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The nature of the antibody response to *Yersinia enterocolitica* serotype IX in cattle

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SUMMARY

The nature of the antibody response of cattle to the antigen of *Yersinia enterocolitica* IX cross-reacting with *Brucella* spp. was examined. Density-gradient ultracentrifugation, ion-exchange chromatography, antibody adsorption and elution and disulphide bond reduction tests showed that both 19 S IgM and 7 S IgG_1 and IgG_2 antibodies were produced in response to the cross-reacting antigen. The highest titres of cross-reacting antibodies were detected by the agglutination and Coombs antiglobulin tests. Production of complement-fixing and precipitating antibodies cross-reacting with *Br. abortus* was transient and high titres were not attained.

In contrast, although infection with *Br. abortus* also evoked cross-reacting antibodies of the IgM and IgG classes, much higher titres were produced in the complement fixation and precipitation tests and these persisted for long periods. At all stages of the serological response to both organisms, the two infections could be differentiated by the quantitative Rose Bengal plate test.

INTRODUCTION

The serological cross-reaction between *Yersinia enterocolitica* serotype IX and *Brucella abortus* was first described by Ahvonen and colleagues (Ahvonen, Jansson & Aho, 1969; Ahvonen & Sievers, 1969). Subsequently this cross-reaction was shown to involve other smooth *Brucella* strains (Corbel & Cullen, 1970; Hurvell, Ahvonen & Thal, 1971). Corbel & Cullen (1970) also showed that antibodies evoked by *Br. abortus* could be distinguished from those evoked by *Y. enterocolitica* IX on the basis of a rapid quantitative slide agglutination test using standardized suspensions of Rose Bengal stained *Br. abortus* and *Y. enterocolitica* IX as antigens.

Although this information is of value in cases where infection with *Y. enterocolitica* IX is suspected, it is still important to know the extent to which cross-reacting antibodies evoked by this organism would be likely to interfere with the diagnosis of bovine brucellosis by the standard system of testing employed for the Brucellosis Incentives and Eradication Schemes of the Ministry of Agriculture, Fisheries and Food.

Fundamental to this problem is the nature of the antibody response to the antigen complex of *Y. enterocolitica* IX which cross-reacts with *Brucella* spp. agglutinogens.

Diaz, Jones, Leong & Wilson (1968) considered the agglutinogen complex of smooth brucellas to be equivalent to the phenol-soluble lipopolysaccharide-protein complex of the organisms. Subsequently Diaz, Lacalle, Medrano & Leong (1970) reported that a phenol-soluble endotoxin component from *Y. enterocolitica* IX cross-reacted serologically with *Brucella* spp. agglutinogens. More recently it has been found that the cross-reacting antigen complex of *Y. enterocolitica* IX contains two components reacting with antisera to *Br. abortus* (M. J. Corbel, to be published). Only one of these appears to be strongly agglutinogenic, however, and this is similar in properties to the lipopolysaccharide described by Diaz *et al.* (1970).

The nature of the antibody response evoked by bacterial lipopolysaccharides varies unpredictably between strains and to some extent with the species of animal used for inoculation. Thus in man, *Salmonella typhi* endotoxin only evokes IgM antibodies even on repeated injection (LoSpalluto, Miller, Dorward & Fink, 1962). Similar observations have been made on other *Salmonella* and *Proteus* strains, although the lipopolysaccharides of some evoke both IgM and IgG antibodies (Bauer & Stavitsky, 1961; Pike & Schulze, 1964; Jonas, 1969; Smith, Barnett & Sanford, 1970).

The serological diagnosis of bovine brucellosis is largely based upon observations that infection is associated with complement-fixing IgG antibodies, whereas residual vaccinal antibodies are of agglutinating, but non-complement-fixing IgM type (Anderson, Jenness, Brumfield & Gough, 1964; Schimmel & Erler, 1967). Thus the nature of the cross-reacting antibodies evoked by *Y. enterocolitica* IX is obviously of potential significance in relation to the diagnosis of bovine brucellosis. The object of the present study was to characterize the antibodies produced by cattle in response to experimental inoculation with *Y. enterocolitica* IX.

