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Differentiation of the serological response to *Yersinia enterocolitica* and *Brucella abortus* in cattle

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SUMMARY

The serological responses of cattle to inoculation with *Brucella abortus* and *Yersinia enterocolitica* type IX were compared. Complete cross-reactions were found in serum agglutination, antiglobulin, complement fixation and Rose Bengal plate tests. The cross-reaction between *Br. abortus* and *Y. enterocolitica* IX was confirmed by immunodiffusion tests. Although antibodies specific for each organism could also be detected by immunodiffusion tests with high titre rabbit or bovine sera, these tests were insufficiently sensitive for routine diagnostic use.

A quantitative Rose Bengal plate test, using Rose Bengal stained *Br. abortus* and *Y. enterocolitica* IX, was developed which enabled the antibody responses to the two organisms to be differentiated. The specificity of this test was confirmed by cross-absorption experiments and its sensitivity was sufficient to permit evaluation of all bovine sera giving positive reactions to the serum agglutination test.

INTRODUCTION

Serological cross-reactions between organisms of the genus *Brucella* and those of other genera, notably *Pasteurella*, *Francisella* and *Vibrio*, have been reported in the past (Mallmann, 1930; Morse, Ristic, Robertstad & Schneider, 1953). Most of these reactions have been marked by quantitatively lower titres to the heterologous organism. However, recently Ahvonen, Jansson & Aho (1969) demonstrated strong serological cross-reactions between *Brucella abortus* and strains of *Yersinia enterocolitica* (Frederiksen, 1964) of serological type IX. Ahvonen & Sievers (1969) also observed the development of high titres of brucella agglutinins in the sera of patients infected with *Y. enterocolitica* IX. These observations were made in Finland, a country from which bovine brucellosis has been eradicated (Huhtala, 1963).

Y. enterocolitica, previously known as *Pasteurella* X or *Bacterium enterocoliticum* (Schleifstein & Coleman, 1943), is distinct from brucella in a number of morphological, cultural and biochemical characteristics (Mollaret & Chevalier, 1964). The organism is widely distributed and has been isolated from man and other animals, including hares, chinchillas, pigs, dogs, cattle and the bush-baby (Dickinson & Mocquot, 1961; Becht, 1962; Daniels, 1963; Mollaret & Lucas, 1965; Niléhn, 1967; Goyon, 1969; Mair, Schubert & Harbourne, 1970). Most isolations

appear to have been reported from Northern Europe and North America. Hitherto the organism has not been recovered from cattle in Great Britain. However, the serological relationship of brucellas to other organisms is obviously of considerable significance in the diagnosis of human and bovine brucellosis, particularly in relation to brucella eradication schemes. It was the object of the present work to examine the cross-reactions between *Y. enterocolitica* IX and brucellas in the various serological tests used in the diagnosis of bovine brucellosis, and if possible to devise a means of differentiating between the serological responses to the two organisms.

MATERIALS AND METHODS

Bacterial strains

Yersinia *strains*. A strain of *Yersinia enterocolitica* type IX was kindly donated by Dr P. Ahvonen, of the Municipal Bacteriological Laboratory, Helskini, Finland, as a freeze-dried culture. On examination it conformed to the biochemical and cultural characters described by Mollaret & Chevalier (1964) and in these respects was identical with another strain of *Y. enterocolitica* (N.C.T.C. 10,461) obtained from the National Collection of Type Cultures, Colindale. Serologically, the Finnish strain was grouped as *Y. enterocolitica* type IX. It did not auto-agglutinate in 0·85% saline nor in acriflavine solutions (Alessandrini & Sabatucci, 1931) and was apparently a smooth strain.

Brucella *strains*. *Br. abortus* strain 99 was used for preparation of the antigens used throughout most of this work. Animal inoculations were performed with *Br. abortus* strain 19 and some serological tests were done with antigens prepared from *Br. melitensis* strain 16M, *Br. neotomae* 5K33 and *Br. suis* strain 1330. All brucella strains were from the *Brucella* type culture collection maintained at this laboratory.

Preparation of antigens

Br. abortus strains 19 and 99 were grown in continuous culture and harvested according to Boyce & Edgar (1966).

