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The serological relationship between *Brucella* spp., *Yersinia enterocolitica* serotype IX and *Salmonella* serotypes of Kauffmann-White group N

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SUMMARY

The serological relationship between *Brucella* spp., *Yersinia enterocolitica* IX, and the group N salmonella serotypes *S. godesberg*, *S. landau*, *S. morehead*, *S. neusdorf*, *S. soerenga* and *S. urbana* was examined using agglutination, antiglobulin, complement fixation, immunodiffusion and fluorescent antibody methods.

Antisera to the group N salmonella serotypes all reacted to significant titres in agglutination and complement fixation, but not antiglobulin or immunodiffusion tests with smooth brucella antigens. These antisera also reacted in agglutination, but not antiglobulin, tests with *Y. enterocolitica* IX. They did not react significantly in any tests with rough brucella antigens.

Conversely, antisera to smooth *Brucella* spp. agglutinated group N salmonellas to low titre and *Y. enterocolitica* IX to titres similar to those given against the homologous strain. Antiserum to *Y. enterocolitica* IX on the other hand reacted with smooth brucella antigens to high titre in agglutination, complement fixation and antiglobulin tests, and with the group N salmonella antigens to substantial titres in agglutination tests.

In direct fluorescent antibody tests, smooth *Brucella* strains and *Y. enterocolitica* IX reacted strongly with FITC-labelled antibody to *Br. abortus* whereas the group N salmonella strains reacted weakly.

In tests with monospecific antisera to the A and M determinants of *Br. abortus* and *Br. melitensis* respectively, *Y. enterocolitica* IX reacted only with the antiserum to the A determinant whereas group N salmonellas reacted to low titre with both A and M antisera.

The results of cross-absorption tests confirmed this relationship and suggested that the O30 antigens of group N salmonella serotypes contained antigenic determinants similar to, but not identical with, the antigenic structure shared by smooth *Brucella* spp. and *Y. enterocolitica* IX.

INTRODUCTION

The serological cross-reaction between smooth *Brucella* spp. and *Yersinia enterocolitica* serotype IX has been well characterized (Ahvonen, Jansson & Aho, 1969; Corbel & Cullen, 1970; Diaz, Lacalle, Medrano & Leong, 1970; Hurvell,

Ahvonen & Thal, 1971; Rusu *et al.* 1970; Akkermans & Hill, 1971; Fribourg-Blanc, 1971; Pop, Cerbu, Pop & Drăghici, 1972). Serological cross-reactions between *Brucella* strains and organisms of other genera have also been described from time to time (Francis & Evans, 1926; Starr & Snider, 1934; Wong & Chow, 1937; Shklair & Stafseth, 1954; Feeley, 1969). Most of these cross-reactions have been low-grade and unlikely to present serious problems in diagnosis.

Of possibly greater significance are serological cross-reactions between *Brucella* spp. and certain *Salmonella* serotypes, particularly those of Kauffmann–White group N (Cioglia, 1950*a*, *b*; Wundt, 1959). At least one of these serotypes, *Salmonella urbana*, has been recently reported to cross-react with *Y. enterocolitica* IX as well as with *Brucella* spp. (Hurvell, 1973). Because of the possible implications of these cross-reactions for the serological diagnosis of brucellosis, the antigenic relationship between brucella, yersinia and salmonella organisms has been examined.

MATERIALS AND METHODS

Bacterial strains

The brucella strains were from the *Brucella* type culture collection maintained at this laboratory. The strain of *Y. enterocolitica* IX used has been described previously (Corbel & Cullen, 1970).

The *Salmonella godesberg* and *S. urbana* strains were from the *Salmonella* reference collection maintained at this laboratory and were provided by Dr C. Wray. The strains of *S. landau*, *S. morehead*, *S. neusdorf* and *S. soerenga* originated from the Salmonella Reference Laboratory of the Central Public Health Laboratory, Colindale. All of these salmonella strains contained the O 30 somatic antigen of Kauffmann–White group N and with the exception of *S. soerenga* which belonged to the $O\,30_{I}$ sub-type, all were of the $O\,30_{I,\,II}$ sub-type.

Antigen preparations

Agglutination suspensions of *Br. abortus* and *Y. enterocolitica* IX were prepared and standardized as described by Corbel (1973*a*). *Br. melitensis* and *Br. suis* agglutination suspensions were prepared from heat-killed smooth *Br. melitensis* 16M and *Br. suis* 1330 cells suspended in 0·15 NaCl containing 0·5 % (w/v) phenol and standardized to give 50 % agglutination with a 1/500 dilution of the International Standard *Br. abortus* antiserum.

Suspensions of *Br. canis* RM6-66 for rough agglutination tests were prepared essentially according to Carmichael & Bruner (1968) but standardized to an optical density of 3·30 at 520 nm wavelength, using an Optica model CF4NI recording spectrophotometer fitted with glass cuvettes of 10 mm. light path (Optica U.K. Ltd, London).

Salmonella suspensions for use as immunizing antigens were grown for 16 hr. at 20° C. on trypticase soy agar, washed off with phosphate buffered saline (PBS: 0·15 M-NaCl, 0·01 M phosphate buffer, pH 7·4) containing 0·1 % (w/v) formaldehyde, allowed to stand at room temperature for 30 min. and then heated at 100° C.

for 5 min. The suspensions were washed by 3 cycles of centrifugation in PBS, resuspended in this medium and adjusted to a concentration of *ca.* 10^9 organisms per ml. OH agglutination suspensions of salmonella strains were prepared by a similar method except that the organisms were harvested in PBS, killed by heating to 60° C. for 1 hr., washed by centrifugation and resuspended in 0·15 M-NaCl containing 0·5 % (w/v) phenol. O antigen suspensions were prepared from the OH suspensions by adding 20 volumes of ethanol, and heating at 50° C. for 30 min. The treated organisms were deposited by centrifugation and resuspended in 0·15 M-NaCl. The suspensions were standardized nephelometrically as described above for *Br. canis*.

Ultrasonic extracts of brucella, yersinia and salmonella organisms for immunodiffusion tests were prepared essentially according to Corbel & Cullen (1970) but with the omission of KCl. Lipopolysaccharide O antigens were extracted from *Salmonella* spp. with diethyl ether-saturated water and purified by ultracentrifugation (Ribi, Milner & Perrine, 1959). The O agglutinogens of *Brucella* spp. and *Y. enterocolitica* IX were extracted with phenol-water (Westphal, Lüderitz & Bister, 1952) and purified as described by Corbel (1973*a*).