MATERIALS AND METHODS

Antigens

The bacterial strains and agglutinating antigens used were as described by Corbel & Cullen (1970) except that the *Y. enterocolitica* IX suspensions were standardized to give 50% agglutination with a 1/500 dilution of the International Standard *Brucella abortus* antiserum. Lipopolysaccharide was extracted from *Y. enterocolitica* IX organisms using 88% (w/v) phenol essentially according to Westphal, Lüderitz & Bister (1952). After prolonged dialysis against distilled water, the soluble material recovered from the phenol phase was chromatographed on Sephadex 4B (Pharmacia, Uppsala) according to Romanowska (1970). Fractions appearing immediately after the void volume were concentrated by drying from the frozen state. Lipopolysaccharide was extracted from *Br. abortus* strain 99 according to Leong *et al.* (1970) and purified as for *Y. enterocolitica* IX lipopolysaccharide.

Antisera

Bovine antisera to *Y. enterocolitica* IX were obtained from cattle inoculated as described by Corbel & Cullen (1970). The anamnestic response to this organism

was examined by injection of a second dose of *ca.* 10^{11} organisms by the subcutaneous route *ca.* 12 weeks after the primary injection. Blood samples were collected at frequent intervals thereafter.

Antisera to *Br. abortus* strain 19 and to virulent field strains of *Br. abortus* biotype 1 were obtained as described previously (Corbel & Cullen, 1970). In addition, bovine antisera to the *Br. abortus* strain 544 were prepared by subcutaneous injection of *ca.* 10^{11} viable organisms followed by collection of blood samples at daily intervals for the first 10 days and twice-weekly intervals thereafter.

Serological methods

The serum agglutination (SA) and complement fixation (CF) tests were performed according to standard procedures (Morgan *et al.* 1971). The Coombs antiglobulin and 2-mercaptoethanol tests were performed as described by Morgan (1967). Immunodiffusion tests against *Y. enterocolitica* IX and *Br. abortus* lipopolysaccharides and ultrasonic extracts were done according to Corbel & Cullen (1970) but using 1 % (w/v) Oxoid No. 1 agar in 1·0 M-NaCl as diffusion medium. Quantitative Rose Bengal plate (QRBP) tests using standard *Y. enterocolitica* IX and *Br. abortus* antigens were also done according to Corbel & Cullen (1970).

Serum fractionation

Density-gradient ultracentrifugation was performed essentially according to Cowan & Trautman (1965). Fractions were dialysed against phosphate-buffered saline (PBS; 0·15 M-NaCl, buffered at pH 7·2 with 0·01 M phosphate buffer) before testing. Ion-exchange chromatography on DEAE-cellulose was done essentially according to Porter & Noakes (1970).

Immuno-adsorption and elution of antibodies was achieved by absorption of 10 ml. volumes of serum with 1·0 ml. volumes of *Y. enterocolitica* IX or *Br. abortus* S99 suspensions (*ca.* 10^{12} organisms/ml.) at 37° C. for 2 hr. followed by overnight incubation at 4° C. After centrifugation at 15,000*g* for 5 min. the deposited organisms were washed by 3 cycles of centrifugation in PBS. Immunoglobulins were eluted by resuspension of the washed organisms in 0·15 M-NaCl buffered at pH 1·0 with formic acid-HCl buffer (Corbel, 1972).

Analytical methods

Protein was estimated according to Sutherland, Cori, Haynes & Olsen (1949) using crystallized bovine serum albumin (Sigma, London) as standard.

Disk electrophoresis was done according to Davis (1964). Immunoelectrophoresis was performed according to Scheidegger (1955).

RESULTS

The serological response to the cross-reacting antigen of Y. enterocolitica *IX*

As shown in Table 1, injection of cattle with *Y. enterocolitica* IX induced, after 6 days, formation of agglutinins reacting with the homologous organisms and with

Table 1. *The serological response of cattle inoculated with* Y. enterocolitica *IX to* Y. enterocolitica *IX and* Br. abortus *antigens*

