A STUDY OF SEROLOGICAL CROSS REACTIONS BETWEEN THE BRUCELLAE AND CERTAIN SALMONELLAE. WITH SPECIAL REFERENCE TO BRUCELLA-SALMONELLA PULLORUM CROSS REACTIONS

By

Irving L. Shklair

AN ABSTRACT

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Felsenfeld et al. in 1951 (2) reported that Brucella antisera cross reacted with Salmonella pullorum. Such cross reactions may constitute a source of error in the serological diagnosis of pullorum disease, as well as in the diagnosis of brucellosis. The present study was undertaken with a view toward further investigation of the serological cross reaction between S. pullorum and Brucella.

Reciprocal agglutination tests were carried out with standard strain S. pullorum #89817, intermediate strain S. pullorum #671, variant strain S. pullorum #BAI, and Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255.

No cross agglutination reactions were observed between S. pullorum #89817 and Brucella. In a number of cases cross reactions were noted between S. pullorum #671 and Brucella. Cross agglutination reactions were observed between S. pullorum #BAI and Brucella. The antigenic structure of S. pullorum was determined by Edwards and Bruner (1) to be IX, XII_1, (XII_2), XII_3. From the above results it appears that the XII_2 antigen, (found in the intermediate and variant strains of S. pullorum) or a part thereof, was responsible for the observed cross reactions.

Reciprocal agglutination tests were also carried out with Brucella and other organisms, Salmonella reading and Proteus (Gwatkin), which contain the XII_2 antigen. The cross reactions observed between these organisms and Brucella were less pronounced than those noted between

S. pullorum #BAI and Brucella. There appears to be a quantitative difference in the amount of the cross reacting antigen (for Brucella) in S. pullorum #BAI, S. reading, and Proteus (Gwatkin). Whether this is a strain characteristic of the organisms involved is not known.

Mono-specific sera containing agglutinins IX, XII_1, XII_2, and XII_3 were prepared. Only the XII_2 mono-specific sera produced cross agglutinations with the Brucella antigens. It was noted that only a small fraction of the XII_2 antigen was involved in these cross reactions.

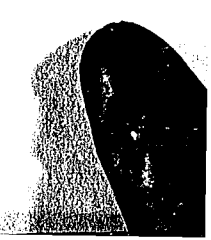
Agglutinin absorption studies carried out with S. pullorum #BAI and Brucella indicated that the antigen common to S. pullorum #BAI, S. reading, and Proteus (Gwatkin) existed in approximately equal amounts in all three species of Brucella tested.

One hundred and seventy-six S. pullorum suspicious and reactor turkeys were tested using Br. abortus #2308 and Br. melitensis #2500 antigens in dilutions of 1:25, 1:50, and 1:100. Of this total, 29 (seventeen percent) showed a cross reaction with the Brucella antigens.

A number of salmonellae, pathogenic for man and animals, contain antigen XII_2. These organisms, if they contain the antigenic factor common for Brucella, may produce agglutinins in sufficient quantity to cause false positive agglutination tests for brucellosis.

1. Edwards, P. R. and D. W. Bruner. Form variation in Salmonella pullorum and its relation to X. strains. Cornell Vet. 32:318-324, 1946.

2. Felsenfeld, O., V. M. Young, E. Loeffler, S. J. Ishichara, and W. F. Schroeder. A study of the nature of brucellosis in chickens. Am. J. Vet. Res. 7:48-54, 1951.



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INTRODUCTION

The incidence of pullorum disease in poultry flocks has steadily decreased during the last few years. However, it is still widespread and potentially very destructive.

Decrease in the incidence of pullorum disease can be attributed to improved methods of detection and removal of infected breeder stock, plus improved sanitary practices in poultry management.

Serological methods are mainly used for the detection of carrier birds. The National Poultry Improvement Plan of 1941 (54) endorses the following serological methods: 1. the standard tube agglutination test; 2. the stained antigen, rapid, whole-blood test; and 3. the rapid serum test. "The choice of method is determined by many factors and objectives peculiar to a state or region. In many states all three methods may be employed, but the whole-blood test is the one most generally used." (55).

