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A quantitative comparison of the sensitivity of serological tests for bovine brucellosis to different antibody classes

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SUMMARY

Brucella-specific antibodies of different immunoglobulin classes were quantitatively evaluated with respect to their efficiency in serological tests for bovine brucellosis.

IgM reacted more efficiently than IgG_1 and IgG_2 in both the Rose Bengal plate test and serum agglutination test. The complement fixation test was found to be slightly more sensitive to IgM than to IgG_1 and did not react to IgG_2. IgM was, however, partly inactivated when heated at 60° C. in the presence of serum.

INTRODUCTION

Antibody produced in response to *Brucella abortus* infection or vaccination with *Br. abortus* strain 19 is found in the IgG_1, IgG_2 and IgM classes (Rice & Boyes, 1971; Beh, 1974). The relative proportions of each of these immunoglobulin classes in the sera of infected and vaccinated animals will vary, and it is important to know the efficiency with which they are measured by the serological tests commonly used to diagnose brucellosis.

The present study quantitates the effects of each of these three types of antibody in three diagnostic tests for bovine brucellosis: the complement fixation test (CFT), Rose Bengal plate test (RBPT) and serum agglutination test (SAT).

MATERIALS AND METHODS

Sera

Sera showing high titres of antibodies against *Br. abortus* by the CFT were obtained from three cows from a commercial herd in which two cows had recently aborted. Sera were also obtained from three adult cows held on the 'Attwood' Veterinary Research Station. These were each vaccinated with 2 ml. of freeze-dried, living *Br. abortus* strain 19 (Commonwealth Serum Laboratories) and bled 11–13 days after vaccination. Bovine sera with no detectable anti-*Brucella* antibody were obtained from cattle held at the Research Station and from commercial herds.

Antigens

Killed *Br. abortus* suspension and Rose Bengal *Br. abortus* antigen were obtained from the Commonwealth Serum Laboratories, Melbourne.

Isolation of bovine immunoglobulins

Each serum was fractionated as follows with the aim of isolating IgG, IgG_1, IgG_2 and IgM. A 50 ml. volume of serum was brought at 4° C. to 37 % saturation with saturated ammonium sulphate, stirred for 30 min. and centrifuged at 10,000 ***g*** for 10 min. The precipitate was dialysed into 30 mM sodium phosphate buffer, pH 8·0, and applied to a 30–46 ml. column of DEAE-Cellulose equilibrated with this buffer. The column was eluted with starting buffer then with an 800 ml. linear gradient from the starting buffer to 200 mM sodium phosphate buffer, pH 6·0. The unretarded peak was considered to be impure IgG. Where fractions eluted after the application of the gradient contained *Brucella*-specific antibody activity, they were pooled and considered to contain IgM.

The pool of impure IgG was usually divided into two. One half was further purified on a 200 ml. column of Sephadex G-200 in phosphate-buffered saline, pH 7·2, the active pool being considered to be purified IgG. The other half was dialysed into 5 mM sodium phosphate buffer, pH 8·0, and applied to a 27–31 ml. column of DEAE-Cellulose equilibrated with this buffer. The column was eluted with starting buffer then with a 500 ml. linear gradient from the starting buffer to 30 mM sodium phosphate buffer, pH 8·0. The unretarded peak was considered to be IgG_2, and active fractions after the application of the gradient were considered to contain IgG_1.

IgM was further purified by twice submitting it to gel filtration on a 200 ml. column of Sephadex G-200 in phosphate-buffered saline, pH 7·2.

Column chromatography, dialysis and centrifugation were performed at 4° C. Columns were monitored for protein by absorbance at 280 nm. They were monitored for antibody activity using the RBPT or, where the sensitivity of this was inadequate, the SAT.

Immunoglobulins were stored in small volumes at −20° C. Solutions used for experiments on antibody activity were thawed and refrozen no more than three times. Where necessary, solutions were concentrated by ultrafiltration before use. Where dilution was necessary, the diluent was phosphate-buffered saline, pH 7·2.