		Reciprocal titres in tests with *Br. abortus* antigen							Reciprocal titres in tests with *Y. enterocolitica* IX antigen						
							Precipitins							Precipitins	
Day	Sample	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps
0	Y 1	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	Y 2	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	Y 3	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	Y 4	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
6	Y 1	40	40	< 10	< 2	< 1	0	0	80	80	10	< 2	4	0	0
	Y 2	40	40	< 10	< 2	< 1	0	0	80	80	< 10	< 2	< 1	0	0
	Y 3	160	160	10	20	8	0	0	1280	1280	80	20	16	0	0
	Y 4	160	160	10	20	1	0	0	160	160	< 10	10	8	0	0
10	Y 1	40	40	< 10	< 2	< 1	0	0	80	80	20	< 2	1	0	0
	Y 2	40	40	< 10	< 2	< 1	0	0	80	80	10	< 2	2	0	0
	Y 3	160	320	20	10	8	0	1	640	2560	80	10	64	0	1
	Y 4	320	320	20	10	8	0	0	640	1280	40	10	64	0	1
14	Y 1	40	40	10	< 2	2	0	0	80	80	40	< 2	4	> 1	0
	Y 2	40	40	20	< 2	< 1	0	0	80	80	40	2	2	1	0
	Y 3	160	320	40	20	8	0	1	640	2560	160	20	256	> 2	1
	Y 4	320	320	40	10	8	0	0	640	2560	160	20	512	> 2	1
28	Y 1	40	80	10	< 2	1	0	0	80	80	10	< 2	1	> 1	0
	Y 2	20	40	10	< 2	< 1	0	0	40	40	10	< 2	< 1	> 1	0
	Y 3	80	160	10	< 2	1	0	0	320	640	40	2	4	> 2	1
	Y 4	40	160	10	< 2	1	0	0	80	160	80	< 2	4	> 2	1
49	Y 1	20	40	10	< 2	1	0	0	40	40	< 10	< 2	1	> 1	0
	Y 2	20	20	10	< 2	< 1	0	0	40	40	10	< 2	2	1	0
	Y 3	40	160	10	10	1	0	0	80	80	10	< 2	2	> 2	0
	Y 4	80	160	10	< 2	1	0	0	80	80	< 10	< 2	2	> 1	0

ssa = subsurface antigens. lps = lipopolysaccharide antigen. * 2-Mercaptoethanol reduction test.

Br. abortus. These agglutinins were largely destroyed by reduction with 2-mercaptoethanol. Antibodies active in the Coombs antiglobulin, RBP, CF and immunodiffusion tests were also detectable at this stage in two of the four sera tested.

On the tenth day after inoculation, the agglutinin titres to both organisms had increased but the agglutinating activity was only partially susceptible to reduction. Antibodies active in the Coombs antiglobulin test and RBP test were detectable to low titre but CF and precipitating activities were absent. The titres in all tests continued to increase until the fourteenth day when they reached their maximum. At this stage precipitating antibodies to both *Y. enterocolitica* IX and *Br. abortus* were detectable by the immunodiffusion test. After this time the antibody titres in all tests steadily declined but the CF and precipitin titres declined more rapidly than the agglutinating antibodies. At this stage and until the titres were finally unmeasurable, the agglutinating activity was partly stable to 2-mercaptoethanol reduction. The agglutination reactions declined to insignificant titres within 2 months of inoculation. The antibodies which persisted for longest were those detectable by the antiglobulin test. However, this may have merely reflected the relatively high sensitivity of this test.

The serological response to the cross-reacting antigen of Br. abortus

The response to infection with both virulent and attenuated strains of *Br. abortus* was very different from that to *Y. enterocolitica* IX with respect to the chronological development of titres in the various tests and in the persistence of these reactions.

As shown in Table 2, cattle inoculated with *Br. abortus* strains 19 or 544 responded by producing agglutinins within 6 days. These were labile to 2-mercaptoethanol but by the tenth day most of the agglutinating activity was reduction-stable. Antibodies active in the CF and antiglobulin tests were detectable by the sixth day, increasing in titre until reaching a maximum at the end of the fourth week after inoculation with *Br. abortus* strain 19. Thereafter the titres declined slowly over a period of 3 months but were still significantly raised at the end of 6 months. The antibodies active in the CF and antiglobulin tests produced in response to inoculation with *Br. abortus* strain 544 showed a similar trend but did not reach a maximum until 6 weeks after inoculation. They underwent only a marginal decline over the succeeding 6 months.

Precipitins became detectable during the second week after inoculation with either *Br. abortus* strain 19 or 544, those reacting with the lipopolysaccharide antigens of *Y. enterocolitica* IX and *Br. abortus* becoming detectable before antibodies to the intracellular antigens of *Br. abortus*. Precipitins to the intracellular antigens of *Y. enterocolitica* IX did not develop. The precipitin patterns to *Br. abortus* antigens increased in complexity over the succeeding 2–3 months and remained apparently unchanged thereafter in animals inoculated with *Br. abortus* strain 544. Sera from cattle vaccinated with *Br. abortus* strain 19 did not possess precipitins when examined 2 years after vaccination.