The diagnostic tests listed above are based on the well-known principle that serum antibodies react specifically with their corresponding antigens. However, it should be remembered that antigens may be shared by several bacterial species which may or may not be otherwise closely related. This has caused confusion in the campaign against pullorum disease.

Recently, Felsenfeld, Young, Loeffler, Ishichara, and Schroeder (21) reported that Brucella antisera produced cross reactions with Salmonella pullorum. Such cross reactions may constitute a source of error in the serological diagnosis of pullorum disease, as well as in the diagnosis of brucellosis.

The present study was undertaken with a view toward further investigation of the serological cross reactions between S. pullorum and Brucella. Serological cross reactions were performed with standard, variant, and intermediate strains of S. pullorum, and Brucella abortus, Brucella melitensis, and Brucella suis. Mono-specific S. pullorum antisera were utilized in these studies. Agglutination absorption studies were also carried out with the above organisms in order to determine the amount of the common antigen or antigens present in them. Cross agglutination studies employing turkeys naturally infected with S. pullorum were also carried out.

REVIEW OF LITERATURE

In 1900 Rettger (45) reported the discovery of the etiological agent of pullorum disease. The disease was first described as a "fatal septicemia of young chicks". In 1909 Rettger (46) called the disease "white diarrhea". Later in the same year Rettger and Stoneburn (47) suggested that the name "bacillary white diarrhea" be given to the disease and that the causitive agent be known as Bacterium pullorum. In 1925 the name of the organism was changed to Salmonella pullorum according to Bergeys Classification. In 1928 the common name of the disease was changed to "pullorum disease".

In 1914 Rettger, Kirkpatrick, and Jones (49) described the complete cycle of pullorum infection in chickens. These investigators stated that to combat the disease successfully the infection cycle had to be broken. The most feasible method of breaking the infection cycle was to detect and remove pullorum carriers.

An early attempt to detect carriers was made by bacteriological examination of fresh eggs from infected flocks. This method was found to be inadequate and impractical in eliminating infected birds (55).

Jones in 1913 (35) reported the use of the macroscopic tube agglutination test as a means of detecting carriers and recommended that serum dilutions of 1:50, 1:100, and

1:200 be used for routine testing.

Rettger, Kirkpatrick, and Jones (50) and Gage, Page, and Hyland (23) confirmed the work of Jones. Gage et al. stated "The macroscopic agglutination test proved to be a good laboratory method for the detection of adult hens harboring Bacterium pullorum."

Runnells, Coon, Farley, and Thorp (51) in 1928 developed the rapid serum test for the detection of pullorum disease carriers. Two serum-antigen dilutions corresponding to the 1:50 and 1:100 dilutions of the tube agglutination test are employed. "Positive reactions may occur quickly but delayed reactions may require several minutes. Gradations of reactions occur in this method as in other methods." (55).

Schaffer, McDonald, Hall and Bunyea (52) and Coburn and Stafseth (10) reported the development of the whole-blood method in which a concentrated stained antigen is employed. In view of its apparent simplicity it is now the most widely used test for the eradication of pullorum carriers.

Other diagnostic tests have been proposed, the intradermal test (57), a precipitin test (16), and the complement fixation test (16), for the detection of carriers. These methods were found to be either unreliable or impractical in control and eradication programs.

The question has often been asked why repeated blood testing of flocks has failed to bring about the elimination of pullorum disease. Sometimes one can find management

practices which are at fault. In other cases, there are certain factors that tend to interfere with the effective application of the agglutination tests employed in the diagnosis of pullorum disease in birds. Horton in 1916 (29) was the first to observe that, occasionally, the sera of birds having pullorum disease failed to agglutinate pullorum antigens. This was subsequently observed by Beach, Halpin, and Lampman (1), Doyle (12), Kaupp and Dearstyne (38), and others. Whether this was due to a prozone phenomenon, or as a result of the organism undergoing form variation, or whether it was due to some unknown physiological factors in the birds is not known.

Hinshaw, Jones, Harr, and Neimeyer (27) and Bunyea, Hall, and Dorset (4) pointed out that the whole-blood test was equivalent to a 1:50 dilution in the tube agglutination test, and that a large number of reactors had titers of only 1:25 and 1:50. It is therefore possible that a large number of birds that were negative to the whole-blood test might be reactors in the 1:25 dilution of the tube test and thus missed. Corpron, Bevins, and Stafseth (11) made a comparative study of the tube agglutination test and rapid whole-blood method applied in testing turkeys and reported that the tube agglutination method was more sensitive and more consistent in reaction than the whole-blood method.

