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Comparison of the results of some serological tests for bovine brucellosis

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SUMMARY

A total of 1887 bovine sera positive to the Rose Bengal plate test were subjected to other serological tests for bovine brucellosis: the complement fixation test using warm fixation (CFTW), the serum agglutination test (SAT) and the radioimmunoassay (RIA).

The SAT was generally much less sensitive than the CFTW. Many sera, however, gave positive reactions in the SAT but no reaction in the CFTW or the RIA. These SAT reactions were attributed to IgM antibody.

Comparison between the results of the CFTW and the RIA led to the conclusion that 200 ng could be used as a minimum diagnostic reaction in the RIA.

INTRODUCTION

Morgan, MacKinnon & Cullen (1969) suggested that the best way to diagnose bovine brucellosis, at least in areas where animals were vaccinated with *Brucella abortus* strain 19, was to use the Rose Bengal plate test (RBPT) as a screening test. Sera positive to the RBPT would then be retested by the complement fixation test (CFT) to obtain a definitive result. The RBPT is believed to give few false negative reactions but comparison with other serological tests suggests that it gives many false positives (Nicoletti, 1967; Morgan *et al.* 1969). Residual antibodies after strain 19 vaccination are believed to cause these false positives. The CFT is performed using either a warm complement fixation step (CFTW) or cold fixation (CFTC). The CFTW, unlike the CFTC, can be completed within a single working day, which is an advantage in routine use.

The serum agglutination test (SAT) was once the most widely used diagnostic test for brucellosis and is still sometimes used in a supplementary role. It is known to give false negative reactions (Alton, Maw, Rogerson & McPherson, 1975*b*) and probably gives false positive reactions, particularly as residual titres after strain 19 vaccination.

Allan, Chappel, Williamson & McNaught (1976) studied the sensitivity of these tests to antibody, using purified immunoglobulins. They found that both the RBPT and the SAT were ten times as sensitive to IgM as to IgG_1 or IgG_2. The CFT measured IgG_1 and also IgM to an uncertain extent. This uncertainty was

caused by the fact that IgM activity was partly destroyed by heating in the presence of serum to inactivate complement. Purified IgM was stable to this degree of heating.

The radioimmunoassay (RIA) is a recently developed test for brucellosis which measures IgG_1 and IgG_2 but not IgM (Chappel *et al.* 1976).

This paper describes the results obtained using the CFTW, the SAT and the RIA, on 1887 sera positive to the RBPT.

MATERIALS AND METHODS

Sera

Sera positive to the RBPT were obtained from 1887 female cattle in 95 herds. They were submitted to this laboratory for routine testing from herds throughout Victoria. Vaccination of calves with strain 19 has been compulsory since 1970.

Serological tests

Rose Bengal plate test

The RBPT was performed as described by Allan *et al.* (1976). Positive reactions were classed subjectively as 'weak' or 'strong' according to the degree of agglutination. Reactions classed as 'strong' include those previously denoted as 3 or 4 (Allan *et al.* 1976).

Serum agglutination test

The SAT was performed as described by Alton, Jones & Pietz (1975*a*), who call it the European tube agglutination test. Results were expressed in international units (i.u.) per millilitre. A reaction of 100 i.u./ml was taken to be positive.

Radioimmunoassay

The RIA was performed as described by Chappel *et al.* (1976). The standard used was IgG_1 from serum H as described in that paper. Results were expressed as nanograms of IgG, equivalent to the standard, in 12·5 μl of serum. They were read over the range 200–10000 ng.

Complement fixation test

The CFTW was performed by the micro-method described by Alton *et al.* (1975*a*). Sera were heated in the plates at 60 °C for 30 min to inactivate complement. Antigen was obtained from the Commonwealth Serum Laboratories, Melbourne, as the Standardized *B. abortus* Agglutination Concentrate (Alton *et al.* 1975*a*) and was added at a dilution of 1/100.

The degree of complement fixation was judged on a scale from 1 to 4 at each twofold dilution from 1/4 to 1/128. A reaction at which all dilutions tested showed complete complement fixation is recorded as 4, 4, 4, 4, 4, 4 whereas a complete reaction to a dilution of 1/8 is recorded as 4, 4. A trace reaction in any well is recorded as *T*.