Comparison of the results of the QRBP test on sera from cattle inoculated with *Br. abortus* with those for sera from *Y. enterocolitica* IX-inoculated animals

Table 2. *The serological response of cattle inoculated with* Br. abortus *strain* 19 *or* 544 *to* Y. enterocolitica *IX and* Br. abortus *antigens*

		Reciprocal titres in tests with *Br. abortus* antigen							Reciprocal titres in tests with *Y. enterocolitica* IX antigen						
							Precipitins							Precipitins	
Day	Sample	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps
0	B 1†	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	B 2†	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	B 3‡	10	< 10	< 10	< 2	< 1	0	0	10	< 10	< 10	< 2	< 1	0	0
	B 4‡	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
6	B 1	80	160	< 10	4	2	0	0	80	80	< 10	< 2	2	0	0
	B 2	160	160	10	10	4	0	0	160	160	< 10	< 2	2	0	0
	B 3	80	160	< 10	4	2	0	0	80	80	< 10	< 2	2	0	0
	B 4	80	80	< 10	10	4	0	0	80	80	< 10	< 2	2	0	0
10	B 1	320	1280	160	40	8	0	1	320	640	40	2	4	0	1
	B 2	320	1280	320	200	16	0	1	320	640	80	40	16	0	1
	B 3	160	320	20	10	8	0	1	160	160	< 10	2	4	0	1
	B 4	80	160	< 10	10	4	0	1	80	80	< 10	2	4	0	0
14	B 1	640	2560	320	200	16	0	1	640	2560	160	80	16	0	1
	B 2	1280	5120	640	200	64	1–2	1	640	2560	160	80	64	0	1
	B 3	640	640	160	80	16	0	1	640	640	40	10	16	0	1
	B 4	160	320	20	10	8	0	1	160	320	< 10	4	8	0	1
28	B 1	640	2560	320	200	32	1–2	1	640	2560	80	80	16	0	1
	B 2	640	5120	320	200	64	2	1	640	2560	160	80	32	0	1
	B 3	320	1280	160	80	32	1	1	320	640	80	20	16	0	1
	B 4	160	320	160	10	16	1	1	160	320	20	4	16	0	1
49	B 1	160	640	160	40	16	> 1	1	160	640	40	10	16	0	1
	B 2	160	1280	160	40	16	2	1	160	640	40	10	16	0	1
	B 3	320	1280	320	200	64	> 2	1	320	1280	160	80	64	0	1
	B 4	320	1280	320	200	64	1	1	320	1280	160	80	64	0	1

* 2-Mercaptoethanol reduction test. ssa = subsurface antigens. lps = lipopolysaccharide antigen.

† B 1; B 2: inoculated with *Br. abortus* strain 19. ‡ B 3; B 4: inoculated with *Br. abortus* strain 544.