Antisera. Rabbit antiserum to *Y. enterocolitica* IX was prepared as described by Corbel & Cullen (1970). Rabbit antiserum to *Br. abortus* 544 was prepared as described by Corbel (1973*a*) and antisera to *Br. melitensis* Rev 1, *Br. suis* Thomsen and *Br. neotomae* 5K33 by a similar method. Rabbit antisera to group N salmonella strains were prepared by subcutaneous injection of 3–4 month old rabbits with *ca.* 10^8 killed organisms in 0·25 ml. PBS. This was combined with 2×10^8 killed organisms given by the intravenous route. Blood samples collected 10 days after inoculation were used for all tests with the exception of the immunodiffusion test. Antisera for use in the latter test were collected 4 days after the rabbits had received a second intravenous injection of 2×10^8 killed organisms given 12 days after primary inoculation.

Ovine antiserum to *Br. ovis* was collected from sheep 4 weeks after intramuscular injection of *Br. ovis* 63/290 emulsified in Freund's complete adjuvant. Antiserum to *Br. canis* RM6-66 was collected from rabbits 4 weeks after intramuscular injection of ultrasonically disrupted organisms in Freund's incomplete adjuvant.

Monospecific antisera to *Br. abortus* A antigen and *Br. melitensis* M antigen were prepared in rabbits according to the procedures recommended by Alton & Jones (1967).

Serological methods

The methods used for the serum agglutination (SA), complement fixation (CF), quantitative Rose Bengal plate (QRBP), Coombs antiglobulin (CAG) and immunodiffusion tests for antibodies to smooth *Brucella* and *Y. enterocolitica* IX strains have been described or referred to elsewhere (Corbel, 1973*a*; Corbel & Cullen, 1970). Agglutination tests using *Br. canis* as antigen were done essentially according to Carmichael & Bruner (1968). Agglutination tests using *Salmonella* antigens were done according to the standard procedure for the SA test but with incubation

Table 1. *Results of cross-agglutination tests on standard suspensions of* Brucella *spp.*, Y. enterocolitica *IX and group N salmonella serotypes*

	Reciprocal agglutination titres* with antigens									
Antiserum	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg* O $30_{I, II}$	*S. landau* O $30_{I, II}$	*S. more-head* O $30_{I, II}$	*S. neusdorf* O $30_{I, II}$	*S. soerenga* O 30_{I}	*S. urbana* O $30_{I, II}$
Br. abortus 544	**10,240**	5,120	10,240	10,240	40	40	80	80	20	80
Br. melitensis Rev 1	5,120	**20,480**	5,120	1,280	160	80	320	160	80	160
Br. suis Thomsen	2,560	1,280	**2,560**	2,560	20	20	20	20	10	20
Y. enterocolitica IX	2,560	1,280	2,560	**10,240**	640	320	640	320	80	320
S. godesberg	160	640	160	320	**5,120**	2,560	2,560	2,560	320	2,560
S. landau	160	160	160	160	2,560	**2,560**	2,560	2,560	640	2,560
S. morehead	320	160	160	160	5,120	5,120	**5,120**	5,120	640	5,120
S. neusdorf	160	320	160	160	2,560	2,560	2,560	**2,560**	640	2,560
S. soerenga	20	20	20	20	640	640	640	1,280	**2,560**	640
S. urbana	160	640	160	640	2,560	5,120	5,120	5,120	1,280	**5,120**

* Results with homologous antigen are in heavy type.

Table 2. *The activity of antisera to* Brucella *spp.*, Y. enterocolitica *IX and group N salmonellas in rapid agglutination tests with acid buffered Rose Bengal stained brucella and yersinia antigens*

Antiserum	Reciprocal titres of acid-stable agglutinins with Rose Bengal antigens		
	Br. abortus	*Br. melitensis*	*Y. enterocolitica IX*
Br. abortus 544	1,024	1,024	1,024
Br. melitensis Rev 1	512	2,048	256
Br. suis Thomsen	512	512	512
Y. enterocolitica IX	256	256	1,024
S. godesberg	8	4	4
S. landau	16	32	16
S. morehead	16	16	16
S. neusdorf	16	32	32
S. soerenga	2	2	2
S. urbana	4	8	8

at 37° C. for 4 hr., followed by 16 hr. at 4° C. The CF test for antibodies to *Br. ovis* was done according to Biberstein & McGowan (1958).

Direct fluorescent antibody (FA) tests, and FA absorption tests using the FITC-conjugated γ-globulin fraction of rabbit antiserum to *Br. abortus* 544, were done on ethanol-fixed smears of *Brucella* spp., *Y. enterocolitica* IX or *Salmonella* spp. according to procedures described previously (Corbel, 1973*b*; Corbel & Day, 1973*a*).

Absorption of sera

Sera were absorbed by incubating 1·0 ml. volumes with equal volumes of a suspension of the absorbing organism containing 0·8 g. of packed cells per ml. The mixture was incubated at 37° C. for 4 hr. followed by 16 hr. at 20° C. The serum was recovered by centrifugation at 10,000 *g* for 20 min.

Bacterial suspensions killed by heating at 60° C. for 1 hr. were used for the absorption in the case of brucella and yersinia strains. O antigen suspensions were used for absorption in the case of salmonella strains.

Absorption of antisera with lipopolysaccharides and lipopolysaccharide-protein complexes was done as described by Corbel & Day (1973*b*).

RESULTS

Examination of rabbit antisera to smooth *Br. abortus*, *Br. melitensis* and *Br. suis* showed that these agglutinated standard suspensions of *Br. abortus*, *Br. melitensis*, *Br. suis* and *Y. enterocolitica* IX to high titre. These antisera also agglutinated O and OH suspensions of the group N salmonella strains, but only to a fraction of the titres produced against the brucella and yersinia antigens (Table 1).

Rabbit antiserum to *Y. enterocolitica* IX reacted in a similar manner, agglutinating standard suspensions of *Y. enterocolitica* IX to high titre and standard suspensions of *Br. abortus*, *Br. melitensis* and *Br. suis* to somewhat lower titres. This

Table 3. *Results of Coombs antiglobulin tests on antisera to brucella, yersinia and group N salmonella strains*

Antiserum	Reciprocal agglutination titres with antigens									
	Br. abortus	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg*	*S. landau*	*S. morehead*	*S. neusdorf*	*S. soerenga*	*S. urbana*
Br. abortus 544	**81,920**	10,240	40,960	20,480	80	40	80	80	20	80
Br. melitensis Rev 1	10,240	**81,920**	10,240	2,560	320	80	320	160	80	160
Br. suis Thomsen	10,240	5,120	**10,240**	5,120	20	10	20	20	< 10	20
Y. enterocolitica IX	5,120	1,280	5,120	**40,960**	640	320	640	320	80	320
S. godesberg	160	640	160	320	**5,120**	2,560	5,120	5,120	320	2,560
S. landau	160	160	160	160	2,560	**5,120**	2,560	2,560	640	2,560
S. morehead	320	160	160	160	5,120	5,120	**5,120**	5,120	640	5,120
S. neusdorf	160	320	160	160	2,560	2,560	2,560	**2,560**	1,280	2,560
S. soerenga	20	20	20	20	320	320	640	640	**1,280**	640
S. urbana	160	640	160	640	5,120	5,120	5,120	5,120	1,280	**5,120**

Results with homologous antigens are in heavy type.
The sera used were as in Table 1.