Other brucella strains and *Y. enterocolitica* IX were grown on serum–dextrose agar in Roux flasks. Brucella cultures were grown at 37° C. for 3 days and *Y. enterocolitica* IX cultures at 22° C. for 18 hr. Organisms were harvested in 0·85% saline, killed by exposure to 0·4% β-propiolactone (LoGrippo & Hartman, 1955) and freed of growth medium by repeated washing with saline.

Standard *Br. abortus* agglutination suspensions were prepared according to WHO monograph no. 19 (1953) and were standardized to give 50% agglutination with 1/500 dilution of the International Standard *Br. abortus* antiserum. *Y. enterocolitica* IX suspensions similarly prepared, were nephelometrically standardized to the same cell density as the standard *Br. abortus* suspensions. OH-antigen suspensions of *Y. enterocolitica* IX were also prepared as described by Winblad, Niléhn & Sternby (1966). O antigen suspensions were prepared from cultures grown at 37° C. and heated at 100° C. for 15 min.

Rose Bengal plate test (RBPT) antigens

Standardized suspensions of *Br. abortus* strain 99 stained with Rose Bengal and buffered at pH 3·65 were prepared according to U.S.D.A., National Animal Disease Laboratory, Diagnostic Reagents Manual 65 C (1965). Similarly stained and buffered suspensions of *Y. enterocolitica* type IX were prepared and standardized to the same packed cell volume as the brucella suspensions.

Soluble antigens

Washed suspensions of *Br. abortus* strain 99 or *Y. enterocolitica* type IX were suspended in 0·5 M-KCl containing 0·1 % cysteine hydrochloride and subjected to ultrasonic vibrations for two periods of 10 min. in a Soniprobe ultrasonic disintegrator (Dawe Instruments Ltd. London). Cell wall debris was removed by centrifugation at 20,000 ***g*** for 2 hr. and the supernatant dialysed against 0·85 % saline for 24 hr. The resulting solutions were adjusted to the same dry-weight concentration and used as antigens in the complement fixation (CF) test. Soluble antigens for immunodiffusion tests were produced by a similar process except that centrifugation of the disrupted organisms was at 20,000 ***g*** for 30 min.

Antisera used

Bovine antisera to *Br. abortus* were prepared by inoculation of two 18-month-old bullocks (B 1 and B 2) with single doses of $1{\cdot}8 \times 10^{11}$ viable *Br. abortus* strain 19 organisms by the subcutaneous route.

In addition anti-brucella sera were obtained from female calves vaccinated at between 3 and 6 months of age with the standard dose of *Br. abortus* strain 19 vaccine prepared at this laboratory. In each case blood samples were taken before inoculation and at weekly intervals after.

Bovine antisera to *Y. enterocolitica* type IX were prepared by inoculation of four 18-month-old bullocks (Y 1, Y 2, Y 3 and Y 4) by the subcutaneous route with 2 ml. volumes of a washed suspension of *Y. enterocolitica* IX in Ringer's solution. This suspension contained 3×10^{10} viable organisms per ml. as determined by the method of Miles & Misra (1938). Blood samples were taken at weekly intervals for 3 months, starting 1 week before inoculation.

Approximately 3 months after inoculation the animals were killed and attempts made to recover *Y. enterocolitica* IX from the viscera and lymph nodes.

Rabbit antisera to *Br. abortus* strain 99 (serum RB 1) and to *Y. enterocolitica* IX (serum RY 1) were prepared by injection of pairs of adult rabbits with *ca.* 10^{10} of the respective organism emulsified in Freund's incomplete adjuvant.

A single dose of antigen was given divided over several intramuscular and subcutaneous sites and the animals exsanguinated 30 days later. Sera from each pair of animals were pooled.

In addition to these experimentally produced sera, samples of bovine diagnostic sera received for testing in connexion with the Brucella (Accredited Herds) scheme were available. Sixty samples of human serum taken at routine examination of laboratory workers were also examined for the presence of antibody to *Brucella* and *Yersinia*.

Absorption of sera

Antibodies reacting with *Br. abortus* were absorbed by mixing 1 ml. of serum with 2 ml. of a washed suspension of *Br. abortus* strain 99, containing *ca.* 10^{12} organisms/ml, and incubating at 37° C. for 1 hr. The absorbed serum was recovered by centrifugation. Antibody reacting with *Y. enterocolitica* IX was absorbed by an identical procedure except that a washed suspension of this organism was used.