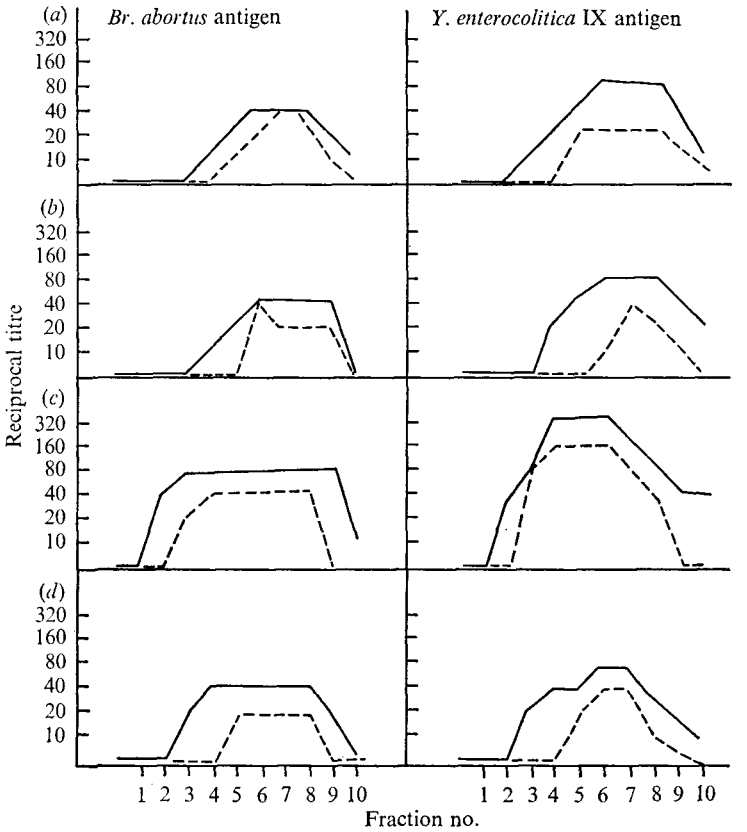

Fig. 1. Density-gradient centrifugation of bovine antisera to *Y. enterocolitica* IX. (*a*) Y 1, (*b*) Y 2, (*c*) Y 3, (*d*) Y 4, at 14 days post-inoculation. ——, SAT; – – – –, 2-Me.

(Tables 1, 2) showed that from the time of the RBPT becoming positive, antibodies to the two organisms could be differentiated on the basis of their titres to the two Rose Bengal stained antigens.

Characterization of the immunoglobulin classes of antibodies to the cross-reacting antigens of Br. abortus *and* Y. enterocolitica *IX*

Density-gradient centrifugation of serum collected from cattle at intervals after injection with *Y. enterocolitica* IX or *Br. abortus* gave results essentially consistent with those of the 2-mercaptoethanol tests. Thus both organisms initially evoked fast-sedimenting 2-mercaptoethanol-labile cross-reacting antibodies, which by the tenth day after inoculation were supplemented by slowly sedimenting 2-mercaptoethanol-stable antibodies. In *Y. enterocolitica* IX-inoculated cattle, the agglutinins remained of the slowly sedimenting type until they became undetectable (Fig. 1*a–d*). Similarly, in *Br. abortus*-inoculated cattle the agglutinins remained of the slowly sedimenting type but did not decline to undetectable levels in the period studied (Fig. 2*a–d*).

Ion-exchange chromatography of bovine antiserum to *Y. enterocolitica* IX

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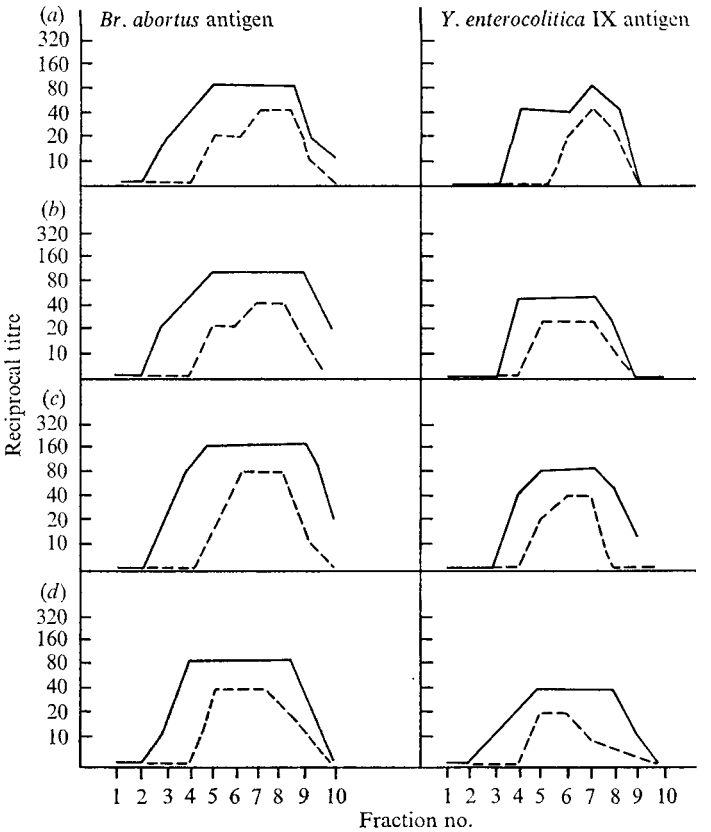

Fig. 2. Density-gradient centrifugation of bovine antisera to *Br. abortus* (*a*) B1, (*b*) B2, (*c*) B3, (*d*) B4 collected 49 days post-inoculation. ——, SAT; – – – –, 2-Me.

collected 14 days after inoculation showed that most of the 2-mercaptoethanol-stable agglutinating activity for *Br. abortus* was distributed between fractions 2 and 3, consisting mainly of IgG_1, with some IgA in fraction 3. Fractions 1, 2 and 3 contained most of the activity detectable in the Coombs antiglobulin test. An appreciable amount of agglutinating activity was eluted in fraction 5, which contained most of the serum IgM. This was largely susceptible to reduction with 2-mercaptoethanol (Table 3).

