, as measured by the brucellosis radioimmunoassay and other tests

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SUMMARY

Serum samples were obtained from 281 heifers vaccinated with *Brucella abortus* strain 19, and from 50 heifers that had received two injections of killed *B. abortus* strain 45/20 adjuvant (K45/20A) vaccine. The serological response measured by the brucellosis radioimmunoassay (RIA) was compared with responses measured by other tests.

The serological responses of cattle during the first weeks after strain 19 vaccination were found to give little guide to the frequency of persistent reactions.

In the case of strain 19, persistent reactions were considered to be those occurring 12 or more months after vaccination. In heifers vaccinated at the recommended age, small numbers of persistent reactions were given by the RIA (four in 374 sera), the complement fixation test using warm fixation (CFTW) (six in 383) and cold fixation (one in 185), the serum agglutination test (two in 222) and the indirect haemolysis test (IHLT) (two in 369). The Rose Bengal plate test gave 74 persistent reactions in 374 sera.

Five of the 50 heifers gave particularly prolonged responses to K45/20A vaccine. In these animals the RIA and IHLT remained positive for longer than the CFTW.

INTRODUCTION

Live strain 19 and killed strain 45/20 adjuvant (K45/20A) vaccines have commonly been used to vaccinate cattle against *B. abortus* (Alton, 1978). There was a diagnostic advantage in vaccinating with strain 45/20 when the serum agglutination test (SAT) was the main routine diagnostic test, as K45/20A vaccines induce little agglutinating antibody irrespective of the age of the animal at vaccination. The situation has changed since the complement fixation test (CFT) replaced the SAT as the definitive diagnostic procedure. K45/20A vaccine can induce persistent non-agglutinating antibody active in the CFT. Whilst the use of strain 19 has largely been confined to calves less than eight months old to minimize the problem of persistent antibody, vaccination of older cattle can be acceptable if the dose is reduced or a different route of administration is used (Alton, 1978).

Vaccination of cattle can reduce the spread of brucellosis in an area but test and

slaughter is necessary for the eradication of the disease. Vaccination adversely affects progress towards eradication in two ways. Firstly, it can inhibit the antibody response to infection, producing false negative reactors (Hayes & Chappel, 1981). Secondly, even when vaccination is performed under recommended conditions, a small proportion of cattle show false positive reactions due to persistent antibody. Such effects become particularly important late in an eradication programme when the incidence of the disease is low, and when problem herds which resist eradication usually appear. At this stage of eradication new approaches may be needed, and anamnestic testing with K45/20A vaccine may be valuable (Cunningham & O'Connor, 1971; Reid & Harvey, 1972).

The brucellosis radioimmunoassay (RIA) (Chappel *et al.* 1981) is a sensitive serological test which may be useful in the eradication of brucellosis from problem herds. In this study the liability of the RIA to false positive reactions due to vaccination was assessed using sera from heifers that had received strain 19 or K45/20A vaccine.

MATERIALS AND METHODS

Sera

Sera were obtained from 230 heifers in 14 Victorian commercial dairy herds believed to be free from brucellosis. The heifers were vaccinated subcutaneously with 4×10^{10} organisms of *B. abortus* strain 19 (Commonwealth Serum Laboratories, Melbourne) between 3 and 8 months of age. Blood samples were collected for up to 27 months after vaccination, at intervals of 1 month or more.

Sera were also obtained from 101 mixed-breed beef heifers on research stations belonging to the Western Australian Department of Agriculture. All were from serologically negative dams and the research stations were free from brucellosis. Fifty-one of these heifers were vaccinated as above with strain 19, 24 at about 6 months of age and 27 at about 15 months. The other 50 received two subcutaneous doses of K45/20A vaccine (Duphavac Batch VO57, Philips Duphar Pty Ltd, North Sydney) 6 to 7 weeks apart. Of these, 24 were first vaccinated at about 8 months of age and 26 at about 15 months. Blood samples were collected for up to 23 months after vaccination, at intervals of 1 week or more, and despatched to our laboratory at regular intervals for RIA testing. Dr S. S. Sutherland kindly provided us with sera and the results of the CFTW, the RBPT and the IHLT.

Serological tests

The RIA was performed as described by Chappel *et al.* (1981). The minimum diagnostic value was taken as 5 u.

The RBPT was performed as described by Allan *et al.* (1976).

The SAT was performed as described by Alton, Jones & Pietz (1975) who describe it as the European tube agglutination test. The minimum diagnostic value was taken as 100 i.u./ml.

The CFTW and CFTC were performed by a micro-method (Anon., 1977), cold fixation being overnight at 4 °C, and the IHLT was as described by Plackett, Cottew & Best (1976). Results were expressed as the reciprocal of the highest dilution at which a 50% reaction occurred. The minimum diagnostic value was taken as 4 for the CFTW and 8 for the CFTC and the IHLT.