Serological tests

Serum agglutination tests. Serum agglutination (SA) tests using 0·5 % phenol-saline as diluent were done according to WHO Monograph 19 (1953). The titres were expressed as reciprocals.

Indirect (antiglobulin) agglutination tests. These were performed according to the Coombs, Mourant & Race (1945) procedure as modified by Brinley-Morgan (1967). Tests with human and bovine sera were performed with rabbit antisera to human and bovine IgG globulins respectively. Tests with rabbit sera were performed with sheep antiserum to crude rabbit globulin fractions.

Rose Bengal plate test. Spot tests with RBPT antigen were performed on 0·03 ml. unit volumes of serum according to Brinley-Morgan, MacKinnon, Lawson & Cullen (1969).

Quantitative tests with RBPT antigens were performed by making serial doubling dilutions of serum in 0·85 % saline and shaking 0.03 ml. volumes with equal volumes of antigen on a white tile. The highest dilution showing visible agglutination within 4 min. was taken as the titre of the serum.

Complement fixation tests. These were done using 0·1 ml. volumes of reagents in WHO pattern agglutination trays. Both soluble and cellular antigens were used, standardized to optimal titre. For the test three 50 % haemolytic doses of complement were used and the sheep erythrocytes were sensitized with four 100 % haemolytic doses of haemolysin. Fixation was carried out at 37° C. for 60 min. or, in some cases, at 4° C. for 18 hr. Tests were read to an end-point of 50 % haemolysis.

All sera for CF tests were inactivated by heating at 56° C. for 30 min.

Immunodiffusion tests. These were performed according to Ouchterlony (1953). The diffusion medium was 0·8% agarose (L'Industrie Biologique Française, S.A., Gennevilliers) in 0·85 % saline containing 0·1 % sodium azide.

Fluorescent antibody staining. The indirect method was used on smears of heat-fixed organisms (Cherry, Goldman & Carski, 1960). Primary staining was done with specific bovine antisera and secondary staining with fluorescein isothiocyanate-labelled rabbit antibovine globulin serum (Difco Laboratories, Detroit). Preparations were examined with a Leitz Ortholux fluorescent microscope.

RESULTS

Examination of a large number of bovine sera taken at random from samples submitted under the Brucellosis (Accredited Herds) scheme showed that all sera containing agglutinins for *Br. abortus* also agglutinated standard *Y. enterocolitica*

IX suspensions to the same or higher titre. In addition a few sera giving negative reactions with brucella antigen agglutinated *Y. enterocolitica* IX suspensions (Table 1). These results parallel those reported by Ahvonen *et al.* (1969) for human sera from patients with confirmed or suspected *Y. enterocolitica* IX infection.

Table 1. *Serum agglutination test titres of* 151 *cattle sera to* Y. enterocolitica *IX and* Br. abortus 99

Serum agglutinating test titres	Number of sera at specified titre	
	Yersinia antigen	Brucella antigen
< 10	49	76
10	19	26
20	31	10
40	12	4
80	7	12
160	4	5
320	12	6
640	4	8
1280	7	3
2560	1	1
5120	5	0
	151	151

Titres are expressed as reciprocals.

Examination of sera from cattle inoculated with *Br. abortus* or *Y. enterocolitica* IX under laboratory conditions also confirmed the reciprocal cross-reactions between the two organisms. Irrespective of which of the two organisms the animals were inoculated with the titre of the agglutinins to *Y. enterocolitica* IX was always equal to or higher than that to *Br. abortus* (Table 2). Similar results were obtained irrespective of whether the agglutinating antigen suspensions were standardized in terms of packed cell volume or turbidity, suggesting that the difference was not simply the result of differences in cell concentration.

Antisera to *Y. enterocolitica* IX, also agglutinated standard suspensions of *Br. melitensis* 16 M, *Br. suis* 1330 and *Br. neotomae* 5K 33 to similar titres to *Br. abortus*. Antisera rendered monospecific for *Br. abortus* by absorption with *Br. melitensis* agglutinated *Y. enterocolitica* IX, but monospecific antisera to *Br. melitensis* did not.

Examination of antisera to *Br. abortus* and *Y. enterocolitica* IX by the antiglobulin test also indicated a reciprocal cross-reaction. As shown in Table 2 the net increase in titre relative to the SA test titre was essentially similar for both the brucella and yersinia inoculated groups when tested against either antigen.