The reports of various Canadian workers concerning the presence of variant strains of S. pullorum offered another

possible explanation concerning the failure of serological tests to eliminate pullorum disease. Younie in 1941 (62) reported that in 1939 a few outbreaks of pullorum disease occurred in chicks hatched from breeder flocks which were negative to pullorum disease tube tests. In 1940 an increase in the number of such outbreaks was noticed. Only a few reactors were found on retesting the flocks. Neither the post-mortem appearance of affected birds nor the cultural characteristics of the organisms isolated from them differed from the usual findings in pullorum disease. However, certain serological differences were noted. In the tube agglutination test, the variant S. pullorum antisera did not react with most of the standard pullorum antigens, but did react with variant antigens in dilutions of 1:800.

In 1946 Edwards and Bruner (14) determined the antigenic components of S. pullorum. The antigenic structure of S. pullorum was found to be IX, XII_1, (XII_2), XII_3. The brackets around the XII_2 indicate that it is subject to form variation. Edwards and Bruner found that the variant strains of S. pullorum contained much XII_2 and very little, if any, XII_3. The standard strains contained little, if any, XII_2 and varying amounts of XII_3. A number of cultures have been isolated which are fairly well balanced in XII_2 and XII_3 antigens. Wright and Edwards (61) designated this type of culture as "intermediate". When an intermediate culture becomes stabilized with either the XII_2 or XII_3 antigen predominating, the culture becomes

either a variant (XII_2) or standard (XII_3) strain.

When testing for either type of S. pullorum it is possible for one type to escape detection if an antigen is prepared from the opposite type. This has resulted in the appearance of a variety of antigens on the market. The tester now has at his disposal antigens prepared from the standard or variant strains alone as well as polyvalent antigen composed of a mixture of standard and variant strains. Since it is not practical to employ double testing the polyvalent antigen has found favor where both standard and variant types of pullorum disease are encountered. Recently, Wright (59, 60) employed a stabilized 'intermediate' type culture in the preparation of a pullorum antigen. He used this antigen successfully for the detection of carrier birds.

Another factor that has to be taken into consideration in the effective application of the pullorum test is the problem of suspicious and "pin-point" reactors. In this category are included those birds whose blood gives a late, very fine agglutination in the whole-blood plate test and a fine, easily dispersed, often low titered reaction in the macroscopic tube test. As Williams pointed out (58) "the reactors may be encountered in certain flocks from year to year or may be demonstrated for only a short period of time. In any event they present a definite problem, especially when they are encountered in great numbers."

It is now recognized that several factors may be

involved in these atypical reactions. Studies carried out so far suggest that cross reacting agglutinins for S. pullorum occur in low dilutions, or, occasionally, in high titers in the blood of such birds. It should also be pointed out that organisms possessing antigens common for S. pullorum can often produce typical agglutination reactions. These typical reactions may be caused by salmonellae other than S. pullorum or by organisms morphologically, culturally and biochemically unrelated to S. pullorum.

It has been only recently that the agglutination of coliform bacilli by Salmonella sera has been examined more closely from the standpoint of antigenic analysis (43). In most instances the serological relationships observed between coliform and Salmonella strains were due to common heat-stable, somatic O antigens. Hobs and Arjona (28) described a culture which contained a portion of antigen XII of the Kauffmann-White classification. Braun, Silberstein, and Welker, cited by Peluffo, Edwards, and Bruner (17), and others, described many coliform cultures which contain group Salmonella somatic antigens.

Johnson and Anderson in 1936 (33) isolated a lactobacillus that agglutinated with S. pullorum antisera in low dilutions. The organism, at the time of isolation, for lack of complete identification was designated as Pl. Later, in 1940, Johnson and Pollard (34) suggested the name Lactobacillus meleagrides for this organism. They also pointed out that Lactobacillus casei could cross agglutinate

with S. pullorum antisera.