Demonstration of identity and purity of bovine immunoglobulins

Immunoelectrophoresis and immunodiffusion were performed in 0·049 M barbitone buffer, pH 8·6. Immunoelectrophoresis was performed against rabbit anti-bovine whole serum and against rabbit anti-bovine immunoglobulin. Immunodiffusion was performed against rabbit anti-bovine IgG, rabbit anti-bovine IgM and rabbit anti-bovine IgA, where appropriate to detect contamination with other classes of immunoglobulin. Polyacrylamide disk gel electrophoresis was performed as described by Davis (1964).

Protein estimations

These were performed by the method of Lowry, Rosebrough, Farr & Randall (1951) using a commercial preparation of bovine immunoglobulin as a standard. Standards and, where possible, unknowns were included in each assay in quadruplicate.

Estimation of Brucella*-specific percentage of immunoglobulin preparations*

Two different methods were used to estimate the percentage of each immunoglobulin preparation with antibody activity against *Br. abortus*.

(*a*) *Radioiodination method.* Immunoglobulins were iodinated with iodine-125 (Marchalonis, 1969), to give specific activities of approximately 0·5 μCi/μg. Iodinated immunoglobulins were diluted in albumin diluent (phosphate-buffered saline, pH 7·2, made 5 mg./ml. with respect to bovine serum albumin).

Br. abortus antigen was washed thoroughly in phosphate-buffered saline, pH 7·2, and resuspended in this buffer at normal strength. Fifty μl. of this antigen was incubated overnight at 37° C. with 1, 0·5 and 0·25 μg. of labelled antibody, the volume being made up to 200 μl. with albumin diluent. Twenty-four tubes were set up for each determination, eight at each concentration of immunoglobulin. Four of each eight were used to assess binding to antigen and the rest were controls to measure adsorption to the walls of the tube. At the end of the incubation all tubes were counted in a well-type scintillation counter. One ml. of albumin diluent was then added and the binding tubes were centrifuged at 16,300 ***g***. Supernatants, including the antigen in the control tubes, were removed, then the procedure was repeated with a further 1 ml. of albumin diluent. Tubes were then recounted. Mean counts in the binding tubes minus mean counts in the control tubes were expressed as a percentage of counts added. The percentages from the three concentrations of immunoglobulin were averaged.

(*b*) *Protein method.* Fifty μl. and 100 μl. volumes of immunoglobulin at a suitable concentration were incubated in the presence or absence of 200 μl. of washed normal strength antigen suspension. Volumes were brought to 300 μl. with buffer. After incubation at 4° C. for two days, tubes were centrifuged at 16,300 ***g*** and 100 μl. samples of each supernatant were removed and the amount of protein estimated. Incubations were set up in quadruplicate.

Test incubations of 50 μl. and 100 μl. of immunoglobulin were designated as T 50 and T 100 respectively. Control incubations (without antigen) were designated as C 50 and C 100.

Control figures to allow for the protein contributed by the antigen and for other undetermined effects were calculated. The test control figure, C_T, was obtained as follows:

$$\text{True protein at } 100\ \mu\text{l.} = 2 \times \text{true protein at } 50\ \mu\text{l.}$$

$$\therefore\ T100 - C_T = 2\,(T50 - C_T)$$

$$\therefore\ C_T = 2\,T50 - T100.$$

Likewise the control figure, Cc, was obtained from:

$$Cc = 2\,C50 - C100.$$

Estimations were performed in groups of at least eight, and C_T and Cc were averaged over all estimations in a group.

The *Brucella*-specific percentage of each batch was calculated for 50 μl. volumes as follows:

$$\left[1 - \frac{T50 - C_T}{C50 - Cc}\right] \times 100.$$

The percentage for 100 μl. volumes was calculated similarly. The mean of the 100 μl. and 50 μl. values from an individual determination was called a 'valid' value if the 100 μl. and 50 μl. values did not differ by more than 10 %. The few values which lay below -5 % were also excluded.