The indirect fluorescent antibody (IFA) test, as expected, gave results compatible with the antiglobulin test. Both organisms showed bright peripheral staining with anti-*Br. abortus* and anti-*Y. enterocolitica* IX sera. There was no staining with pre-inoculation sera at the same dilution.

The results of CF tests with bovine antisera did not indicate complete reciprocal

cross-reaction between the two organisms. As shown in Table 3, sera from animals infected with either *Br. abortus* or *Y. enterocolitica* IX reacted with brucella antigens in CF tests. However, some cattle sera from both these groups failed to react with yersinia antigen in CF tests. In the case of high-titre rabbit antisera, positive reactions were obtained with yersinia antigens but titres were lower than those obtained with brucella antigens and titres to the soluble antigen were lower than those to the intact cell suspension. The use of the CF test did not allow the antibody responses to the two organisms to be distinguished.

Table 2. *Serum agglutination and antiglobulin test titres of anti-Brucella and anti-Yersinia sera to* Br. abortus *and* Y. enterocolitica *IX antigens*

	Serum agglutination test		Antiglobulin test	
Serum	Brucella antigen	Yersinia antigen	Brucella antigen	Yersinia antigen
S19 calves			ND	ND
S1	10	40	ND	ND
S2	20	20	ND	ND
S3	320	640	ND	ND
S4	160	640	ND	ND
S5	80	640	ND	ND
S6	80	160	ND	ND
S7	320	640	ND	ND
S8	640	2,560	2,560	> 10,240
S9	1,280	5,120	5,120	> 10,240
S10	160	160	640	5,120
S19 bullocks				
B1	320	640	1,280	5,120
B2	320	320	1,280	5,120
Field samples				
F1	20	40	20	80
F2	10	20	40	40
F3	20	40	20	160
F4	80	160	80	1,280
F5	640	640	5,120	5,120
F6	320	640	1,280	10,240
F7	80	640	1,280	5,120
F8	80	160	1,280	5,120
F9	160	320	640	5,120
F10	80	320	160	1,280
Yersinia bullocks				
Y1	20	160	80	5,120
Y2	40	640	320	> 10,240
Y3	80	1,280	640	> 10,240
Y4	80	640	320	> 10,240
Rabbit sera				
RB1 (anti-Brucella)	640	1,280	2,560	5,120
RY1 (anti-Yersinia)	2,560	10,240	> 10,240	> 10,240

ND = Not done.
Titres are expressed as reciprocals.

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Sera from animals injected with either *Br. abortus* or *Y. enterocolitica* IX both gave positive reactions to the RBP test. Similar results were obtained using a Rose Bengal stained *Y. enterocolitica* IX antigen (RBY). However, it was thought that quantitative differences might exist in the reaction of *Brucella* and *Yersinia* infected animals to these tests.

Tests performed on serial doubling dilutions of sera from these groups of animals confirmed this (Table 4). All samples of sera from cattle and rabbits inoculated with *Br. abortus* gave titres to RBBr. and RBY which were either identical or showed a slightly higher titre for the RBBr. antigen. All samples of sera from cattle and rabbits inoculated with *Y. enterocolitica* IX gave titres to RBY antigen which were significantly higher than those to RBBr. Examination of

Table 3. *Complement-fixation titres of anti-Brucella and anti-Yersinia sera to* Br. abortus *and* Y. enterocolitica *IX antigens*

	Whole cell antigen		Soluble antigen	
Serum	Brucella	Yersinia	Brucella	Yersinia
S 19 calves				
S 1	2	—	—	—
S 2	2	—	—	—
S 3	32	16	16	4
S 4	16	8	4	—
S 5	64	32	16	4
S 6	64	32	64	8
S 19 bullocks				
B 1	128	64	32	8
B 2	128	64	16	8
Yersinia bullocks				
Y 1	2	—	—	—
Y 2	8	4	8	—
Y 3	16	8	8	4
Y 4	16	16	16	8
Rabbit sera				
RB 1	512	32	256	64
RY 1	512	256	512	256

Titres are expressed as reciprocals.

approximately 150 sera from cattle with positive titres for *Brucella* ($> 1/40$ in the SA test or $> 50\ \%$ fixation at 1/4 in the CF test) and which were in some cases confirmed by isolation of the organism, gave results similar to those obtained in this test with sera from animals experimentally infected with *Br. abortus*. No false positive reactions were given by either RBBr or RBY antigen in tests on approximately 100 brucella-negative cattle sera or on 60 brucella-negative human serum samples.