Gwatkin (26) reported a strain of Proteus isolated from a hen whose antiserum reacted to variant, but not to regular or standard pullorum antigens. The organism is very rich in XII_2; it has been found to be useful in differentiating between the standard and variant strains of S. pullorum.

Edwards, Bruner, Doll, and Hermann (15) isolated a culture of Staphylococcus that reacted with XII_2 antisera. However, repeated intravenous injections of dead and living cultures into chickens failed to give rise to any agglutinins of XII_2 cultures of S. pullorum. They doubted if this organism played any part in false positive reactions in the diagnosis of pullorum disease.

In 1946, Garrard, McDermott, Burton, and Carpenter (24, 25) carried out post-mortem studies on 87 fowl exhibiting non-specific pullorum reactions. They isolated many strains of staphylococci, coliforms, and enterococci. These organisms were classified as follows:

A. Gram positive cocci -- Micrococci, staphylococci, and enterococci

B. Coliform group -- Escherichia coli, Aerobacter aerogenes, and some intermediate types

C. Miscellaneous group -- Proteus, Alcaligines, and other unidentified genera

"The majority of the cocci were isolated from the ovaries and liver, while most of the coliform types were found in the intestine. Many representatives of each group

gave non-specific reactions with pullorum and polyvalent Salmonella sera." (25).

An enterococcus isolated from the liver of a hen was inoculated intravenously into a group of 20 White and Barred Rock fowl. Agglutination tests were run using both standard and variant S. pullorum antigens. The majority of reactions were observed with the variant type antigen. In some cases titers as high as 1:640 and 1:1280 were recorded with the variant S. pullorum antigen. Further tests showed that the XII_2 antigen was involved, as absorption of the XII_2 antibody produced negative results with Proteus (Gwatkin), Salmonella reading, and standard and variant S. pullorum antigens. Antigens IX and XII_3 could not be detected by colony typing (6).

Burton and Garrard (5) carried out additional experiments on the organisms mentioned above. They inoculated two Colobactrum, and three Paracolobactrum into pullets. All the inoculated pullets reacted with either the standard or variant pullorum antigens. Absorption tests showed that antigen XII_2 was common to all organisms except Paracolon intermedium which possessed antigen XII_3. P. intermedium produced titers as high as 1:2560 when tested against standard pullorum antigen.

"The fact that representatives of the enterococci and coliform groups of organisms isolated from various organs of non-pullorum reacting fowl have been shown to cause cross reactions with pullorum antigen does not mean

that their presence is the complete answer to non-pullorum reactions. There is sufficient evidence to suspect, however, that organisms common to the intestinal content of fowl are more commonly being found in other organs, where they cause low-grade infections and induce the production of agglutinins strong enough to cause cross reactions with pullorum antigen." (5).

In 1951 Felsenfeld et al. (21) reported that Brucella antiserum produced a cross agglutination with S. pullorum. They did not specify whether S. pullorum standard, variant or intermediate, strain was used.

It is now known that Brucella will cross agglutinate with a number of unrelated organisms. Foshey (22) pointed out "It is apparently not widely recognized that anti-brucella serum may have very broad agglutinating properties for other genera and families. The antibrucella serums that we have made. . . . agglutinate an extraordinarily large number of different bacteria, most of the colon-typhoid dysentery group, and some Salmonella, some Aerogenes, almost all strains of Proteus and Alkalegenes, B. tularense, and even H. influenzae." Foshey, unfortunately, did not specifically mention any of the salmonellae that cross agglutinated, and it is not known whether S. pullorum was one of the organisms tested.

Cioglia (8, 9) described common H and O antigens in Brucella melitensis and a number of salmonellae. However, no mention was made of cross agglutination reactions between

the *Brucella* group and *S. pullorum*.

Huddleson (30), Stafseth (53), and others have reported that the first cases of brucellosis in chickens were observed by Fiorentini in Italy in 1907. Evidence of *Brucella* infection was based on the fact that 55 percent of the birds reacted positively to the brucella agglutination test, and that *Br. melitensis* was isolated from the spleen of those that were ill.

Zweck and Zeller in 1913 (63) injected *Brucella abortus* into chickens in an attempt to produce the disease. The birds showed no evidence of the disease other than the production of agglutinins. Many other investigators have shown that chickens can produce agglutinins against the *Brucella* group of organisms. An excellent review of the history of brucellosis in fowl will be found in Biester and Schwarte, *Diseases of Poultry* by Stafseth (53).