Serological tests

(*a*) *Rose Bengal plate test.* Twenty-five μl. of test substance was added to 25 μl. of Rose Bengal antigen on a haemagglutination tray and mixed on a rotary agglutinator for 4 min. Agglutination was assessed on a scale from 1+ to 4+. To assess the activities of immunoglobulin preparations, each was diluted with phosphate-buffered saline, pH 7·2, through a series of concentrations varying by about 20 %. The solution which just gave a 1+ reaction was retained for protein estimation. To reduce subjective error, a single worker assessed all such RBPT endpoints.

(*b*) *Complement fixation test.* The CFT was performed by a micro-method (Alton, Maw, Rogerson & McPherson, 1975).

Immunoglobulin preparations were heated at 60° C. for 30 min. and assayed using warm fixation (37° C., 40 min.), this being the standard diagnostic method in our laboratory. To compare immunoglobulin preparations, each was diluted until it gave a 4+ reaction at a dilution of 1/2 (i.e. a 4/2 reaction). The diluted solution was then retained for protein estimation. If two or more dilutions of one preparation both gave a 4/2 reaction in the same assay, the solution with the highest dilution was retained.

Diluted preparations giving the desired 4/2 reaction were simultaneously assayed in three other ways: by warm fixation without prior inactivation, and by cold fixation (4° C., 14–18 hr.) with and without inactivation.

(*c*) *Serum agglutination test.* The SAT (Alton & Jones, 1967) was standardized to read in international units using the International Standard Anti-*Brucella abortus* Serum.

Immunoglobulin preparations were diluted to give readings between a 1+ reaction and a 4+ reaction at a dilution of 1/20, i.e. 34–53 i.u., and retained for protein estimation. Results were expressed as i.u. for a 1 mg./ml. protein solution. To reduce subjective error, the same worker read all these SAT reactions.

RESULTS

Immunoglobulins

Twenty batches of purified immunoglobulin were used: six IgG, six IgG_1, five IgG_2 and three IgM. These were derived from the sera of three infected and three

Table 1. *Reproducibility of methods for determining the* Brucella*-specific percentage of batches of immunoglobulin*

Immunoglobulin batch	Replicates by radioiodination method	Replicates by protein method (valid values)
a (IgG_1)	29·9, 29·8, 28·4, 28·3, 25·2	20·8, 20·2
b (IgG)	5·6, 5·6, 5·6, 5·4, 5·3	5·4, 0·4
c (IgG)	0·43, 0·42, 0·38, 0·22	9·4, 5·0, −2·7, −3·8

Table 2. Brucella*-specific percentage of preparations of immunoglobulin used in this study*

	IgG	IgG_1	IgG_2	IgM
Sera from infected cows				
I1	5·4	21·6	0·87	—
I2	—	11·8	2·2	—
I3	17·9	28·3	4·5	—
Sera from vaccinated cows				
V1	3·7, 4·6	25·0	—	6·2
V2	0·91	1·2	0·33	6·4
V3	1·3	2·5	0·33	9·8

Table 3. *Effectiveness of immunoglobulin preparations in the serum agglutination test*

Immunoglobulin preparation		I.u./mg./ml. protein total			*Brucella*-specific percentage	I.u./mg./ml. protein specific
		1	2	Mean		
IgM	V1	392	442	417	6·2	6726
	V2	482	505	494	6·4	7719
	V3	534	815	675	9·8	6888
IgG	I1	62	63	63	5·5	1145
	I3	142	134	138	17·9	771
	V1 (*a*)	72	59	66	3·7	1784
	V1 (*b*)	57	74	66	4·6	1435
	V2	19	29	24	0·91	2637
	V3	26	33	30	1·3	2308
IgG_1	I1	214	196	205	21·6	949
	I2	157	147	152	11·8	1288
	I3	230	196	213	28·3	753
	V1	294	288	291	25·0	1164
	V2	27	34	31	1·2	2583
	V3	49	66	58	2·5	2320
IgG_2	I1	5	4	5	0·87	575
	I2	12	12	12	2·2	545
	I3	38	94	66	4·5	1467
	V2	13	9	11	0·33	3333
	V3	10	10	10	0·33	3030

recently vaccinated cows. *Brucella*-specific IgM was only obtained in usuable quantities from sera of vaccinated cows.