The results obtained on fractionation of bovine antiserum to *Br. abortus* collected 14 days after inoculation were qualitatively similar to those for the anti-*Y. enterocolitica* IX serum, with a high proportion of the agglutinins reacting with *Y. enterocolitica* IX being eluted in fractions 3, 4 and 5 (Table 3). The elution profiles for the two sera (Fig. 3) were essentially similar.

Attempts were also made to characterize the immunoglobulins involved in the cross-reaction by eluting antibodies absorbed by the heterologous organisms. No success was obtained with sera collected before the tenth day after inoculation, probably because of the low antibody concentrations present, but immunoglobulins were recovered in eluates from organisms treated with high-titre sera.

Eluates prepared from *Br. abortus* cells used to absorb antiserum to *Y. entero-*

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Table 3. *Serological activity of fractions separated from bovine antisera to* Y. enterocolitica *IX and* Br. abortus *by ion-exchange chromatography on* DEAE-*cellulose*

		Reciprocal titres in tests with *Br. abortus* antigen							Reciprocal titres in tests with *Y. enterocolitica* IX antigen						
							Precipitins							Precipitins	
Serum	Fraction no.	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps
Y 3,	1	< 10	160	< 10	< 2	< 1	0	0	< 10	320	< 10	< 2	< 1	1	1
14 days	2	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	3	80	640	80	20	4	0	1	320	1280	320	20	32	2	1
	4	10	80	< 10	10	2	0	0	10	80	< 10	< 2	2	0	0
	5	20	80	10	< 2	< 1	0	0	40	160	10	< 2	< 1	0	0
	6	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	7	40	80	10	< 2	< 1	0	0	80	160	10	< 2	< 1	0	0
B 2,	1	< 10	640	< 10	< 2	< 1	1	1	< 10	640	< 10	< 2	< 1	0	1
14 days	2	< 10	10	< 10	< 2	< 1	0	0	< 10	10	< 10	< 2	< 1	0	0
	3	160	2560	160	200	64	2–3	1	160	1280	160	80	32	0	1
	4	10	80	10	20	8	1	1	10	80	10	10	8	0	1
	5	40	160	10	4	2	0	0	40	160	< 10	< 2	2	0	0
	6	< 10	< 10	< 10	< 2	< 1	0	0	< 10	< 10	< 10	< 2	< 1	0	0
	7	80	80	10	2	< 1	0	0	80	80	< 10	< 2	< 1	0	0

* 2-Mercaptoethanol reduction test. ssa = sub-surface antigens. lps = lipopolysaccharide antigen.

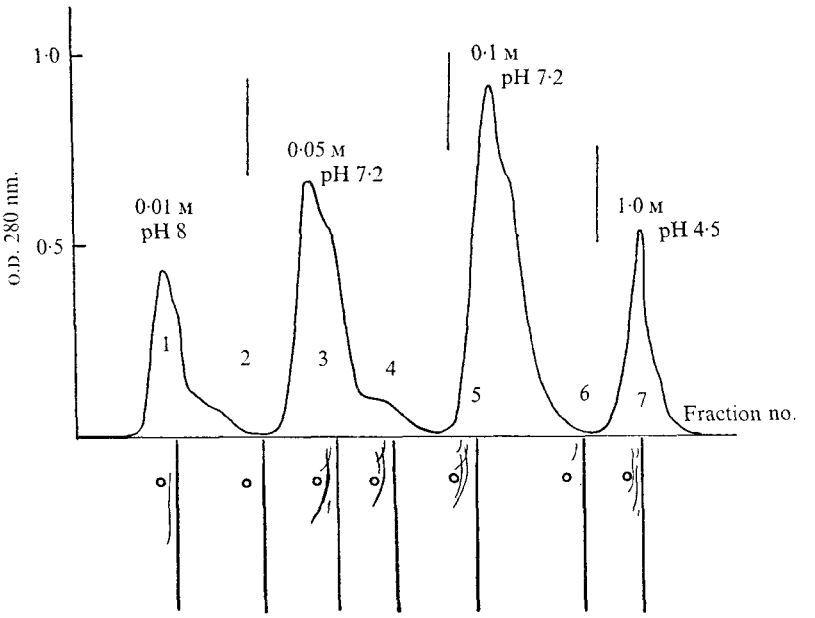

Fig. 3. Ion-exchange chromatography on DEAE-cellulose of bovine antiserum to *Y. enterocolitica* IX.