Table 4. *Agglutinating activity of antiserum to* Br. abortus *towards homologous and cross-reacting antigens following absorption with brucella, yersinia and salmonella suspensions*

	Reciprocal agglutination titres with antigens									
Absorbing agent	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg* O 30$_{I,II}$	*S. landau* O 30$_{I,II}$	*S. morehead* O 30$_{I,II}$	*S. neusdorf* O 30$_{I,II}$	*S. soerenga* O 30$_{I}$	*S. urbana* O 30$_{I,II}$
0·15 M-NaCl	**5,120**	2,560	5,120	2,560	20	20	40	40	10	20
Br. abortus 544	**< 10**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Br. melitensis 16M	**80**	< 10	40	10	< 10	< 10	< 10	< 10	< 10	< 10
Br. suis Thomsen	**10**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Y. enterocolitica IX	**40**	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
S. godesberg	**2,560**	2,560	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. landau	**2,560**	2,560	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. morehead	**2,560**	2,560	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. neusdorf	**2,560**	2,560	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. soerenga	**2,560**	2,560	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. urbana	**2,560**	2,560	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10

Results with the homologous antigen are in heavy type.

antiserum also agglutinated O and OH suspensions of the group N salmonellas but to slightly higher titres than were produced by the anti-*Brucella* sera (Table 1).

The rabbit antisera prepared against the group N salmonella serotypes all agglutinated standard *Br. abortus*, *Br. melitensis*, *Br. suis* and *Y. enterocolitica* IX suspensions to significant titres. These titres were much lower however than those produced against the homologous and cross-reacting salmonella strains (Table 1).

It was notable that *S. soerenga* showed less cross-reactivity with brucella and yersinia antigens than did the other group N salmonella strains. The titres produced by antiserum to *S. soerenga* were also lower when tested against the other salmonella strains than when tested against the homologous antigen. A similar relationship applied when antisera to the other salmonella strains were tested against *S. soerenga* O antigen (Table 1).

Essentially similar results were obtained with the Rose Bengal plate test, using *Br. abortus*, *Br. melitensis* or *Y. enterocolitica* IX antigens. Acid-stable agglutinins for Rose Bengal stained *Br. abortus*, *Br. melitensis* and *Y. enterocolitica* IX were present in antisera prepared against smooth *Br. abortus*, *Br. melitensis*, *Br. suis*, *Y. enterocolitica* IX and group N salmonellas. With the exception of the antisera to *Y. enterocolitica* IX and *Br. melitensis*, which reacted to higher titre with their homologous Rose Bengal stained antigens, all of the sera gave similar reactions with the three antigens. In each case the titres obtained with the anti-*Salmonella* sera were much lower than those given by the antisera to *Brucella* spp. and *Y. enterocolitica* IX (Table 2).

When the antisera to smooth *Brucella* spp. were tested in the CAG test (Table 3) the agglutinin titres were considerably enhanced in tests with standard *Br. abortus*, *Br. melitensis* or *Br. suis* suspensions. A more moderate rise of titre was produced when these sera were tested against *Y. enterocolitica* IX antigen. No rise was observed in the titres given by these sera when tested against salmonella antigens.

The agglutinin titre of the antiserum to *Y. enterocolitica* IX showed a marginally significant rise in the CAG test when tested against *Brucella* antigens, a definite rise when tested against the homologous antigen and no rise when tested against salmonella antigens.

No rise of agglutinating activity was observed when the anti-*Salmonella* sera were tested against brucella, yersinia or salmonella suspensions in the CAG test.

The antigenic relationship between brucella, yersinia and group N salmonella strains was further confirmed by cross-absorption tests. The results of these tests are summarized in Tables 4, 5, 6, 7 and 8. Thus absorption of the antisera to smooth brucella strains, *Y. enterocolitica* IX or group N salmonellas with *Br. abortus* antigen removed all antibodies reacting with *Br. abortus*.

Absorption with *Br. abortus* greatly reduced the agglutinin titre of antiserum to *Br. melitensis* for its homologous antigen but residual titres of *Br. melitensis*-specific antibodies remained.

Absorption with *Br. abortus* had a similar effect on the activity of antiserum to *Y. enterocolitica IX* towards its homologous antigen. Thus absorption with *Br. abortus* reduced the agglutinin titre of this antiserum towards *Y. enterocolitica* IX, but high titres of yersinia-specific antibodies remained.

Table 5. *Agglutinating activity of antiserum to* Br. melitensis *towards homologous and cross-reacting antigens following absorption with brucella, yersinia and salmonella suspensions*

	Reciprocal agglutination titres with antigens									
Absorbing agent	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg* O $30_{I, II}$	*S. landau* O $30_{I, II}$	*S. morehead* O $30_{I, II}$	*S. neusdorf* O $30_{I, II}$	*S. soerenga* O 30_{I}	*S. urbana* O $30_{I, II}$
0·15 M-NaCl	2,560	**10,240**	2,560	5,120	40	40	40	40	10	40
Br. abortus 544	< 10	**80**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Br. melitensis 16M	< 10	**< 10**	< 10	< 10	< 10	< 10	< 10	10	10	< 10
Br. suis Thomsen	< 10	**80**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Y. enterocolitica IX	40	**160**	40	< 10	< 10	< 10	< 10	< 10	< 10	< 10
S. godesberg	2,560	**5,120**	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. landau	2,560	**10,240**	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. morehead	2,560	**10,240**	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. neusdorf	2,560	**5,120**	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10
S. soerenga	2,560	**10,240**	2,560	5,120	< 10	< 10	< 10	< 10	< 10	< 10
S. urbana	2,560	**10,240**	2,560	2,560	< 10	< 10	< 10	< 10	< 10	< 10

Results with the homologous antigen are in heavy type.