Each batch of immunoglobulin showed the characteristic behaviour of its class or subclass on immunoelectrophoresis and polyacrylamide gel electrophoresis. Immunoelectrophoresis against anti-bovine immunoglobulin serum showed each

to be free from contamination by other immunoglobulins. Each was also shown by immunodiffusion to be free from IgA: IgM batches were shown to be free from IgG, and batches of IgG and its subclasses were free from IgM by this method.

Several batches of immunoglobulin showed a single non-immunoglobulin contaminant on polyacrylamide gel electrophoresis or immunoelectrophoresis against anti-bovine whole serum. Densitometry indicated that this contamination comprised only 8 % of the worst-contaminated batches.

Brucella-*specific percentages*

The *Brucella*-specific percentages of most batches of immunoglobulin were measured at least once by the radioiodination method. They were also tested by the protein method until two or more valid values were obtained. The reproducibility of the radioiodination method was found to be superior (Table 1) and so the radioiodination values were accepted.

Values obtained by the two methods were, however, related (Fig. 1). The correlation coefficient, calculated after angular transformation, was 0·85 ($P < 0{\cdot}001$). A commercial preparation of bovine immunoglobulin, which was negative to the RBPT and CFT, gave a value of 0·15 % by the radioiodination method and valid values of 0·2 % and 2·2 % by the protein method.

Brucella-specific percentages for the twenty batches of immunoglobulin used are shown in Table 2. V1 IgG consisted of two batches, (*a*) and (*b*), which were separated only for the final gel filtration. No differences could be detected in the *Brucella*-specific percentage of batches of IgG_1 or IgM after freezing and thawing up to three times.

Serum agglutination test

Table 3 shows the effectiveness of immunoglobulin classes in the SAT. In terms of international units/mg./ml. of specific immunoglobulin protein IgM was about five times as effective as IgG_1 and IgG_2. The figures obtained differed between batches of immunoglobulin of a particular class or subclass, but this does not necessarily indicate a real difference according to the serum of origin of the immunoglobulin batch. Greatest variation occurred at the lowest *Brucella*-specific percentages, where small errors in estimating these percentages could greatly influence the results.

The SAT activity in terms of the total protein concentration of IgG_1 showed significant regression ($P < 0{\cdot}01$) on the *Brucella*- specific percentage (Fig. 2). This illustrates the importance of taking into account the *Brucella*-specific percentage of each batch of immunoglobulin. Variation in the *Brucella*-specific percentage of different batches accounted for 88 % of the variance of SAT activity in terms of total immunoglobulin protein.

Complement fixation test

Table 4 shows the effectiveness of immunoglobulin classes in the CFT, using warm fixation after heat inactivation for 30 min. at 70° C. This is expressed in terms of the minimum concentrations of total and specific immunoglobulin required to

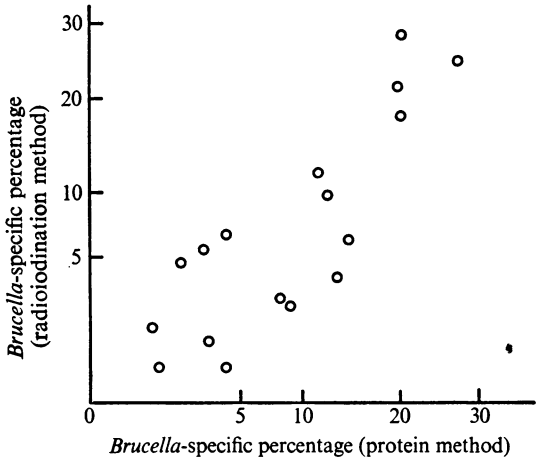

Fig. 1. Comparison of *Brucella*-specific percentages estimated by the radioiodination and protein methods. Values obtained by the radioiodination method are either single estimates, or means where more than one estimation was performed. Values obtained by the protein method are means of at least two valid values.