A reaction of 2 (at a dilution of 1/4) was taken to be positive.

Atypical reactions in the complement fixation test

For simplicity, sera giving 'atypical' reactions in the CFTW were excluded from comparisons between tests. An 'atypical' reaction was arbitrarily defined as any reaction which does not fall into the normal series: 0; *T*; 1; 2; 3; 4; 4, *T*; 4,1; 4, 2 etc. Prozones fall into the atypical category. Examples of atypical CFT reactions observed in this study were:

1, 1; 2, 2, *T*; 4, 4, 3, 1; 1, 1, *T*, *T*, *T*; 0, 0, *T*, 4, 4, 4; 0, 0, 0, 0, 4, 4.

One reason for excluding atypical reactions is that some are ambiguous, that is they may or may not represent true positives. However, the terms 'atypical' and 'ambiguous' must not be confused. A small but uncertain proportion of atypical reactions are ambiguous (Chappel, McNaught, Bourke & Allan, 1978).

RESULTS

Tables 1–3 compare the results of the CFTW, the SAT and the RIA. Atypical reactions, as defined in the Materials and Methods, were given by 257 out of 1887 sera (14%): these sera were excluded from the tables.

For three sera there was insufficient volume for the SAT. One of these sera gave an atypical reaction in the CFTW.

Comparison between the complement fixation test (warm fixation) and the serum agglutination test

Table 1 shows that sera giving CFTW reactions of 4, 4, 4, 1 and below usually gave SAT reactions below 100 i.u./ml.

Sera giving SAT reactions above 200 i.u./ml usually gave strong reactions in the CFTW while sera giving SAT reactions below 100 i.u./ml usually gave negative CFTW reactions.

The group of 111 sera giving SAT reactions of 100–200 i.u./ml were bimodal in their CFTW reactions. About half were negative to the CFTW but most of the others gave strong reactions. Table 4 shows that, within this group of sera, the results of the CFTW correlated closely with those of the RIA.

Comparison between the complement fixation test (warm fixation) and the radioimmunoassay

It can be calculated from Table 2 that 94% of sera positive to the CFTW gave RIA reactions of 200 ng or more. On the other hand, 15% of sera negative to the CFTW gave RIA reactions of 200 ng or more and 6% gave RIA reactions of 500 ng or more.

The lowest category of reactions positive to the CFTW, 2 to 4, 1, corresponded most closely to the RIA range 500–900 ng.

Comparison between the radioimmunoassay and the serum agglutination test

It can be calculated from Table 3 that only 82% of sera positive to the SAT gave RIA reactions of 200 ng or more, while 24% of sera negative to the SAT gave reactions of 200 ng or more. In general, therefore, the RIA related less closely to the SAT than to the CFTW.

Table 1. *Comparison between the results of the complement fixation test (warm fixation) and the serum agglutination test*

(Sera giving atypical results in the CFTW were excluded.)

	SAT reaction (i.u./ml.)					
CFTW reaction	0–50	50–100	100–200	200–400	> 400	Totals
Negative	940	194	54	17	5	1210
2 to 4, 1	54	11	4	1	0	70
4, 2 to 4, 4, 1	28	13	5	2	1	49
4, 4, 2 to 4, 4, 4, 1	20	11	8	6	1	46
4, 4, 4, 2 to 4, 4, 4, 4, 1	9	8	13	12	5	47
4, 4, 4, 4, 2 to 4, 4, 4, 4, 4, 1	2	3	6	6	10	27
4, 4, 4, 4, 4, 2 or above	5	6	21	35	112	179
Totals	1058	246	111	79	134	1628

Table 2. *Comparison between the results of the complement fixation test (warm fixation) and the radioimmunoassay*

(Sera giving atypical reactions in the CFTW were excluded.)