Table 6. *Agglutinating activity of antiserum to* Y. enterocolitica *IX towards homologous and cross-reacting antigens following absorption with brucella, yersinia and salmonella suspensions*

	Reciprocal agglutination titres with antigens									
Absorbing antigen	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg* O $30_{I, II}$	*S. landau* O $30_{I, II}$	*S. morehead* O $30_{I, II}$	*S. neusdorf* O $30_{I, II}$	*S. soerenga* O 30_{I}	*S. urbana* O $30_{I, II}$
0·15 M-NaCl	2,560	640	1,280	**5,120**	160	160	160	160	40	160
Br. abortus 544	< 10	< 10	< 10	**1,280**	< 10	< 10	< 10	< 10	< 10	< 10
Br. melitensis 16M	40	< 10	10	**1,280**	< 10	< 10	< 10	< 10	< 10	< 10
Br. suis Thomsen	< 10	< 10	< 10	**1,280**	< 10	< 10	< 10	< 10	< 10	< 10
Y. enterocolitica IX	< 10	< 10	< 10	**< 10**	< 10	< 10	< 10	< 10	< 10	< 10
S. godesberg	2,560	640	1,280	**1,280**	< 10	< 10	< 10	< 10	< 10	< 10
S. landau	1,280	640	1,280	**5,120**	< 10	< 10	< 10	< 10	< 10	< 10
S. morehead	2,560	640	640	**5,120**	< 10	< 10	< 10	< 10	< 10	< 10
S. neusdorf	1,280	640	1,280	**2,560**	< 10	< 10	< 10	< 10	< 10	< 10
S. soerenga	2,560	640	1,280	**5,120**	< 10	< 10	< 10	< 10	< 10	< 10
S. urbana	1,280	640	1,280	**2,560**	< 10	< 10	< 10	< 10	< 10	< 10

Results with the homologous antigen are in heavy type.

As in the case of *Br. abortus*, absorption with *Br. melitensis* eliminated agglutinins for group N salmonellas from antiserum to *Brucella* spp. and *Y. enterocolitica* IX.

Absorption with *Br. abortus* produced a marginal reduction in the titre of antisera to *S. godesberg*, *S. landau*, *S. neusdorf*, *S. morehead* and *S. urbana* for salmonella $O\,30_{I,II}$ antigen but had no effect on the titre of anti-*S. soerenga* serum for its homologous antigen.

Absorption with *Br. melitensis* removed all antibodies reacting with *Br. melitensis* from antisera to *Brucella* spp., *Y. enterocolitica* IX and group N salmonellas. It also removed most of the antibodies reacting with *Br. abortus* from these sera but left appreciable titres of *Br. abortus*-specific agglutinins in the antisera prepared against this organism and *Y. enterocolitica* IX.

Similarly, although a significant reduction in the agglutinin titre of *Y. enterocolitica* IX antiserum for its homologous antigen was produced by absorption with *Br. melitensis*, high titres of yersinia-specific agglutinins remained. As in the case of *Br. abortus*, absorption with *Br. melitensis* eliminated agglutinins for group N salmonellas from antisera to *Brucella* spp., and *Y. enterocolitica* IX.

Absorption of antisera to brucella, yersinia and salmonella strains with smooth *Br. suis* and *Br. neotomae* organisms produced results essentially similar to those given by absorption with *Br. abortus*.

Absorption of anti-brucella, yersinia and salmonella sera with *Y. enterocolitica* IX eliminated agglutinins for that organism. It also substantially reduced the brucella agglutinin titre of antisera to smooth *Brucella* spp., but left low titres of brucella-specific antibodies in antisera to *Br. abortus* and *Br. melitensis*. It eliminated the brucella agglutinins from antisera to *Y. enterocolitica* IX and group N *Salmonella* spp. *Y. enterocolitica* IX also absorbed the salmonella agglutinins of antisera to brucella and yersinia organisms. It had no effect on the $O\,30_{I,II}$ agglutinin titres of antisera to *S. godesberg*, *S. landau*, *S. morehead*, *S. neusdorf* and *S. urbana*. It slightly reduced the $O\,30_{I,II}$ agglutinin titre of antisera to *S. soerenga* but had no significant effect on its titre for *S. soerenga* $O\,30_{I}$ antigen.

Absorption of antisera to brucella or yersinia organisms with group N *Salmonella* spp. eliminated the salmonella agglutinins but had little effect on the titres of agglutinins for *Brucella* spp. or *Y. enterocolitica* IX. Absorption of antisera to group N salmonellas with salmonella $O\,30_{I}$ or $O\,30_{I,II}$ antigens eliminated agglutinins for brucella and yersinia organisms. Absorption of antisera to *S. soerenga* with homologous antigen eliminated all agglutinins for the $O\,30_{I}$ and $O\,30_{I,II}$ salmonella sub-types. Absorption of anti-*S. soerenga* serum with any of the $O\,30_{I,II}$ antigens eliminated agglutinins for the salmonella strains of this sub-type but still left appreciable titres of agglutinins for *S. soerenga*. Similarly, absorption of antisera to any of the $O\,30_{I,II}$ salmonellas with $O\,30_{I,II}$ antigens eliminated the salmonella agglutinins whereas absorption with *S. soerenga* antigen reduced the salmonella agglutinin titres and left agglutinins for the $O\,30_{I,II}$ sub-type.

Absorption of antisera to smooth *Brucella* spp. with *Brucella* lipopolysaccharide-protein extracts removed agglutinins for brucella, yersinia and group B salmonella organisms. Absorption with *Y. enterocolitica* IX lipopolysaccharide-protein extract

Table 7. *Agglutinating activity of antiserum to* S. landau *towards homologous and cross-reacting antigens following absorption with brucella, yersinia and salmonella suspensions*

	Reciprocal agglutination titres with antigens									
Absorbing antigen	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg* O $30_{I,II}$	*S. landau* O $30_{I,II}$	*S. morehead* O $30_{I,II}$	*S. neusdorf* O $30_{I,II}$	*S. soerenga* O 30_{I}	*S. urbana* O $30_{I,II}$
0·15 M-NaCl	80	40	80	80	1,280	**1,280**	1,280	1,280	320	1,280
Br. abortus 544	< 10	< 10	< 10	< 10	640	**640**	640	640	320	1,280
Br. melitensis 16M	< 10	< 10	< 10	< 10	640	**640**	640	640	320	640
Br. suis Thomsen	< 10	< 10	< 10	< 10	640	**640**	640	640	320	640
Y. enterocolitica IX	20	40	20	< 10	1,280	**1,280**	1,280	1,280	320	1,280
S. godesberg	< 10	< 10	< 10	< 10	< 10	**< 10**	< 10	< 10	< 10	< 10
S. landau	< 10	< 10	< 10	< 10	< 10	**< 10**	< 10	< 10	10	< 10
S. morehead	< 10	< 10	< 10	< 10	< 10	**< 10**	< 10	< 10	< 10	< 10
S. neusdorf	< 10	< 10	< 10	< 10	< 10	**10**	< 10	< 10	10	< 10
S. soerenga	10	< 10	< 10	< 10	160	**320**	160	160	< 10	160
S. urbana	< 10	< 10	< 10	< 10	< 10	**10**	< 10	< 10	< 10	< 10

Results with the homologous antigen are in heavy type.