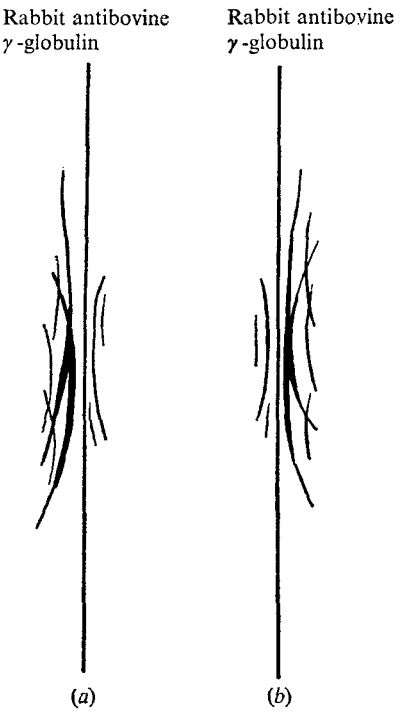

Fig. 4. (*a*) Immunoelectrophoresis of bovine serum (left) and eluate from *Br. abortus* cells used to absorb bovine antiserum to *Y. enterocolitica* IX (right). (*b*) Immunoelectrophoresis of eluate from *Y. enterocolitica* IX cells used to absorb bovine antiserum to *Br. abortus* (left) and bovine serum (right).

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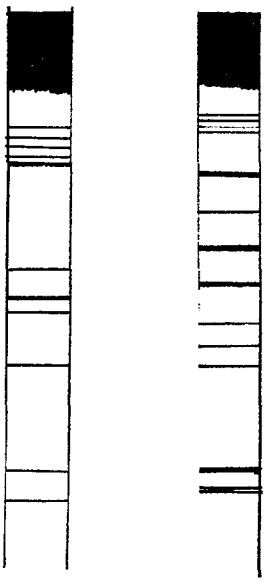

Fig. 5. Disk electrophoretic patterns of (*a*) eluate from *Br. abortus* cells used to absorb bovine antiserum to *Y. enterocolitica* IX. (*b*) eluate from *Y. enterocolitica* IX cells used to absorb bovine antiserum to *Br. abortus*.

colitica IX, contained immunoglobulins of IgA, IgG_1 and IgG_2 classes (Fig. 4*a*). IgM may have been present but was in too low concentration to be detectable by immunoelectrophoresis. A very similar immunoelectrophoretic pattern was obtained with eluates from the *Y. enterocolitica* IX cells used to absorb antiserum to *Br. abortus* (Fig. 4*b*).

Disk electrophoresis of the eluates confirmed their complexity (Fig. 5). The fast-migrating components were probably extracted from the bacterial cells by the acid buffer.

The anamnestic response to the cross-reacting antigen of Y. enterocolitica *IX*

Injection of cattle previously inoculated with *Y. enterocolitica* IX with a second dose of organisms after the initial antibody response had declined to insignificant titres, produced a rapid response with 2-mercaptoethanol-stable antibodies predominating at the sixth day. Precipitins and CF antibodies to both *Y. enterocolitica* IX and *Br. abortus* antigens were also detectable at this time. However, even though quite high antibody titres to *Br. abortus* were obtained, these declined rapidly as in the primary response (Table 4).

DISCUSSION

The nature of the serological response to the cross-reacting antigen of *Y. enterocolitica* IX was considered significant in relation to its possible effects on diagnostic tests for brucellosis. It was clear from both the present results and earlier studies,

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Table 4. *The serological response of cattle previously inoculated with* Y. enterocolitica *IX to secondary stimulation with* Y. enterocolitica *IX cells*