Table 1. *Response of cattle to strain* 19 *vaccination*

(*a*) Response of 254 heifers to vaccination between 3 and 8 months of age. No animal is represented more than once in any time interval

	Weeks after vaccination						
	4–7	13–15	21–29	32–44	48–58	68–78	85–94
	Number of reactor animals (number of animals tested)						
RIA	64(182)	23(150)	18(194)	11(197)	1(183)	2(63)	1(65)
RBPT	178(183)	129(166)	67(196)	42(197)	23(182)	11(64)	8(65)
CFTW	171(183)	82(165)	25(194)	7(197)	1(179)	0(64)	0(66)
CFTC	150(158)	72(142)	24(173)	12(174)	4(127)	0(54)	0(50)
IHLT	6(180)	3(149)	4(194)	7(174)	0(180)	1(63)	1(60)
SAT	108(142)	17(141)	10(153)	11(174)	6(159)	2(54)	0(55)

(*b*) Response to vaccination between 3 and 8 months of age in 14 Victorian herds

	Weeks after vaccination						
	4–7	13–15	21–29	32–44	48–58	68–78	85–94
	Number of herds with one or more reactor (number of herds tested)						
RIA	8(11)	5(9)	7(11)	4(12)	1(11)	1(5)	0(7)
RBPT	11(11)	10(10)	7(11)	7(12)	5(11)	1(5)	3(7)
CFTW	11(11)	8(10)	6(11)	2(12)	0(10)	0(5)	0(7)
CFTC	11(11)	8(10)	6(11)	6(12)	1(9)	0(5)	0(6)
IHLT	2(11)	0(9)	2(11)	3(11)	0(11)	0(5)	0(6)
SAT	10(10)	5(10)	3(10)	4(12)	1(11)	1(5)	0(7)

(*c*) Response of 27 heifers to vaccination at about 15 months of age

	Weeks after vaccination					
	5	10	22	34	55	63
	Number of reactor animals (number of animals tested)					
RIA	22(25)	16(27)	7(25)	4(12)	3(15)	2(15)
RBPT	27(27)	27(27)	10(14)	13(15)	12(15)	13(15)
CFTW	27(27)	27(27)	7(26)	1(15)	0(15)	0(15)
IHLT	16(26)	14(26)	2(26)	0(15)	0(15)	0(15)

RESULTS

Strain 19 *vaccine*

Table 1*a* shows the response to strain 19 vaccine in heifers between 3 and 8 months of age, which is the recommended time for vaccination (Alton *et al.* 1975). The results for Victorian and Western Australian heifers were similar and have been combined. Almost all animals became positive to the RBPT, CFTW, CFTC and SAT, less than half became RIA-positive, and few became positive to the IHLT. The RBPT gave the most prolonged responses. Table 1*b* shows the numbers of Victorian herds with one or more reactors among these heifers.

The more limited data for animals vaccinated at about 15 months of age generally showed higher proportions of reactors (Table 1*c*).

One heifer, negative to all tests 9 months after vaccination at 23 weeks of age, became positive to the RIA, IHLT and RBPT several months later (Table 2). She was pregnant when the secondary rise occurred. The herd has been monitored as

Table 2. *Serology of a heifer with an unusual response to strain* 19 *vaccination*

(The animal was vaccinated at 23 weeks of age. She calved 101 weeks after vaccination.)

		Weeks after vaccination						
	pre-vaccination	4	26	39	55	68	84	121
RIA (units)	0	3	8	2	0	10	9	4
RBPT	0	2	1	0	0	1	0	1
CFTW	0	4	4	0	ND	0	0	0
CFTC	0	4	4	0	ND	0	0	0
IHLT	0	0	8	0	0	8	8	0
SAT(i.u./ml)	<34	47	40	<34	34	<34	40	<34

ND = not done.

Table 3. *Persistent reactions in heifers, vaccinated between* 3 *and* 8 *months of age,* 12 *to* 27 *months later*

(*a*) Number of heifers giving one or more positive reaction (number of heifers tested)

RIA	3(199)
RBPT	31(196)
CFTW	2(198)
IHLT	1(198)
CFTC	1(140)
SAT	2(175)

(*b*) Number of positive sera (number of sera tested)

RIA	4(374)
RBPT	74(374)
CFTW	6(383)
IHLT	2(369)
CFTC	1(185)
SAT	2(222)

part of a national eradication programme since before the heifer was born, and is believed to have been free from brucellosis throughout this period.