The specificity of the quantitative RB tests in distinguishing antibodies to *Brucella* and *Y. enterocolitica* IX was confirmed by cross-absorption tests performed on antisera to the two organisms. The results summarized in Table 4 showed that absorption of anti-yersinia sera with *Br. abortus* removed antibodies

reacting to this organism in the quantitative RBT but did not eliminate the reaction to RBY antigen although the titres were substantially reduced. Absorption of the sera with *Y. enterocolitica* IX eliminated antibody to both organisms.

Absorption of anti-brucella sera with *Y. enterocolitica* IX removed antibody to both organisms as did absorption with *Brucella*. Thus the cross-reactions were not completely reciprocal although compatible with the results obtained in quantitative RBP tests with the two antigens.

Table 4. *Quantitative Rose Bengal test titres of absorbed and unabsorbed antisera to* Br. abortus *and* Y. enterocolitica *IX*

	Unabsorbed		Brucella absorbed		Yersinia absorbed	
Serum	RB Br. titre	RBY titre	RB Br. titre	RBY titre	RB Br. titre	RBY titre
Cattle						
Anti-brucella sera						
B1	32	16	—	—	—	—
B2	32	32	—	—	—	—
F4	4	4	—	—	—	—
F5	64	32	—	—	—	—
F6	32	32	—	—	—	—
F8	4	4	—	—	—	—
S5	4	4	—	—	—	—
S6	4	4	—	—	—	—
S9	64	64	—	—	—	—
S10	8	4	—	—	—	—
Anti-yersinia sera						
Y1	2	4	—	1	—	—
Y2	2	32	—	4	—	—
Y3	8	256	—	16	—	—
Y4	8	512	—	16	—	—
Rabbit						
RB1 anti-brucella	32	32	—	—	—	—
RY1 anti-yersinia	32	512	—	32	—	—

Titres are expressed as reciprocals.

Brucella-absorbed anti-yersinia sera still agglutinated OH-suspensions of *Y. enterocolitica* IX indicating that the residual agglutinins not absorbed by *Br. abortus* were H-specific (Table 5).

Attempts were also made to distinguish the antibody response to the two organisms by immunodiffusion tests. These results shown in Pl. 1, figs. 1 and 2 confirmed the cross-reaction between *Brucella* spp. and *Y. enterocolitica* IX. In fig. 1 the reaction of soluble extracts of *Br. abortus* (*Br. ab.*) and *Y. enterocolitica* IX (*Y.e.*IX) is studied. The line pattern components (l.p.c.) 1 and 2 appear specific to the *Y.e.*IX–anti-*Y.e.*IX system, whereas l.p.c. 3 is common to the *Y.e.*IX–anti-*Y.e.*IX and *Y.e.*IX–anti-*Br. ab.* systems. L.p.c. 4, 5 and 6 appear specific to the *Br. ab.*–anti-*Br. ab.* system but l.p.c. 7 is common to both this and the *Br. ab.*–

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anti-*Y.e.*IX systems. L.p.c. 3 and 7 appear to merge but do not give reactions of complete identity.

In fig. 2, the reaction of soluble extracts of *Y.e.*IX, *Br. ab.*, *Br. melitensis* (*Br. mel.*) and *Br. suis* with anti-*Y.e.*IX serum is studied. Multiple l.p.c. 1, 2, 3, 4, are seen in the *Y.e.*IX–anti-*Y.e.*IX systems; single l.p.c. 5, 6 and 7 are seen in the *Br. ab.*–anti-*Y.e.*IX, *Br. mel.*–anti-*Y.e.*IX and *Br. suis*–anti-*Y.e.*IX systems respectively. The l.p.c. in *Br. ab.* and *Br. suis* appear to correspond to components of low diffusibility, that in *Br. mel.* to a component similar to the cross-reacting antigen of *Y.e.*IX.

