J. Hyg., Camb. (1982), **88**, 21
Printed in Great Britain

A comparison of the results of the brucellosis radioimmunoassay and other serological tests in experimentally infected cattle

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(*Received* 15 *January* 1981)

SUMMARY

The serology of 27 heifers found to be positive to culture after inoculation with *Brucella abortus* strain 544, was studied. Eighteen heifers had previously been vaccinated with strain 19 or strain 45/20 and nine were unvaccinated. Post-infection serum samples were tested for *Brucella* antibodies by radioimmunoassay (RIA), complement fixation test (CFT), indirect haemolysis test (IHLT) and Rose Bengal plate test (RBPT).

All of the unvaccinated heifers showed strong humoral responses to experimental infection in the RIA, CFT, IHLT and RBPT. The CFT and RBPT became positive sooner after infection than the other tests in the unvaccinated heifers. However, in vaccinated heifers the RIA was the most sensitive test early in infection and the results of the RBPT were variable. Three of the vaccinated heifers showed weak and inconsistent humoral responses and, in these animals, the RIA gave fewer false negative reactions than the other tests.

INTRODUCTION

Progress towards the eradication of bovine brucellosis by test and slaughter slows as the incidence of disease declines (Nelson *et al.* 1966; Nicoletti & Muraschi, 1966). The persistence of infection in some herds after eradication procedures have been applied may be due to the presence of infected animals that are negative to some or all of the serological tests used (Cordes & Carter, 1979). No serological test is completely free from false negative reactions, particularly in the early stages of infection before antibody levels rise and in chronic infection when titres are very low (Morgan, 1971).

The brucellosis radioimmunoassay (RIA) was developed to try to increase the frequency of detection of infected animals in problem herds. Radioimmunoassays are potentially more sensitive than other serological tests and may have an advantage over existing tests in identifying animals with low levels of specific antibody (Chappel *et al.* 1976).

In this study, sera from experimentally infected, culture-positive cattle were used to compare the number of false negative reactions given by the RIA with the numbers given by other serological tests.

MATERIALS AND METHODS

Sera from heifers in an experimental vaccination and infection trial were kindly provided for RIA testing by Dr S. Sutherland of the Western Australian Department of Agriculture. The heifers were of mixed breeds, from dams believed to be free from brucellosis and were serologically negative for brucellosis at the beginning of the trial. The 27 heifers selected for this study were those from which virulent *B. abortus* organisms were recovered at parturition or abortion and/or necropsy.

Five heifers were vaccinated subcutaneously with 4×10^{10} *B. abortus* strain 19 organisms (Batch 320–1, Commonwealth Serum Laboratories, Melbourne). Four were vaccinated at about 6 months of age and one at about 15 months. Thirteen heifers received two subcutaneous doses of killed strain 45/20 adjuvant (K45/20A) vaccine (Duphavac Batch V057, Philips Duphar Pty Ltd, North Sydney) 6 to 7 weeks apart. Five heifers were given their first injection at about 8 months of age and eight at about 15 months. Nine heifers were unvaccinated.

When the heifers were 15 to 18 months old, a bull was run in the same paddock for about 2 months. Six unvaccinated and 14 vaccinated heifers became pregnant. Three months after the bull was removed, pregnant heifers were inoculated with $1{\cdot}5 \times 10^7$ viable *B. abortus* strain 544 organisms via the conjunctiva. Non-pregnant heifers received $1{\cdot}5 \times 10^8$ viable organisms by the same route. All but two of the vaccinated heifers were serologically negative by the time of experimental infection (Table 1).

Abortions occurred in five unvaccinated and three vaccinated heifers; weak calves were born to one unvaccinated and three vaccinated heifers and the calf of one vaccinated heifer was stillborn (Table 1). *B. abortus* strain 544 was isolated from the milk of 15 heifers shortly after parturition or abortion, from the stillborn foetus, from the two aborted foetuses that were recovered and from three of the four weak calves which were necropsied shortly after birth (Table 1). Strain 544 organisms were also recovered from the tissues of 25 of the 27 heifers at necropsy, approximately 9 months after experimental infection.

Serum samples were collected nearly every week for at least 29 weeks from the time of experimental infection and despatched to our laboratory at regular intervals for RIA testing. The results of bacteriological examinations and of serological tests other than the RIA were provided by Dr Sutherland. Only samples for which the results of all four tests were available were included in this study.

The complement fixation test (CFT) using warm fixation, the indirect haemolysis test (IHLT), the Rose Bengal plate test (RBPT) and the RIA were performed as described previously (Chappel *et al.* 1981). Minimum diagnostic values were 4 for the CFT, 8 for the IHLT and 5 u. for the RIA.