Persistent reactions to strain 19 vaccination were considered for the purposes of this study to be those occurring 12 or more months after vaccination. Late vaccination is known to increase the persistence of serum antibody (Lambert *et al.* 1961). Serum samples collected after 12 months or more were available from 15 of the 27 heifers vaccinated at about 15 months of age. Persistent reactions were given in 14 of these animals by the RBPT, in five by the RIA, in a different five by the CFTW, and in none by the IHLT.

The numbers of heifers and sera that showed persistent reactions after vaccination at the recommended age are shown in Table 3. The RBPT gave by far the most persistent reactions but each of the other tests used was positive in one or more cases. The reactions of the RIA, CFTW, CFTC and SAT were all in different animals.

K45/20A vaccine

Neither the RIA nor the IHLT became positive in any heifer after the first dose of vaccine (Table 4). Two animals reached 4 u in the RIA but most sera gave values

Table 4. *Serological response to a single dose of* K45/20A *vaccine*

(The numbers of heifers that reached the minimum diagnostic value at any time during the 6 weeks after the first dose of vaccine are shown)

Age at first injection (months)	Total number of heifers	Number of heifers positive to			
		RIA	RBPT	CFTW	IHLT
8	24	0	2	1	0
15	26	0	5	6	0
Total	50	0	7	7	0

Table 5. *Serological response to two doses of* K45/20A *vaccine*

(The numbers of heifers that reached the minimum diagnostic value at any time during the 6 weeks after the second dose of vaccine are shown.)

Age at first vaccination (months)	Total number of heifers	Number of heifers positive to			
		RIA	RBPT	CFTW	IHLT
8	24	15	18	17	10
15	26	5	11	12	7
Total	50	20	29	29	17

of 0 to 2 u. The CFTW and the RBPT each became positive in seven of the 50 heifers, both becoming positive in four. The highest CFTW titre reached was 32.

During the 6 weeks following the second dose of vaccine, more heifers became positive to the CFTW and the RBPT than to the RIA and IHLT (Table 5). There were less positive reactions in the group of heifers first vaccinated at 15 months. All RIA reactions were less than 20 u. and all CFTW and IHLT titres were 32 or less.

The serological response of most heifers was largely confined to 6 to 8 weeks after the second dose, but five heifers gave responses that persisted much longer. Overall in these five animals the RIA and the IHLT stayed positive much longer than the CFTW (Table 6).

DISCUSSION

This study illustrates the fact that the serological responses of a group of cattle during the first weeks after vaccination cannot reliably be used to predict the frequency of false positive reactions that interfere with subsequent diagnosis. A minority of individual animals cause diagnostic difficulties. The response to vaccination is complicated by the fact that there is progressive change not only in the concentration of serum antibody, but also in its antigen specificity, isotype composition and avidity. These characteristics of the antibody response are likely to vary from animal to animal, and to affect the relative sensitivities of different serological tests.

The RIA, CFTW, CFTC, SAT and IHLT all gave small numbers of persistent reactions, approximately 0·5% to 1·5%, after strain 19 vaccination at the recommended age, compared with 20% given by the RBPT. No test was free from

Table 6. *Serological responses of five heifers that reacted strongly to two doses of* K45/20A *vaccine*

	RIA			CFTW			IHLT			RBPT		
Heifer	93	97	98	93	97	98	93	97	98	93	97	98
	Vaccinated at about 8 months of age											
7*	8	5	9	—	—	8	8	–	32	–	–	2
11	NT	4	15	4	—	4	16	—	16	1	—	—
15	22	1	11	4	—	4	—	—	—	—	—	—
20	16	5	15	—	—	—	16	8	16	—	—	—
24	7	10	20	4	—	4	NT	NT	NT	—	—	—
28	NT	NT	NT	—	—	4	16	8	8	—	—	—
32	4	10	23	—	—	—	8	8	16	—	—	—
36	0	0	3	—	—	—	—	8	16	—	—	—
40	3	5	10	—	—	—	—	8	16	—	—	—
44	3	5	5	—	—	—	—	—	—	NT	NT	NT

	RIA		CFTW		IHLT		RBPT	
Heifer	105	109	105	109	105	109	105	109
	Vaccinated at about 15 months of age							
8*	18	5	4	8	NT	16	—	—
12	30	8	4	4	16	32	—	—
16	26	5	—	—	32	16	—	—
20	15	5	—	NT	16	—	—	—
24	13	5	—	—	32	8	—	—
28	14	5	—	—	16	8	—	—
32	15	5	—	—	16	—	—	—
36	12	5	—	—	16	—	—	—
40	12	2	—	—	—	—	—	—
44	5	NT	—	—	—	—	—	—
49	7	3	—	—	8	—	—	—
53	5	2	—	—	8	—	—	—

NT = not tested.
* Weeks since 2nd injection

persistent reactions nor, except in the case of the RBPT, were the numbers of such reactions very different. The initial IHLT response to strain 19 was low compared with other tests, in agreement with the findings of Plackett *et al.* (1976) and Plackett *et al.* (1980). Nevertheless the IHLT gave two persistent reactions, both in the heifer whose serology is shown in Table 2. Plackett *et al.* (1980) reported that 0·5 % of calves showed persistent IHLT reactions after strain 19 vaccination at the recommended age.