The results obtained with antisera to *S. godesberg*, *S. morehead*, *S. neusdorf* and *S. urbana* were essentially similar to those presented in this table.

Table 8. *Agglutinating activity of antiserum to* S. soerenga *towards homologous and cross-reacting antigens in tests with brucella, yersinia and salmonella suspensions*

	Reciprocal agglutination titres with antigens									
Absorbing antigen	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Y. entero-colitica* IX	*S. godesberg* O $30_{I, II}$	*S. landau* O $30_{I, II}$	*S. morehead* O $30_{I, II}$	*S. neusdorf* O $30_{I, II}$	*S. soerenga* O 30_{I}	*S. urbana* O $30_{I, II}$
0·15 M-NaCl	10	10	10	10	320	320	320	320	**1,280**	320
Br. abortus 544	< 10	< 10	< 10	< 10	320	160	160	320	**1,280**	320
Br. melitensis 16M	< 10	< 10	< 10	< 10	160	320	160	160	**1,280**	160
Br. suis Thomsen	< 10	< 10	< 10	< 10	320	320	160	320	**1,280**	160
Y. enterocolitica IX	< 10	< 10	< 10	< 10	10	10	20	10	**1,280**	10
S. godesberg	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	**320**	< 10
S. landau	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	**640**	< 10
S. morehead	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	**320**	< 10
S. neusdorf	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	**320**	< 10
S. soerenga	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	**< 10**	< 10
S. urbana	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	**320**	< 10

Results with the homologous antigen are in heavy type.

Table 9. *Complement fixing activity of antisera to* Brucella *spp.*, Y. enterocolitica IX *and group N salmonellas in tests with* Br. abortus *antigen*

Antiserum	Reciprocal complement fixation titres
Br. abortus 544	4,000
Br. melitensis Rev 1	4,000
Br. suis	2,000
Y. enterocolitica IX	200
S. godesberg	80
S. landau	200
S. morehead	200
S. neusdorf	40
S. soerenga	40
S. urbana	200

Table 10. *The effects of absorption with brucella, yersinia and salmonella antigens on the activity of antisera to rough and smooth brucella strains in the* Br. ovis *CF test*

	Reciprocal titres given by antisera					
Absorbing antigen	*Br. abortus*	*Br. melitensis*	*Br. suis*	*Br. neotomae*	*Br. canis*	*Br. ovis*
0·15 M-NaCl	80	40	40	< 40	1,280	2,560
Br. abortus 544	**80**	40	40	< 40	1,280	2,560
Br. melitensis 16M	80	**40**	40	< 40	1,280	2,560
Br. suis Thomsen	80	40	**40**	< 40	1,280	2,560
Br. neotomae 5K33	80	40	40	< **40**	1,280	2,560
Br. canis RM6-66	< 40	< 40	< 40	< 40	< **40**	40
Br. ovis 63/290	< 40	< 40	< 40	< 40	< 40	< **40**
Y. enterocolitica IX	80	40	40	< 40	1,280	2,560
S. godesberg	80	40	40	< 40	1,280	2,560
S. landau	80	40	40	< 40	1,280	2,560
S. morehead	80	40	40	< 40	1,280	2,560
S. neusdorf	80	40	40	< 40	1,280	2,560
S. soerenga	80	40	40	< 40	1,280	2,560
S. urbana	80	40	40	< 40	1,280	2,560

Results with the homologous antigen are in heavy type.

Antisera to *Y. enterocolitica* IX and group N salmonella serotypes did not react significantly in the *Br. ovis* CF test.

had a similar effect although some residual brucella-specific agglutinins were left in the antisera to *Br. abortus*, *Br. melitensis* and *Br. suis*.

Absorption of antisera to *Y. enterocolitica* IX with the purified O agglutinogen preparations from brucella, yersinia or group N salmonella strains produced essentially similar effects to those resulting from absorption with intact organisms. Similarly, absorption of antisera to group N salmonellas with these preparations had an effect virtually indistinguishable from that produced by intact organisms.

Rabbit antisera to *Br. abortus*, *Br. melitensis*, *Br. suis*, *Y. enterocolitica* IX or group N salmonella strains all reacted to substantial titres in the CF test with *Br. abortus* antigen. The titres given by the anti-yersinia and anti-salmonella sera

were substantial but considerably lower than those produced by the antisera to *Brucella* spp. (Table 9).

The antisera to smooth *Br. abortus*, *Br. melitensis*, *Br. neotomae*, *Br. suis*, *Y. enterocolitica* IX and group N salmonellas did not react to significant titres in agglutination tests with *Br. canis* antigen. Thus in no case was an agglutinin titre > 1/10 observed. On the other hand, antisera to *Br. canis* and *Br. ovis* produced agglutinin titres of 1/160 and 1/80 respectively when tested against *Br. canis* antigen. These sera did not react with smooth brucella antigens nor with yersinia or salmonella antigens.

Similar results were given in CF tests with *Br. ovis* antigen. The specificity of these reactions was confirmed by cross-absorption tests. Thus smooth brucella, yersinia and salmonella suspensions did not significantly absorb agglutinins for *Br. canis*, nor complement fixing antibodies to *Br. ovis*, from antisera to rough brucella strains. Absorption with either *Br. ovis* or *Br. canis* on the other hand, was almost equally effective in eliminating agglutinating or CF antibodies from these sera (Table 10).

When the undiluted FITC-conjugated γ-globulin fraction of antiserum to smooth *Br. abortus* was used in direct FA tests performed on smears of smooth *Br. abortus*, *Br. melitensis*, *Br. suis*, *Y. enterocolitica* IX or group N salmonella organisms, fluorescence was observed in each case. At conjugate dilutions > 1/2 the salmonella strains failed to fluoresce, although fluorescence of the brucella and yersinia strains continued to be produced even at high conjugate dilutions.