The culture method of Alton, Jones & Pietz (1975) was followed using a serum dextrose agar culture medium containing antibiotics (Farrell, 1974).

Table 1. *Summary of the experimental trial history of the culture-positive heifers studied*

Heifer no.	Age and type of vaccination	Pregnancy status and outcome	Serology when experimentally infected	Isolation of *B. abortus*		
				Foetal/neonatal tissues	Dam	
					Parturition	Necropsy
1	NV	NP	Neg.			+
2	NV	Abortion	Neg.	+	+	+
4	NV	Abortion	Neg.	NT	+	−
5	NV	NP	Neg.			+
6	NV	Weak calf	Neg.	+	+	+
7	NV	NP	Neg.			+
8	NV	Abortion	Neg.	NT	+	+
10	NV	Abortion	Neg.	NT	+	+
11	NV	Abortion	Neg.	NT	+	+
39	S19, ≃ 6 m	Healthy calf	Neg.		+	+
41	S19, ≃ 6 m	Abortion	Neg.	NT	−	+
43	S19, ≃ 6 m	Healthy calf	Neg.		+	+
46	S19, ≃ 6 m	Healthy calf	Neg.		−	+
66	S19, ≃ 15 m	Healthy calf	RBPT 2		−	+
89	K45/20A, ≃ 8 m	Abortion	Neg.	NT	+	+
91	K45/20A, ≃ 8 m	NP	Neg.			+
95	K45/20A, ≃ 8 m	Weak calf	Neg.	−	−	+
96	K45/20A, ≃ 8 m	Weak calf	Neg.	+	+	+
99	K45/20A, ≃ 8 m	NP	Neg.			+
115	K45/20A, ≃ 15 m	Healthy calf	Neg.		+	+
116	K45/20A, ≃ 15 m	Healthy calf	Neg.		−	+
117	K45/20A, ≃ 15 m	Stillbirth	Neg.	+	+	+
118	K45/20A, ≃ 15 m	Abortion	RIA 5	+	+	+
120	K45/20A, ≃ 15 m	Healthy calf	Neg.		+	+
122	K45/20A, ≃ 15 m	NP	Neg.			+
123	K45/20A, ≃ 15 m	Weak calf	Neg.	+	+	−
124	K45/20A, ≃ 15 m	NP	Neg.			+

NV, not vaccinated. NP, not pregnant. NT, not tested. m, months. Neg., negative to tests.

Table 2. *False negative reactions in sera from nine culture-positive, unvaccinated heifers that gave persistent responses to experimental infection*

Serological reaction			No. of sera
RIA	CFT	IHLT	(no. of heifers)
+	+	−	4 (4)
+	−	+	0
+	−	−	0
−	+	+	5 (5)
−	+	−	5 (4)
−	−	+	1

RESULTS

The serological responses of pregnant and non-pregnant heifers to experimental infection with *B. abortus* strain 544, were similar in size and duration. Vaccinated heifers tended to become positive more slowly after infection than unvaccinated ones. Apart from some RBPT negative reactions in a few of the strain 45/20 vaccinated heifers, little difference was seen in the test responses given by strain 19 and strain 45/20 vaccinated heifers.

All unvaccinated and 15 vaccinated heifers became consistently positive to the RIA, CFT and IHLT, and usually to the RBPT. The remaining three vaccinated heifers had short-lived serological responses. The 27 heifers will be considered in three groups defined as follows:

	No. of heifers	No. of sera
Unvaccinated, persistent serological responses to infection	9	165
Vaccinated, persistent serological responses to infection	15	341
Vaccinated, transient serological responses to infection	3	69

Unvaccinated heifers with persistent serological responses to infection

The nine heifers in this group each had at least one serum sample for which the results of the RIA, CFT and IHLT did not agree. There were 15 such sera of which 14 were positive to the CFT, six to the IHLT and four to the RIA (Table 2). These sera were all collected between 4 and 10 weeks after infection when antibody levels were rising. The RBPT was positive for every serum positive to one or more of the RIA, CFT or IHLT and one serum positive to the RBPT was negative to the other three tests. Thus, early in infection in these unvaccinated heifers, the RBPT and CFT gave fewer false negative reactions than the IHLT or RIA.

Vaccinated heifers with persistent serological responses to infection

Ten of the 15 heifers in this group had one or more serum sample for which the results of the RIA, CFT and IHLT did not agree. Although heifer 118 had an RIA reaction of 5 when inoculated with strain 544, it was RIA negative before and just after experimental infection and did not become positive to the RIA before the other tests. All but two of the 19 sera with disparate results in the RIA, CFT and

Table 3. *False negative reactions in sera from* 15 *culture-positive, vaccinated heifers that gave persistent responses to experimental infection*

Serological reaction			No. of sera
RIA	CFT	IHLT	(no. of heifers)
+	+	−	6 (5)
+	−	+	3 (3)
+	−	−	5 (4)
−	+	+	0
−	+	−	2 (2)
−	−	+	3 (2)

Table 4. *Comparison of the Rose Bengal plate test with the other tests in sera from* 15 *culture-positive, vaccinated heifers that gave persistent responses to experimental infection*

			RBPT	
RIA	CFT	IHLT	+	−
All +			235 (15)	33 (5)
Results disagree			13 (7)	6 (4)
All −			9 (4)	45 (15)

The number of heifers from which the samples were collected are shown in brackets.