Table 5. *Serum agglutination test titres of absorbed and unabsorbed anti-yersinia and anti-brucella sera to O and OH* Y. enterocolitica *IX antigens*

	Unabsorbed titre		Brucella absorbed		Yersinia absorbed	
Serum	OH	O	OH	O	OH	O
Cattle						
Anti-brucella						
S 8	2,560	2,560	—	—	—	—
F 5	640	640	—	—	—	—
B 1	640	640	—	—	—	—
S 9	5,120	5,120	—	—	—	—
Anti-yersinia						
Y 1	160	20	40	—	—	—
Y 2	640	40	160	—	—	—
Y 3	1,280	80	320	—	—	—
Y 4	640	80	160	—	—	—
Rabbit						
RB 1 anti-brucella	1,280	1,280	20	10	—	—
RY 1 anti-yersinia	10,240	2,560	1,280	10	20	—

Titres are expressed as reciprocals.
Negative results = < 10.

DISCUSSION

The results of the SA tests showed that the observations of Ahvonen *et al.* (1969) on human sera were applicable to bovine sera. They also confirmed that complete cross-agglutination occurs between *Br. abortus* and *Y. enterocolitica* IX. The tests performed with various *Brucella* species showed that the cross-reaction with *Y. enterocolitica* IX is common to all smooth strains of the genus *Brucella*. Thus the serological response to *Y. enterocolitica* IX cannot be distinguished from that to brucellas on the basis of SA tests. Similarly the results of the antiglobulin tests indicated that both organisms evoked cross-reacting 'incomplete' antibody thus rendering them indistinguishable on the basis of Coombs or IFA tests. The results of the CF tests were also inconclusive in this respect.

These results clearly indicated that the standard serological tests employed for the diagnosis of *Brucella* infections failed to differentiate the serological response to these from that to *Y. enterocolitica* IX. For this reason an attempt was made to

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devise a test which would differentiate between the serological responses to the two organisms.

The results of immunodiffusion tests confirmed the cross-reaction between *Y. enterocolitica* IX and brucella strains but also showed that l.p.c. specific to each group could be detected with homologous antisera. Unfortunately this test was insufficiently sensitive to be of general use in evaluating field sera.

Spot tests performed with RBBr. and RBY antigens also confirmed the cross-reaction between the two groups. However, by performing the test on serial dilutions of serum it was possible to detect differences in titre between the *Brucella* and *Yersinia* groups. From the results obtained, it appeared that brucella-infected individuals gave titres to RBY and RBBr. antigen which were either equal or marginally higher for the brucella antigen. In no confirmed case of brucella infection was the reverse result obtained. On the other hand, *Y. enterocolitica* IX inoculated individuals gave titres which were invariably higher with the RBY antigen. Although the number of yersinia-inoculated animals studied was small, the results obtained were consistent and suggested that this test might be of value in differentiating the serological response to *Y. enterocolitica* IX from that due to *Brucella* spp. in cases of doubtful aetiology.

Hitherto there have been no reports of isolation of *Y. enterocolitica* IX from cattle in Great Britain and there is no evidence to suggest that such cross-reactions are likely to be encountered in field samples. With the exception of the examples cited by Mollaret (1968) and Goyon (1969) there seems little evidence to implicate *Y. enterocolitica* IX as a common cause of infection in cattle. In the present study, injection of *Y. enterocolitica* IX into cattle failed to produce any significant pathological changes apart from transient pyrexia and no organisms could be recovered post-mortem. Mollaret & Guillon (1965) also failed to produce any significant changes on inoculating a large number of strains of *Y. enterocolitica* into a wide range of animals.

However, in specific cases of doubt, the use of the quantitative Rose Bengal plate tests with RBY and RBBr. antigens, in combination with H-agglutination tests performed on brucella-absorbed sera, would enable a differential diagnosis to be made.