On absorbing the conjugate with *Br. abortus*, *Br. melitensis*, *Br. suis* or *Y. enterocolitica* IX, all fluorescent staining of brucella, yersinia or salmonella organisms was eliminated. Absorption of the conjugate with one or other of the group N salmonella strains only removed antibodies reacting with these organisms and did not prevent fluorescent staining of brucella or yersinia organisms.

The relationship of the cross-reacting antigens of brucella, yersinia and salmonella strains to the A and M antigens of smooth *Brucella* spp. was determined by agglutination reactions using antisera made monospecific for these antigens.

In slide tests, the A monospecific serum agglutinated *Br. abortus* 544 and *Br. suis* 1330 but not *Br. melitensis* 16M. This antiserum also agglutinated *Y. enterocolitica* IX and the group N salmonella strains but less strongly than the brucella strains.

The M monospecific serum did not agglutinate *Br. abortus* 544, *Br. suis* 1330 or *Y. enterocolitica* IX but did agglutinate *Br. melitensis* 16M and the group N salmonellas, the latter weakly. The specificity of these results was confirmed by tube agglutination and cross-absorption tests with monospecific antisera and standard *Br. abortus*, *Br. melitensis*, *Br. suis*, *Y. enterocolitica* IX and salmonella suspensions (Table 11).

On diffusion of ultrasonic extracts of *Br. abortus* 544, *Br. melitensis* 16M, *Br. suis* 1330 or *Br. neotomae* 5K33 against antisera to *Br. abortus*, *Br. melitensis*, *Br. neotomae* or *Br. suis*, numerous precipitation lines were produced. These included the line pattern component (lpc) corresponding to the O agglutinogen of each of these strains. This was identical with the single lpc produced by purified prepara-

Table 11. *The effect of absorption with brucella, yersinia and salmonella antigens on the agglutinating activity of antisera monospecific for the A determinant of* Br. abortus *and the M determinant of* Br. melitensis

		Reciprocal agglutinin titres following absorption with the antigens										
Antiserum	Agglutinating antigen	0·15 M-NaCl	*Br. abortus* 544	*Br. melitensis* 16M	*Br. suis* 1330	*Y. enterocolitica* IX	*S. godesberg*	*S. landau*	*S. morehead*	*S. neusdorf*	*S. soerenga*	*S. urbana*
A mono-specific	*Br. abortus*	160	< 10	80	< 10	10	80	160	160	160	160	80
	Br. melitensis	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	Y. enterocolitica IX	10	< 10	10	10	< 10	< 10	10	10	< 10	10	10
	S. godesberg	10	< 10	10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	S. landau	10	< 10	10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	S. morehead	10	< 10	10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	S. neusdorf	10	< 10	10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	S. soerenga	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	S. urbana	10	< 10	10	10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
M mono-specific*	*Br. melitensis*	80	80	< 10	80	40	80	80	80	80	80	80

* This antiserum agglutinated *B. melitensis* strongly and the group N salmonellas weakly when undiluted. It did not agglutinate any antigen other than *Br. melitensis* at dilutions of 1/10 or greater.

tions of the brucella lipopolysaccharide-protein O agglutinogens on diffusion against antisera to smooth brucella strains. Except in the case of *Br. melitensis* preparations this lpc was always formed as a semi-lunar arc immediately adjacent to the antigen well.

Ultrasonic extracts of *Br. canis* and *Br. ovis* also produced complex precipitation patterns on diffusion against antisera to smooth brucellas but the lpcs corresponding to the smooth lipopolysaccharide-protein agglutinogens of these strains were not produced. Similarly, antisera to *Br. canis* and *Br. ovis*, although producing several precipitation lines on diffusion against extracts of smooth brucella strains, did not produce any corresponding to the O agglutinogens of these organisms.

Antisera to the smooth brucella strains all produced a single line of precipitation on diffusion against extracts of *Y. enterocolitica* IX. This lpc corresponded to the O agglutinogen and was identical with the lpc produced by these sera on diffusion against the purified lipopolysaccharide-protein complex of this organism. The antisera to *Br. canis* and *Br. ovis* did not react with *Y. enterocolitica* IX antigens.

None of the antisera to smooth or rough brucella strains reacted in immunodiffusion tests with ultrasonic extracts of group N salmonellas or with their purified lipopolysaccharides.

Antiserum to *Y. enterocolitica* IX produced numerous precipitation lines on diffusion against ultrasonic extracts of the homologous organism. One of these lpcs was identical with the single lpc given by the *Y. enterocolitica* IX lipopolysaccharide-protein extract. This lpc cross-reacted with the single lpc produced by extracts of smooth brucella strains on diffusion against antiserum to *Y. enterocolitica* IX. No reaction was produced by extracts of *Br. canis* or *Br. ovis* on diffusion against anti-*Y. enterocolitica* IX serum.

Antiserum to *Y. enterocolitica* IX produced between 2 and 3 precipitation lines on diffusion against ultrasonic extracts of group N salmonellas but did not precipitate with the purified lipopolysaccharides of these organisms. It seems probable that the cross-reacting components were antigens common to the Enterobacteriaceae.

The antisera to the group N salmonellas produced extensive cross-precipitation between ultrasonic extracts of all these strains. They also produced from 1 to 3 precipitation lines with ultrasonic extracts of *Y. enterocolitica* IX but did not cross-react with extracts of brucella strains. No definite reaction between the purified lipopolysaccharide-protein antigens of brucella or yersinia strains and antisera to group N salmonellas could be detected by immunodiffusion.

DISCUSSION

The results of the agglutination, CF and cross-absorption tests on the antisera prepared against group N salmonellas showed that these contained antibodies to smooth *Brucella* organisms and to *Y. enterocolitica* IX, as well as to the somatic O 30 somatic antigen. No antibodies to brucella or yersinia organisms were detected in the pre-inoculation sera and it must be concluded that those present in the post-inoculation sera were produced in response to the salmonella antigens. This conclusion is in agreement with the observations of Cioglia (1948; 1950*a*, *b*, *c*) and Wundt (1959).

Allowing for individual differences between the animals used for antiserum production, the degree of cross-reaction observed with brucella and yersinia antigens was similar for antisera prepared against the five group N salmonella strains of the O $30_{I, II}$ sub-type studied. A weaker, though definite, cross-reaction was observed with antiserum to the single O 30_{I} sub-type strain studied, *S. soerenga*. This suggested that the O 30 somatic antigen common to this *Salmonella* group was responsible for eliciting the cross-reacting antibodies. Furthermore, it would seem that the antigenic determinants present in the O $30_{I, II}$ sub-group bore a closer resemblance to the common antigenic components of *Brucella* spp. and *Y. enterocolitica* IX than did those of the O 30_{I} sub-type. Confirmation of this was given by the absorption tests using salmonella O antigens.