IHLT were collected between 1 and 22 weeks after infection when antibody levels were rising. Fourteen of the sera were positive to the RIA, eight to the CFT and six to the IHLT (Table 3). In these vaccinated animals, the RIA gave fewer false negative reactions than the CFT or IHLT.

The RBPT was negative in 33 sera positive to the other three tests (Table 4). Twenty-five of these sera came from one strain 45/20 vaccinated heifer (no. 122) which was negative to the RBPT for nearly the entire experimental period, although it showed a strong persistent humoral response as measured by the RIA, CFT and IHLT. A further five sera in this category came from another strain 45/20 vaccinated heifer (no. 115), but most of her serum samples were positive to the RBPT. The remaining three sera came from one strain 19 and two strain 45/20 vaccinated heifers.

Six of the nine sera positive to the RPBT but negative to the other three tests came from heifer 66 which was vaccinated with strain 19 when 15 months old. This heifer was positive to the RBPT from the time of vaccination, so the apparent good performance of the RBPT immediately after infection may have been due to a persistent vaccination titre.

Vaccinated heifers with transient serological responses to infection

There were three heifers in this category (nos. 46, 95 and 116). Two were vaccinated at about 6 months of age, one with strain 45/20 and one with strain 19. The other was vaccinated with strain 45/20 when about 15 months old. The serological responses of these heifers were transient although for each animal there was a period of at least 4 weeks when sera were positive to one or more of