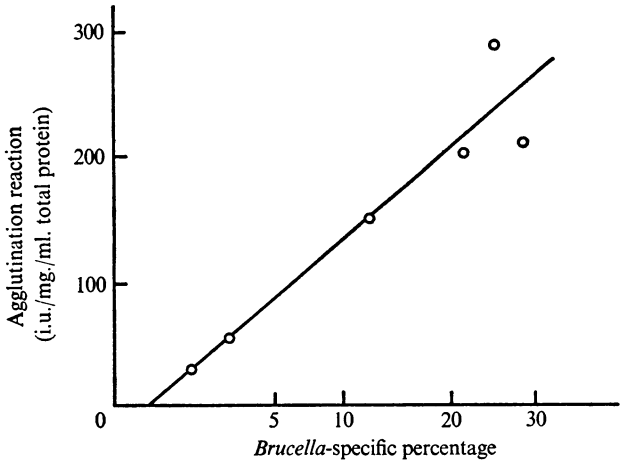

Fig. 2. Dependence of serum agglutination test activity of IgG_1 preparations on the *Brucella*-specific percentage. The regression line is described by the equation $Y = 8{\cdot}50x - 19{\cdot}1$, where Y is the international units per mg. per ml. of total protein and x is the *Brucella*-specific percentage after angular transformation.

give a 4/2 reaction, the lowest concentrations indicating the greatest effectiveness. IgM appeared more active than IgG_1. IgG_2 did not fix complement at any of the concentrations used. IgG was as effective as IgG_1 despite its component of IgG_2.

Varying the method of performing the assay as described in the Materials and Methods made little difference to the results obtained. Anti-complementary reactions were obtained on a few occasions, always with IgG_1 preparations assayed using cold fixation. Heat inactivation in buffer did not affect the activity of any

Table 4. *Effectiveness of immunoglobulin preparations in the complement fixation test using warm fixation after inactivation at 60° C. for 30 min*

Immunoglobulin preparation		μg./ml. protein total*			*Brucella*-specific percentage	μg./ml. protein specific*
		1	2	Mean		
IgM	V1	83	108	96	6·2	6
	V2	83	70	77	6·4	5
	V3	53	30	42	9·8	4
IgG	I1	100	74	87	5·5	5
	I3	49	40	45	17·9	8
	V1 (*a*)	120	120	120	3·7	4
	V1 (*b*)	103	150	127	4·6	6
	V2	1300	1800	1550	0·91	14
	V3	375	345	360	1·3	5
IgG_1	I1	40	39	40	21·6	9
	I2	73	39	56	11·8	7
	I3	41	30	36	28·3	10
	V1	40	33	37	25·0	9
	V2	2563	1440	2002	1·2	24
	V3	245	168	207	2·5	5
IgG_2	All negative.					
	I1		5700†		0·87	50
	I2		6450		2·2	142
	I3		2550		4·5	115
	V2		9300		0·33	31
	V3		18,750		0·33	62

* To give a 4+ reaction at a dilution of 1/2.

† Concentrations given are the highest at which preparations were tested.

batch of purified immunoglobulin. However, preliminary experiments indicated that if serum was present at a dilution of 1/4, IgM but not IgG_1 was partly inactivated by heating at 60° C.

Rose Bengal plate test

Table 5 shows the effectiveness of immunoglobulin classes in the RBPT. This is expressed in terms of the minimum concentrations of total and specific immunoglobulin required to give a 1+ reaction. In terms of specific protein, IgM was about five times as effective as IgG_1 and IgG_2.

The effect of adding diluting buffer on the pH of the reacting mixture was tested, in order to show whether it was comparable to the effect of serum, with the following results: Rose Bengal antigen, pH 3·65; Rose Bengal antigen + 1 volume serum, pH 3·80; Rose Bengal antigen + 1 volume buffer, pH 3·68.

Quantitative considerations

The approximate concentrations of specific antibody of each immunoglobulin class required to give positive reactions in each test have been estimated and are shown in Table 6.