It is not clear why some cattle produce sufficient antibody to give positive serological reactions 12 months or more after strain 19 vaccination. Persistent infection with strain 19 after calfhood vaccination is apparently very rare (Meyer & Nelson, 1969; Cordes & Carter, 1979). Non-living antigen might remain in the body for different periods, and there may be other reasons why individual cattle respond differently to a similar antigenic stimulus. Late vaccination is a well-recognized factor (Lambert *et al.* 1961; Alton *et al.* 1975) but this does not in itself provide an explanation. It has been suggested that virulent *B. abortus* itself, or other micro-organisms bearing a degree of antigenic similarity, could induce weak

anamnestic responses in vaccinated cattle in the absence of infection (Morse, Schneider & McNutt, 1955). A non-specific stimulus to the immune system might have a similar effect of elevating serum antibody to detectable levels. The heifer whose serology is shown in Table 2 showed a small secondary rise in serum antibody although no second exposure to *B. abortus* antigen was suspected.

It could be valuable to know the immunoglobulin isotypes and antigen specificities of antibody responsible for persistent vaccination reactions, as this could help to explain the susceptibility of different serological tests to vaccination-induced false positive reactions. The isotype, the antigen specificity and the avidity of antibody associated with persistent vaccination reactions are likely to vary not only with the serological test concerned, but also with the circumstances leading to the production of the antibody in the particular animal. The nature of the antibody involved in persistent reactions to strain 19 seems to have received only isolated attention (Corbel, 1972; Patterson, Deyoe & Stone, 1976).

It has been suggested that IgM antibody is responsible for persistent strain 19 vaccination reactions, at least in the case of agglutination tests (Elberg, 1973; Alton, 1978). However the studies on which these conclusions are apparently based have involved only small groups of animals, followed for a few weeks or months after vaccination, with no direct examination of persistent antibody (Rose & Roepke, 1964; Rose, Lambert & Roepke, 1964; Beh, 1974). Most persistent reactions to strain 19 in the RBPT may well be due to IgM antibody, to which this test is very sensitive (Allan *et al.* 1976). However, it does not follow that the far rarer reactions given by other serological tests involve the same isotype.

Strain 45/20 is a rough strain of *B. abortus*. It has little or none of the serologically-important lipopolysaccharide of smooth strains, but has its own distinct polysaccharide surface antigen (Diaz & Jones, 1973). Cattle given K45/20A vaccine consistently give minimal responses of agglutinating antibody (Alton, 1978).

Five of the 50 heifers gave particularly prolonged responses to strain 45/20, and it is in these cattle that the relative behaviour of different tests is of greatest interest. The longest CFTW response was for 28 weeks after the second injection: the length of the response has been reported to vary widely in different studies (Alton, 1978). The RIA and the IHLT both remained positive for months longer than the CFTW, so these tests would be at a disadvantage when K45/20A vaccination is used.

The antibody response to strain 45/20, with or without prior exposure of the animal to smooth *B. abortus* organisms, has been partly defined by Beh (1975) and Corbel (1976). It is notable that the production of IgM antibody is very brief. It is not yet known why the rough antigen induces so little agglutinating antibody. As with strain 19, little attempt has been made to directly study the serum antibody of individual animals that give unusually prolonged responses.

The efficiency of both the RIA and the IHLT appears to be greater for detecting antibody produced later in an immune response. Because the RIA is based on antibody competition it is most sensitive to antibodies of higher avidity, which are produced increasingly as the immune response progresses (Chappel *et al.*1976). The IHLT is not very sensitive to antibody produced in initial response to strain 19, and is less sensitive than the CFTW in early infection (Hayes & Chappel, 1981).

An increase in the sensitivity of the IHLT during the antibody response may be related to a change in antigen-specificity, since the IHLT used a lipopolysaccharide-containing extracted antigen rather than the whole cells employed in the RIA and the CFT.

The RIA has been shown to be more sensitive than the CFTW or the IHLT early in infection in vaccinated cattle (Hayes & Chappel, 1981). It is likely to be even more sensitive in chronically infected animals with serum antibody of high avidity. The results of this study indicate that this sensitivity is not associated with a greater number of persistent reactions after strain 19 vaccination. However, vaccination with strain 45/20 may interfere with the results of both the RIA and the IHLT.

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