	RIA reaction (ng)						
CFTW reaction	< 200	200–400	500–900	1000–1900	2000–10000	> 10000	Totals
Negative	1026	115	41	9	10	11	1212
2 to 4, 1	17	16	25	5	5	2	70
4, 2 to 4, 4, 1	7	4	14	14	9	1	49
4, 4, 2 to 4, 4, 4, 1	2	3	5	7	25	4	46
4, 4, 4, 2 to 4, 4, 4, 4, 1	0	6	2	6	20	13	47
4, 4, 4, 4, 2 to 4, 4, 4, 4, 4, 1	0	0	2	1	12	12	27
4, 4, 4, 4, 4, 2 or above	1	2	3	7	39	127	179
Totals	1053	146	92	49	120	170	1630

Relationship between serological results and the strength of the reaction in the Rose Bengal plate test

Sera giving 'strong' RBPT reactions were typically strongly positive to the other tests. Most sera giving 'weak' reactions were negative to the CFTW and to the SAT and most gave RIA reactions of less than 200 ng.

On the other hand, of sera positive to other tests, many gave 'weak' RBPT reactions. 'Weak' RBPT reactions were obtained with 36% of sera positive to the CFTW and with 39% of sera giving RIA reactions of 500 ng or more.

DISCUSSION

The reaction given by a serological test is a function of the sensitivity of the test to antibody of different immunoglobulin classes and of the serum concentrations of antibody of each class. Elberg (1973) stated that the sera of cattle with brucellosis contain antibody predominantly of the IgG class, while residual titres

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Table 3. *Comparison between the results of the radioimmunoassay and the serum agglutination test*

(Sera giving atypical reactions in the CFTW were excluded.)

	SAT reaction (i.u./ml)					
RIA reaction (ng)	0–50	50–100	100–200	200–400	> 400	Totals
< 200	818	176	46	13	0	1053
200–400	113	17	9	7	0	146
500–900	64	11	8	3	4	90
1000–1900	27	9	4	5	4	49
2000–10000	30	21	18	19	32	120
> 10000	6	12	26	32	94	170
Totals	1058	246	111	79	134	1628

Table 4. *Relationship between the results of the radioimmunoassay and the complement fixation test (warm fixation) on sera giving reactions of* 100–200 *i.u./ml in the serum agglutination test*

(Sera giving atypical results in the CFTW were excluded.)

	CFTW reaction			
RIA reaction (ng)	Negative	2 to 4, 1	4, 2 or above	Totals
< 200	43	1	2	46
200–400	4	1	4	9
⩾ 500	7	2	47	56
Totals	54	4	53	111

after strain 19 vaccination are attributable to IgM. This statement is probably at least broadly correct, although quantitative studies are needed to confirm it.

Allan *et al.* (1976) studied the sensitivity of three serological tests for brucellosis to antibody of different immunoglobulin classes. The RBPT was positive to about 5 μg/ml of IgM antibody but required 50 μg/ml of *Brucella*-specific IgG_1 or IgG_2 to react. An SAT reaction of 100 i.u./ml could be produced by about 10 μg/ml of specific IgG_1 or IgG_2.

The CFT measures both IgG_1 and IgM but not IgG_2. IgM appears to react as well as or better than IgG_1 but in practice it may be partly or completely destroyed when serum is heated to inactivate complement. Allan *et al.* (1976) found that this heat treatment had no effect on purified IgM but appeared partly to destroy IgM activity in the presence of serum.

Insensitivity of the serum agglutination test

The SAT is, on average, much less sensitive than the CFTW (Table 1). This is as expected from the results of Allan *et al.* (1976), assuming that the sera of most infected animals contain predominantly IgG antibody.

Allan *et al.* (1976) showed that approximately 100 μg/ml of *Brucella*-specific IgG antibody will give an SAT reaction of 100 i.u./ml. From their results it can also be seen that about 12 μg/ml of specific IgG_1 should give a CFTW reaction of 2.

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Table 5. *Prediction of the median reaction in the serum agglutination test which corresponds to each category of results in the complement fixation test (warm fixation)*

(The median SAT reaction was predicted from the results of Allan *et al.* (1976), as described in the text.)

CFTW reaction	IgG antibody concentration required to give CFTW reaction (μg/ml)	Mean IgG antibody concentration required to give CFTW reaction (μg/ml)	Predicted SAT reaction (i.u./ml)	Observed median SAT range* (i.u./ml.)
Negative	0–15	7·5	7·5	0–50
2 to 4, 1	15–30	22·5	22·5	0–50
4, 2 to 4, 4, 1	30–60	45	45	0–50
4, 4, 2 to 4, 4, 4, 1	60–120	90	90	50–100
4, 4, 4, 2 to 4, 4, 4, 4, 1	120–240	180	180	100–200
4, 4, 4, 4, 2 to 4, 4, 4, 4, 4, 1	240–480	360	360	200–400
4, 4, 4, 4, 4, 2 or above	$\geqslant$ 480	$\geqslant$ 480	$\geqslant$ 480	$\geqslant$ 400

* Derived from Table 1.