		Reciprocal titres in tests with *Br. abortus* antigen							Reciprocal titres in tests with *Y. enterocolitica* IX antigen						
							Precipitins							Precipitins	
Day*	Sample	SAT	Anti-globulin (Coombs)	2-ME†	CFT	QRBPT	ssa	lps	SAT	Anti-globulin (Coombs)	2-ME*	CFT	QRBPT	ssa	lps
1	Y 1	10	20	< 10	< 2	< 1	0	0	40	40	< 10	< 2	1	1	0
	Y 2	20	40	< 10	< 2	< 1	0	0	40	40	10	< 2	1	1	0
	Y 3	40	40	10	4	1	0	0	80	80	10	< 2	2	≥ 1	0
	Y 4	80	160	< 10	< 2	< 1	0	0	80	80	< 10	< 2	2	1	0
6	Y 1	20	80	10	2	2	0	0	80	160	40	4	4	1–2	1
	Y 2	40	160	10	10	2	0	0	80	320	80	10	8	1–2	1
	Y 3	80	160	40	20	8	0	1	640	2560	320	20	32	2	1
	Y 4	160	320	40	20	8	0	1	640	2560	320	20	64	1–2	1
30	Y 1	20	40	10	< 2	< 1	0	0	80	640	40	< 2	1	1	0
	Y 2	20	80	10	< 2	< 1	0	0	80	320	40	< 2	2	1	0
	Y 3	40	160	20	< 2	1	0	0	160	640	80	4	8	2	1
	Y 4	40	80	20	2	2	0	0	320	1280	160	4	16	1	1

* Day after second inoculation with *Y. enterocolitica* IX cells. ssa = subsurface antigens. lps = lipopolysaccharide antigens.

† 2-Mercaptoethanol reduction test.

(Ahvonen *et al.* 1969; Corbel & Cullen, 1970; Hurvell *et al.* 1971; Akkermans & Hill, 1971) that, in all species studied, *Y. enterocolitica* IX evoked antibodies which superficially appeared indistinguishable from those provoked by infection with *Br. abortus*.

However, it was apparent from the present results that, although *Y. enterocolitica* IX could stimulate production of high titres of agglutinins to *Br. abortus*, it was relatively less effective in evoking CF and precipitating antibodies. Furthermore, unlike those provoked by *Br. abortus* infection, these cross-reacting antibodies were transient and rapidly declined to insignificant levels. Thus it was unlikely that *Y. enterocolitica* IX would cause an animal to become a persistent reactor to diagnostic tests for brucellosis. This meant that, with the present system of repeated tests, it would be unlikely to produce serious difficulty in the assessment of cattle for accreditation to brucellosis-free herds.

The nature of the antibodies produced in response to the cross-reacting antigen of *Y. enterocolitica* IX was clearly not significantly different in type from those evoked by *Br. abortus*. In both instances the fractionation results showed that 19 S IgM and 7 S IgG_1, IgG_2 and probably IgA antibodies were produced. This was not entirely unexpected in view of the similar serological activities which could be demonstrated in response to both antigens. However, as already indicated, the nature of the antibody response engendered by the agglutinogens of even closely related organisms cannot be predicted *a priori*. Thus the results of Smith *et al.* (1970) showed that the carbohydrate residues of lipopolysaccharide antigens, although largely determining serological specificity, did not determine the nature of the antibody response. Recent evidence (Lüderitz, Galanos & Rietschel, 1971) has suggested that the lipid A component may be significant in this respect.

Some evidence is available which suggests that brucella strains may vary in the pattern of the immunoglobulin response they elicit. Thus Howe (1970) observed that, in *Br. canis* infection in the dog, antibodies to the lipopolysaccharide antigen were almost entirely of the IgM class, whereas antibodies to the nucleoprotein and intracellular antigens were of IgG class. In *Br. suis* infections in man the antibodies produced against the lipopolysaccharide agglutinogen were predominantly IgG at all stages of infection (Howe, 1970). In *Br. abortus* infections in cattle, sequential production of IgM and IgG antibodies to the agglutinogen has been observed in both this and other studies (Rose, Lambert & Roepke, 1964; Rice, Tailyour & Cochrane, 1966). It appears from the present results that the cross-reacting antigen of *Y. enterocolitica* IX elicits a qualitatively similar response, the transient nature of which may be attributed to the minimal persistence of this organism. Ahvonen *et al.* (1969) noted that in human infections with *Y. enterocolitica* IX, the agglutinins cross-reacting with brucellas usually underwent rapid decline.

Although *Y. enterocolitica* IX has not been shown to infect cattle under natural conditions, it would appear that even should this occur persistent false positive reactions would not arise in the animals infected.

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