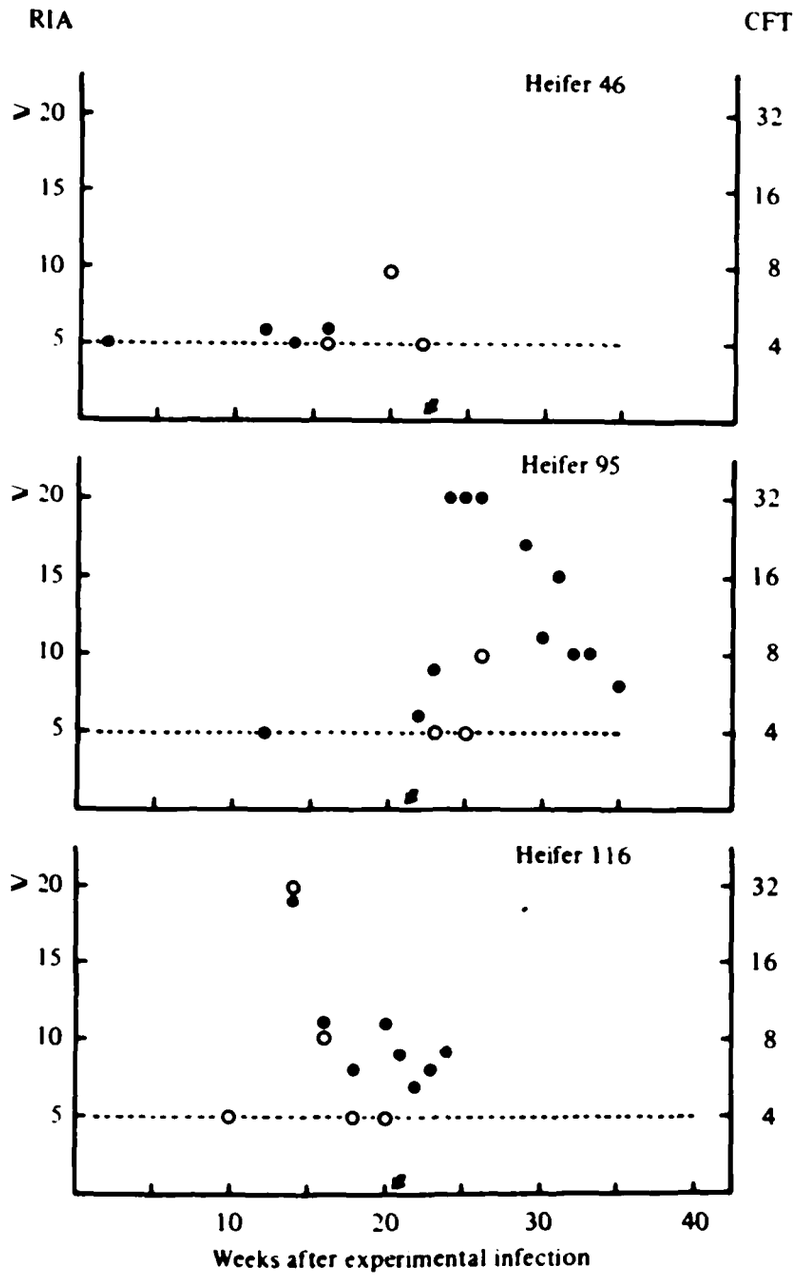

Fig. 1. Sera from vaccinated heifers with transient serological responses to infection, giving RIA (●) or CFT (○) reactions at or above the minimum diagnostic value (---). ✐ = parturition.

Table 5. *Comparison of tests in three culture-positive, vaccinated heifers that gave transient responses to experimental infection*

Heifer no.	No. of sera tested	No. of sera positive to test			
		RIA	CFT	IHLT	RBPT
46	22	4	3	0	4
95	20	12	3	8	0
116	27	8	5	2	2
Total	69	24 (35%)	11 (16%)	10 (15%)	6 (9%)

https://doi.org/10.1017/S0022172400069850 Published online by Cambridge University Press

the tests. Figure 1 shows the RIA and CFT reactions that reached or exceeded the minimum diagnostic value. Overall, the RIA was positive in more than twice as many sera as the CFT or IHLT and four times as many as the RBPT (Table 5).

The RBPT was positive in weeks 8, 12, 16 and 22, as many times as the RIA, in heifer 46 vaccinated with strain 19. The IHLT was negative. All samples from heifer 95 were negative to the RBPT and only three were positive to the CFT, while the RIA and IHLT were both positive for some time. A high proportion of IgG_2 antibody in the heifer could explain this result. The RBPT is less sensitive to IgG than to IgM antibody (Allan *et al.* 1976) and IgG_2 antibody can interfere in the CFT but not the IHLT (Plackett, Cottew & Best, 1976). The RIA measures both IgG subclasses. Samples from heifer 116 were positive to the RBPT and IHLT in weeks 14 and 16 when the RIA and CFT gave their highest reactions.

DISCUSSION

The RBPT gave fewer false negative reactions than the other tests in unvaccinated heifers early in the course of infection, as might be expected from its high sensitivity to IgM antibody (Allan *et al.*1976). However, in some heifers vaccinated with strain 45/20, the RBPT gave many more false negative reactions than the other tests. Strain 45/20 vaccines are known to produce little persistent agglutinating antibody capable of reacting in tests such as the RBPT (Alton, 1978). The results suggest that these vaccines may also influence the nature of the antibody response of *some* animals to antigenic exposure some months later.

The CFT gave fewer false negative reactions than the RIA or IHLT in unvaccinated heifers early after infection, but the RIA gave least false negative reactions in vaccinated heifers. The RIA measures IgG rather than IgM antibody and, being based on competition for antigen between different populations of antibody, preferentially measures higher avidity antibody (Chappel *et al.* 1976). The RIA is expected to be at its least sensitive in sera collected soon after an initial exposure to *Brucella* antigens, when IgM and low avidity IgG antibody predominate. Animals with the prior antigenic experience of vaccination may produce higher avidity IgG antibody in response to later stimulation. The RIA may have a similar advantage in sensitivity over the other tests in cattle previously exposed to *B. abortus* in the field.

The three vaccinated heifers whose serological responses to infection were transient are interesting as they are of a type likely to cause difficulties in serological diagnosis. None of these heifers were positive to culture at parturition and they may have been reinfected by other cows aborting. Naturally infected cattle with similar serological behaviour have been reported by Cordes & Carter (1979). They found that some serologically negative, culture-positive cattle were unvaccinated, so it appears that vaccination is not solely responsible for the occurrence of problem animals.

The RIA was positive for 35% of the sera from the heifers with transient responses to infection, compared with 16% for the CFT; 15% for the IHLT and 9% for the RBPT. There is evidence (Williams & Halliday, 1980) that cattle which produce antibody in the lowest concentrations, produce it at highest avidity. Thus,

the RIA may have its greatest advantage in sensitivity over the other tests in cases where serological diagnosis is most difficult.

All serological tests are subject to a proportion of false negative reactions and infected cattle with little or no detectable antibody will occur, so the limitations of serological diagnosis must be recognized. A strategy based solely on serological testing is unlikely to succeed in eradicating infection from all herds. However, the results of this study support the view that the use of the RIA as a supplementary test may improve the serological detection of infection in herds which are not responding to eradication procedures as quickly as expected.

We are grateful to Ellen Frew and Denise Young for skilled technical assistance.

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