Assuming that 80% of *Brucella*-specific IgG is IgG_1, about 15 μg/ml of specific IgG should give a CFTW reaction of 2. Table 5 shows that one could correctly predict, from the results of Allan *et al.* (1976), the median SAT result observed for each category of CFTW results.

Sensitivity of the serum agglutination test to IgM

Despite the general insensitivity of the SAT, which relates to its insensitivity to IgG (Table 5), it gave many reactions of 50–200 i.u./ml with sera which are negative to the CFTW (Table 1). For the SAT ranges 100–200 i.u./ml and 50–100 i.u./ml, the median CFTW results observed were much lower than would be predicted for sera containing predominantly IgG antibody.

It is likely that a substantial proportion of SAT results less than 200 i.u./ml were due to IgM, to which the SAT is probably more sensitive than the CFTW. This possibility was supported by the results of the RIA, which is insensitive to IgM, on sera giving SAT reactions of 100–200 i.u./ml (Table 4).

IgM antibodies can predominate either in early infection or long after vaccination with strain 19 (Elberg, 1973). However, the period in early infection during which IgM antibody is present in serum to the exclusion of IgG is relatively short. On the other hand, residual IgM antibodies are common in sera of cattle from areas where strain 19 vaccine is used, as indicated by the many RBPT-positive sera which are negative to all other tests.

Radioimmunoassay

The results of the RIA showed a general relationship with those of the CFTW (Table 2). Most sera which gave RIA reactions of 500 ng or more were positive to the CFTW (Table 2), although sera giving reactions of up to at least 2000 ng were usually negative to the SAT (Table 3).

Although an RIA reaction of 500 ng corresponded the most closely to the minimum diagnostic value accepted for the CFTW, it is nevertheless suggested that 200 ng be tentatively taken as a minimum diagnostic value for the RIA. An RIA value of 200–400 ng with a positive result in the CFTW could indicate early infection.

The RIA appears to be at least as sensitive as the CFTW and to be worth considering as an alternative or supplementary diagnostic method. It has not yet, however, been evaluated under field eradication conditions.

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REFERENCES

ALLAN, G. S., CHAPPEL, R. J., WILLIAMSON, P. & MCNAUGHT, D. J. (1976). A quantitative comparison of the sensitivity of serological tests for bovine brucellosis to different antibody classes. *Journal of Hygiene* **76**, 287.

ALTON, G. G., JONES, L. M. & PIETZ, D. E. (1975*a*). *Laboratory Techniques in Brucellosis*, 2nd ed. World Health Organization Monograph Series, no. 55. Geneva.

ALTON, G. G., MAW, J., ROGERSON, B. A. & MCPHERSON, G. G. (1975*b*). The serological diagnosis of bovine brucellosis: an evaluation of the complement fixation, serum agglutination and Rose Bengal tests. *Australian Veterinary Journal* **51**, 57.

CHAPPEL, R. J., MCNAUGHT, D. J., BOURKE, J. A. & ALLAN, G. S. (1978). The diagnostic efficiency of some serological tests for bovine brucellosis. *Journal of Hygiene* **80**, 373.

CHAPPEL, R. J., WILLIAMSON, P., MCNAUGHT, D. J., DALLING, M. J. & ALLAN, G. S. (1976). Radioimmunoassay for antibodies against *Brucella abortus*: a new serological test for bovine brucellosis. *Journal of Hygiene* **77**, 369.

ELBERG, S. S. (1973). Immunity to *Brucella* infection. *Medicine, Baltimore* **52**, 339.

MORGAN, W. J. B., MACKINNON, D. J. & CULLEN, G. A. (1969). The Rose Bengal plate agglutination test in the diagnosis of brucellosis. *Veterinary Record* **85**, 636.

NICOLETTI, P. (1967). Utilization of the card test in brucellosis eradication. *Journal of the American Veterinary Medical Association* **151**, 1778.