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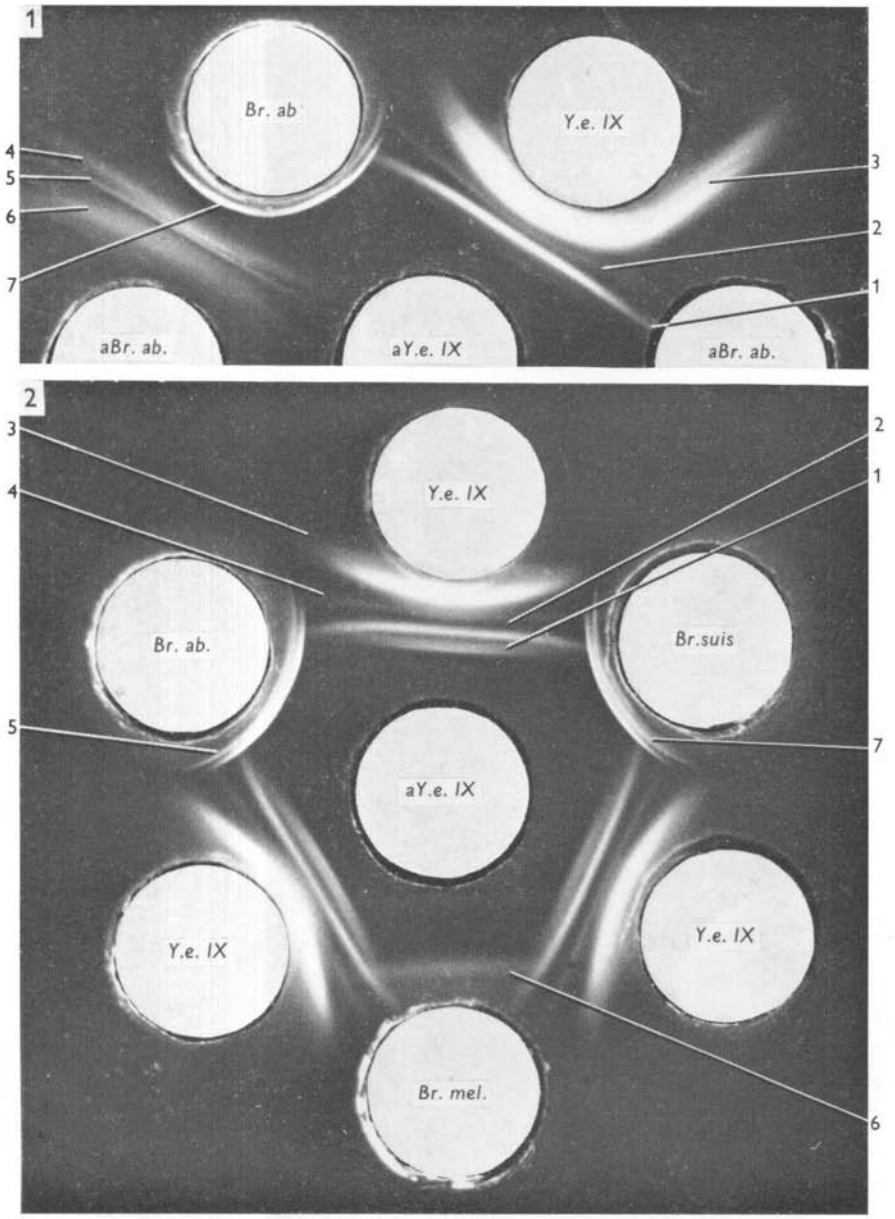
EXPLANATION OF PLATE

PLATE 1

Fig. 1. This shows the reaction of *Br. abortus* (*Br. ab.*) and *Y. enterocolitica* IX (*Y.e.* IX) antigens with anti-*Br. abortus* serum (anti-*Br. ab.*) and anti-*Y. enterocolitica* IX serum (anti-*Y.e.* IX). L.p.c. 1 and 2 appear specific to *Y.e.* IX and l.p.c. 4, 5 and 6 appear specific to *Br. ab.* L.p.c. 3 is common to both the *Y.e.* IX–anti-*Y.e.* IX and *Y.e.* IX–anti-*Br. ab.* systems. L.p.c. 7 is similarly common to the *Br. ab.*–anti-*Br. ab.* and *Br. ab.*–anti-*Y.e.* IX systems. Thus l.p.c. 3 and 7 apparently correspond to serologically related but not identical components.

Fig. 2. This shows the reactions of *Br. ab.*, *Br. melitensis* (*Br. mel.*) and *Br. suis* antigens with anti-*Y.e.* IX serum. L.p.c. 1, 2 and possibly 4 are identified as specific to *Y.e.* IX. L.p.c. 5, 6 and 7 are given by reaction of *Br. ab.*, *Br. mel.* and *Br. suis* with a *Y.e.* IX serum and appear related to l.p.c. 3 of the *Y.e.* IX–anti-*Y.e.* IX system.



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