Table 5. *Effectiveness of immunoglobulin preparations in the Rose Bengal plate test*

Immunoglobulin preparation		μg./ml. protein total*			*Brucella*-specific percentage	μg./ml. protein specific*
		1	2	Mean		
IgM	V1	82	195	139	6·2	9
	V2	85	80	83	6·4	5
	V3	63	80	72	9·8	7
IgG	I1	900	900	900	5·5	50
	I3	474	720	587	17·9	105
	V1 (*a*)	589	640	615	3·7	23
	V1 (*b*)	794	780	787	4·6	36
	V2	1800	1800	1800	0·91	16
	V3	1425	2175	1800	1·3	23
IgG_1	I1	223	260	242	21·6	52
	I2	309	420	365	11·8	43
	I3	303	380	342	28·3	97
	V1	149	230	190	25·0	48
	V2	1075	810	943	1·2	11
	V3	750	780	765	2·5	19
IgG_2	I1	3700	3500	3600	0·87	31
	I2	4300	2250	3275	2·2	72
	I3	1050	790	920	4·5	41
	V2	2667	2700	2684	0·33	9
	V3	4800	4975	4888	0·33	16

* To just give a 1+ reaction.

Table 6. *Approximate concentration of each immunoglobulin class required to give positive serological reactions*

Serological test (diagnostic level)	Specific protein concentration* (μg./ml.)		
	IgG_1	IgG_2	IgM
CFT (1+ reaction at a dilution of 1/4)	10	—	5
SAT (100 i.u.)	100	100	10
RBPT (1+ reaction)	50	50	5

* Figures are approximate and are estimated from the data given in Tables 3, 4 and 5.

DISCUSSION

This is, to our knowledge, the first quantitative study of the efficiency of different immunoglobulin classes in serological tests for bovine brucellosis. To obtain quantitative information of this type it is necessary to use purified immunoglobulin preparations. It is also necessary to estimate, for each batch of immunoglobulin used, the percentage of the antibody which is directed against the antigens in question. This must be measured using the primary antigen–antibody reaction.

Of the two methods used for estimating the *Brucella*-specific percentage, the radioiodination method proved to be the more precise. This was probably due to the fact that the bound antibody was, in this method, measured directly, whereas in the protein method a small amount of bound antibody was measured as the

difference between two larger amounts of free antibody. The protein method did, however, provide evidence (Fig. 1) that the radioiodination method gave values which were approximately correct and that iodination does not appreciably reduce antibody activity.

Serum agglutination test

It is widely believed that the SAT is unsatisfactory as a sole diagnostic method for bovine brucellosis, due partly to a high proportion of non-specific reactions (Alton *et al.* 1975). Non-specificity in this context means diagnostic non-specificity, where an SAT-positive animal is not infected with the disease. There are, however, at least two types of diagnostic non-specificity in agglutination reactions, and these have been widely confused.

Firstly, agglutination can be immunologically non-specific, due to non-specific agglutinins which can agglutinate a variety of unrelated bacterial antigens (Hess, 1953*a*, *b*; Rose & Roepke, 1957). Hess (1953*b*) claimed that these cause about 60 % of non-specific agglutination reactions to brucellosis.

Secondly, agglutination may be immunologically specific but diagnostically non-specific. The usual reason for this is prior vaccination, especially with the attenuated *Br. abortus* strain 19. Vaccination with strain 19 is usually performed before 8 months of age, so that little or no antibody will be detected if testing is performed at least 12 months later. Residual vaccination titres do, however, occur, especially if vaccination is carried out later than recommended.

Infection with virulent strains of *Br. abortus*, even in the chronic phase, is believed to stimulate predominantly the production of IgG, while residual vaccination titres are attributed mainly to IgM (Morgan, 1969; Elberg, 1973). From this it is sometimes wrongly assumed that IgM is responsible for all non-specific agglutination.

The agglutination test has been regarded as more sensitive to IgM than to IgG and therefore as particularly prone to interference by residual vaccination titres (Kulshreshtha, Atal & Wahi, 1973). We have confirmed that IgM is measured more efficiently than IgG_1 or IgG_2 by the SAT. The SAT gives, therefore, diagnostically non-specific reactions of both the immunologically specific and the immunologically non-specific kinds.