It was also evident from these results that, although the salmonella O 30 antigen elicited antibodies cross-reacting with brucella and yersinia antigens and was effective in absorbing these, it was less effective in absorbing those antibodies cross-reacting with these antigens produced in response to either smooth *Brucella* or *Y. enterocolitica* IX organisms. This suggested that although the salmonella O 30 antigen contained structural components bearing some relationship to the common antigenic determinants of *Brucella* spp. and *Y. enterocolitica* IX it was largely distinct from these.

In contrast, it was evident from the results of both the present and previous studies, that the cross-reacting antigens of smooth brucellas and *Y. enterocolitica* IX were very similar. Thus in cross-absorption experiments, although the presence of brucella-specific and yersinia-specific agglutinins could be demonstrated, the agglutinin titre of anti-brucella or anti-yersinia serum for the homologous antigen was greatly reduced by absorption with the heterologous organism.

The limited antigenic relationship of brucella and yersinia organisms to the group N salmonellas was confirmed by the results of the cross-agglutination and cross-absorption tests performed on antisera to these organisms.

The low titre of agglutinins for group N salmonellas present in the anti-*Brucella* sera and the somewhat higher titres of salmonella agglutinins present in the anti-*Yersinia* serum suggested a closer antigenic relationship between the salmonella O 30 antigen and the cross-reacting antigen of *Y. enterocolitica* IX than between the O 30 antigen and *Brucella* agglutinogens. It is also possible that the closer antigenic similarity of the salmonella and yersinia antigens resulted from the presence of antigenic determinants common to Enterobacteriaceae and unrelated to the antigen cross-reacting with *Brucella* spp. However, the complete absorption of salmonella agglutinins from antisera to *Y. enterocolitica* IX by *Brucella* spp. did not support this interpretation.

There was, however, no evidence that the brucella-yersinia-salmonella cross-reaction was attributable to a common antigen of the Kunin type (Kunin, 1963). Antibodies to this are not detectable by direct agglutination and, as shown by Le Minor, Chalon & Veron (1972), the distribution of the Kunin antigen among bacterial genera, although including *Salmonella* and *Yersinia*, does not extend to *Brucella*. Furthermore, the results of the absorption tests using purified O antigen extracts indicated that the cross-reacting antigenic determinants of brucella,

yersinia and group N salmonella strains were associated with the lipopolysaccharide-containing agglutinogen complexes.

The failure to demonstrate any cross-reaction between the agglutinogens of the salmonella strains and those of the brucella and yersinia strains in immunodiffusion tests was probably attributable to the relatively low titres of the cross-reacting antibodies involved. It may also have been related to the immunoglobulin class of these antibodies however. Thus further studies have shown that the antibodies involved in the cross-reaction of brucella or yersinia strains with group N salmonellas are exclusively of IgM type (Corbel, to be published). This is in contrast with the cross-reaction between brucella and yersinia agglutinogens which involves antibodies of both IgM and IgG classes (Corbel, 1973*a*, *c*; Hurvell, 1973).

The results of fluorescent antibody tests confirmed the distant antigenic relationship between the group N salmonellas and smooth *Brucella* spp. compared with the closer antigenic relationship between the latter and *Y. enterocolitica* IX.

Some indication of the possible relationship of the Salmonella O 30 antigen to the cross-reacting antigens of *Brucella* spp. and *Y. enterocolitica* IX was apparent from the results of the cross-agglutination tests with A and M monospecific sera. It seems probable that the O 30 antigen contains antigenic determinants resembling structures common to both the A and M antigens but only forming a minor part of the structure. On the other hand, the cross-reacting antigen of *Y. enterocolitica* IX evidently contains antigenic determinants similar to those of the main structure of the smooth *Brucella* agglutinogens and in addition, determinants common to the A but not the M antigen (Corbel & Cullen, 1970; Corbel & Phillip, 1972; Hurvell, 1973).

The precise nature of the antigenic determinants involved in the brucella-yersinia-salmonella cross-reaction has not been determined. However, the cross-reacting antigenic determinants of *Brucella* spp. and *Y. enterocolitica* IX have been shown to reside in the polysaccharide component of the lipopolysaccharide-protein agglutinogen complexes (Diaz *et al.* 1970; Corbel, 1973*c*; Hurvell, 1973) and it seems probable that the cross-reacting determinants of group N salmonellas comprise part of the polysaccharide fraction of the O 30 lipopolysaccharide somatic agglutinogen. According to Hurvell (1973) the only monosaccharides shared by the *Brucella* spp. and *Y. enterocolitica* IX lipopolysaccharides, other than those comprising the core structure, are glucose and galactose. The O 30 side chains of group N salmonella lipopolysaccharides have been shown to contain glucose residues in β 1–3 and β 1–4 linkage to *N*-acetylgalactosamine (Simmons, Lüderitz & Westphal, 1965). It seems possible that the antigenic determinants involved in the brucella-yersinia-salmonella cross-reaction contain terminal glucose units, perhaps linked through galactosyl residues to the core of the lipopolysaccharide agglutinogen. It may be significant that a serological cross-reaction has also been observed between *Brucella* spp., group N salmonellas and *Francisella tularensis* (Wundt, 1959). *F. tularensis* has also been reported to contain, *inter alia*, glucose and galactose as structural components (Parnas, Mierzejewski, Feltynowski & Lazuga (1955). Clearly further studies are required to determine the precise structure of the antigenic determinants involved in the cross-reactions between *Brucella* spp. and other organisms.

REFERENCES

AHVONEN, P., JANSSON, E. & AHO, K. (1969). Marked cross-agglutination between Brucella and a sub-type of *Yersinia enterocolitica*. *Acta pathologica et microbiologica scandinavica* **75**, 291.

AKKERMANS, J. P. W. M. & HILL, W. K. W. (1971). *Yersinia enterocolitica* serotype 9 infectie als storend element bij de serologische diagnostiek van brucella-infecties bij het varken. *Tijdschrift voor Diergeneeskunde* **96**, 1654.

ALTON, G. G. & JONES, L. M. (1967). Laboratory techniques in brucellosis. *WHO Monograph Series* No. 55.

BIBERSTEIN, E. L. & MCGOWAN, B. (1958). Epididymitis in rams. Studies on laboratory diagnosis. *Cornell Veterinarian* **48**, 31.

CARMICHAEL, L. E. & BRUNER, D. W. (1968). Characteristics of a newly-recognized species of *Brucella* responsible for infectious canine abortions. *Cornell Veterinarian* **58**, 579.

CIOGLIA, L. (1948). Agglutinazione della S. urbana nelle brucellosi e suo valore diagnostico. *Bolletina della Societa Italiana di Biologia sperimentale* **24**, 1117.

CIOGLIA, L. (1950*a*). Antigeni communi a brucelle e salmonelle. *Giornale di Batteriologia e Immunologia* **42**, 81.

CIOGLIA, L. (1950*b*). Sulla diagnosi sierologica nelle brucellosi. *Giornale di Batteriologia e Immunologia* **42**, 91.

CIOGLIA, L. (1950*c*). Sulla constituzione antigene della brucelle melitensis e Bang. *Giornale di Batteriologia e Immunologia* **42**, 346.

CORBEL, M. J. (1973*a*). The nature of the antibody response to *Yersinia enterocolitica* serotype IX in cattle. *Journal of Hygiene* **71**, 309.

CORBEL, M. J. (1973*b*). The direct fluorescent antibody test for detection of *Brucella abortus* in bovine abortion material. *Journal of Hygiene* **71**, 123.

CORBEL, M. J. (1973*c*). Immunological properties of an antigen from *Yersinia enterocolitica* serotype 9 cross-reacting with *Brucella* species agglutinogens. In *Contributions to Microbiology and Immunology* **2**, Yersinia, Pasteurella and Francisella, pp. 150–6.

CORBEL, M. J. & CULLEN, G. A. (1970). Differentiation of the serological response to *Yersinia enterocolitica* serotype IX and *Brucella abortus* in cattle. *Journal of Hygiene* **68**, 519.

CORBEL, M. J. & DAY, C. A. (1973*a*). Assessment of fluorescent antibody absorption procedures for differentiation of the serological response to *Yersinia enterocolitica* serotype IX and *Brucella abortus* in cattle. *British Veterinary Journal* **129**, lxvii.

CORBEL, M. J. & DAY, C. A. (1973*b*). Assessment of indirect haemagglutination procedures for the serological diagnosis of bovine brucellosis. *British Veterinary Journal* **129**, 480.

CORBEL, M. J. & PHILLIP, J. I. H. (1972). The relationship of *Brucella abortus* agglutinogenic antigens to the receptor sites for Tbilisi phage. *Research in Veterinary Science* **13**, 91.

DIAZ, R., ⊢ACALLE, R., MEDRANO, M. P. & LEONG, D. (1970). Immunobiological activities of the endotoxin from *Yersinia enterocolitica* strain M.Y. 79. *Proceedings of the Vth International Congress on Infectious Diseases, Vienna; Bacteria* **2**, 11.

FEELEY, J. C. (1969). Somatic O antigen relationship of Brucella and Vibrio cholerae. *Journal of Bacteriology* **99**, 645.

FRANCIS, E. & EVANS, A. C. (1926). Agglutination, cross-agglutination and agglutinin absorption in tularaemia. *Public Health Reports, Washington* **41**, 1273.

FRIBOURG-BLANC, A. (1971). Étude par immunofluorescence des antigènes somatiques de *Yersinia enterocolitica*. *Annales Biologiques et Cliniques* **29**, 263.

HURVELL, B. (1973). Serological cross-reactions between different *Brucella* species and *Yersinia enterocolitica*. An immunological and immunochemical study. *Thesis*, Stockholm.

HURVELL, B., AHVONEN, P. & THAL, E. (1971). Serological cross-reactions between different brucella species and *Yersinia enterocolitica*. *Acta veterinaria scandinavica* **12**, 86.

KUNIN, C. M. (1963). Separation, characterization and biological significance of a common antigen in Enterobacteriaceae. *Journal of Experimental Medicine* **115**, 565.

LE MINOR, L., CHALON, A. M. & VERON, M. (1972). Recherches sur la présence de l'antigène commun des 'Enterobacteriaceae' (Antigène Kunin) chez les 'Yersinia', 'Levinea', 'Aeromonas' et 'Vibrio'. *Annales de l'Institut Pasteur* **123**, 761.

PARNAS, J., MIERZEJEWSKI, T., FELTYNOWSKI, A. & LAZUGA, K. (1955). Comparative studies on properties of bacteria: *Pasteurella tularemiae*, *Pasteurella multocida*, *Pasteurella rodentium* and *Brucella brucei*. *Annals of the Maria Curie-Sklodowska University* **10**, 207. Cited by Olitski, A. In *Immunological Methods in Brucellosis Research*: Part I. *In vitro* procedures. Basel and New York: S. Karger.

POP, A., CERBU, A., POP, A. & DRĂGHICI, D. (1972). Parentés antigéniques entre les espèces classiques de *Brucella* et de l'espèce *Yersinia enterocolitica* sérotype 9. I. Agglutinogènes et récepteurs phagique. *Archives roumaines de Pathologie experimentale et de Microbiologie* **31**, 45.

RIBI, E., MILNER, K. C. & PERRINE, T. D. (1959). Endotoxic and antigenic fractions from the cell wall of *Salmonella enteritidis*. Methods for separation and some biologic activities. *Journal of Immunology* **82**, 75.

RUSU, V., STANESCU, C., MUSCAN, A., LĂZĂROAE, D. & POPESCU, M. (1970). Infection humaine a *Yersinia enterocolitica* sérotype 9. Implications épidémiologiques. *Archives roumaines de Pathologie experimentale et de Microbiologie* **29**, 507.

SHKLAIR, I. L. & STAFSETH, H. J. (1954). A study of serological cross-reactions between the *Brucella* and certain salmonellae. I. The antigen common to Brucella and Salmonella pullorum. *Michigan State College Veterinarian* **14**, 130.

SIMMONS, D. A. R., LÜDERITZ, O. & WESTPHAL, O. (1965). The immunochemistry of Salmonella chemotype VI O-antigen. The structure of oligosaccharides from Salmonella group N (O 30) lipopolysaccharides. *Biochemical Journal* **97**, 815.

STARR, L. E. & SNIDER, G. E. (1934). Serologic relationship of Brucella and Pasteurella. *Journal of Infectious Diseases* **55**, 384.

WESTPHAL, O., LÜDERITZ, O. & BISTER, F. (1952). Über die Extraktion von Bakterien mit Phenol/Wasser. *Zeitschrift für Naturforschung* **7B**, 148.

WONG, D. H. & CHOW, C. H. (1937). Group agglutinins of *Brucella abortus* and *Vibrio cholerae*. *Chinese Medical Journal* **52**, 591.

WUNDT, W. (1959). Zur Frage der Antigengemeinschaften zwischen Brucellen und Bakterien anderer Gattungen. *Zeitschrift für Hygiene* **145**, 556.