Complement fixation test

Morgan (1969) stated that the CFT for bovine brucellosis detects both IgG and IgM, the former being the more effective. The CFT is therefore often regarded as superior to the SAT in distinguishing infected animals from those which have been vaccinated with strain 19. The complement-fixing ability of bovine IgG has been attributed entirely to IgG_1 (Curtain, 1971; Cho & Ingram, 1972).

We have confirmed that IgG_2 does not fix complement. We found that IgM probably fixed complement about twice as efficiently as IgG_1 on a weight basis, which on a molar basis would make it about ten times as efficient. However, although IgM is stable to 60° C. in buffer, it seems to be partially labile in serum under conditions used in our laboratory. Because of this, the CFT probably

measures IgG_1 in serum more effectively than IgM. In other laboratories, however, the complement inactivating procedure may vary with respect to temperature, dilution of serum, and diluting buffer. Further research is needed into the effect of these conditions on the stability of IgM.

Rose Bengal plate test

The proposed Australian eradication scheme for bovine brucellosis is to be based on the use of the RBPT as a screening test, with Rose Bengal positive sera tested subsequently by CFT. This approach was suggested by Davies (1971).

Our data show that the RBPT detects IgM much more efficiently than IgG_1 and IgG_2. In this respect it behaves more like the SAT than like the CFT, and as the RBPT is an agglutination test, this is not surprising. The RBPT is a plate agglutination test in which the *Br. abortus* cells are bound to a dye and suspended in a lactate buffer of pH 3·65. This acidic buffer is believed to inhibit immunologically non-specific reactions (Rose & Roepke, 1957; Davies, 1971).

Corbel (1972*a*, *b*) has claimed that the RBPT detects only IgG_1 and fails to react to either IgM or IgG_2. His studies were, however, non-quantitative. His approach was to fractionate a number of sera, and he found that RBPT activity remained entirely in fractions containing IgG_1. Because the proportions of each immunoglobulin class in the sera he used were unknown, he was unable to correctly determine the efficiency of each class in the test. Corbel (1973) subsequently showed that fewer sera negative to the CFT and fewer sera overall agglutinate Rose Bengal antigen at pH 3·65 than at pH 7·0. He interpreted this as showing that the acidic buffer gives the RBPT specificity for IgG_1 by preventing the action of other immunoglobulin classes, although he accepted the probable additional involvement of immunologically non-specific agglutinins. It is equally possible from his results, however, that inactivation of immunologically non-specific agglutinins could wholly explain the effect of the acidic buffer.

The RBPT is more likely than the CFT to produce false positive reactions due to residual vaccination titres because of its sensitivity to IgM. A large number of false positive Rose Bengal reactions do occur, as judged by negative results in the CFT (Alton *et al.* 1975). This situation may, however, be tolerable in an inexpensive screening test, as long as few false negative reactions occur.

Quantitative considerations

The approximate concentrations of specific antibody of each immunoglobulin class required to give positive diagnostic reactions in each test are shown in Table 6. Interactions between antibodies of different classes or subclasses, interference by other serum components, and differences in the effectiveness of different antibody populations of the same class or subclass may complicate the interpretation of these findings. These possibilities, however, can be investigated using a quantitative experimental approach.

The RBPT is less sensitive than the CFT for IgG_1 (Table 6). Some CFT-positive sera containing predominantly IgG_1, perhaps from chronic carriers, may therefore be missed by the RBPT. Sera reacting in this way have been observed by Miller,

Nettleton & Robertson (1973). It is important that this be further investigated before the RBPT is given unqualified recognition as a suitable screening test.

Studies such as this one should lead to improved diagnostic accuracy in serological tests for bovine brucellosis, with substantial economic benefit where test-and-slaughter eradication programmes are undertaken. There is, however, a need for quantitative information on the absolute level of *Brucella*-specific antibodies of each class present in sera from cattle under different circumstances of infection and vaccination.

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