MATERIALS AND METHODS

This section is divided into four groups of experiments. Experiment I deals with the agglutination reactions between S. pullorum and the Brucella species. Experiment II covers the preparation and agglutination reactions of monospecific sera. Experiment III covers agglutinin-absorption studies using S. pullorum, variant strain, and Brucella. Experiment IV includes the cross agglutination studies on turkeys naturally infected with S. pullorum.

Experiment I

Specific and cross agglutination reactions between S. pullorum and Brucella.

a. Cultures

Salmonella pullorum cultures		Somatic structure
S. pullorum #89817	standard strain	IX, XII_1, XII_3
S. pullorum #671	intermediate	IX, XII_1, (XII_2), XII_3
S. pullorum #BAI	variant	IX, XII_1, XII_2

S. pullorum #89817 and #671 were isolated at the Michigan State College Poultry Pathology Laboratory. S. pullorum #BAI was obtained from the Bureau of Animal Industry. From this original culture a number of sub-cultures were prepared and lyophilized. The culture used in this experiment is one of the lyophilized sub-cultures.

Brucella cultures

Br. abortus #2308

Br. melitensis #2500

Br. suis #1255

Br. abortus #2308 and *Br. melitensis* #2500 were obtained from the Brucella laboratory at Michigan State College. *Br. suis* #1255 was obtained from the stock culture collection of the Department of Bacteriology and Public Health at Michigan State College.

All the cultures used were in the smooth phase.

b. Groups of animals

White Leghorn and White Barred Rock roosters and pullets, 3 to 8 months old, were used in this experiment. All the birds were negative for *S. pullorum* and *Brucella* agglutinins.

Group 1:	6 birds	Inoculated with *S. pullorum* #89817
Group 2:	8 birds	Inoculated with *S. pullorum* #671
Group 3:	7 birds	Inoculated with *S. pullorum* #BAI
Group 4:	8 birds	Inoculated with *Br. abortus* #2308
Group 5:	10 birds	Inoculated with *Br. melitensis* #2500
Group 6:	6 birds	Inoculated with *Br. suis* #1255

c. Antigen preparation

S. pullorum #89817, #671, and #BAI antigens were prepared according to the directions set forth by the Livestock Sanitary Association in 1932 (44). Other *S. pullorum* antigens used in this experiment included the University of New

Hampshire standard antigen, pullorum stained antigen K, regular and polyvalent, (Lederle) and Redigen regular and polyvalent antigen (Columbus Vaccine Co.).

Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255 antigens were prepared using smooth colonies of the above organisms according to the directions of Huddleson (30).

d. Preparation of antisera

The S. pullorum cultures listed above were grown on nutrient agar slants for 24 hours. The growth was removed with sterile saline and the suspension was prepared, the turbidity corresponding to a reading of 4 to 6 on the McFarland nephelometer scale.

The Brucella were grown on tryptose agar slants for 48 to 72 hours. The organisms were removed with sterile saline, the concentration corresponding to a reading of 4 to 6 on the McFarland nephelometer scale.

The birds were injected intramuscularly (pectoral muscles) with 1 to 2 ml of the above concentrations. Three to four injections were given to each bird. The injections were given a week apart. Seven to ten days after the last injection 10 to 20 ml of blood was removed from each animal by cardiac puncture. The clotted blood was centrifuged and the serum removed.

e. Agglutination tests

Tube agglutination tests were set up in serial dilutions of 1:20 to 1:5160. The tests were incubated for 24 hours

at 37° C and then read. Reciprocal agglutination tests were set up between the organisms listed previously.

The S. pullorum antigens were adjusted to a pH of 8.2. The Brucella antigens were adjusted to a pH of 7.6. This figure was used as it closely approximated the pH of chicken blood serum, and it was believed that the most uniform results could be obtained using this pH.

Experiment II

Mono-specific sera were prepared to aid in finding the factor or factors responsible for the cross agglutination between S. pullorum and Brucella.

a. Cultures

	Somatic antigens
Salmonella paratyphi A, var. durazzo	II, XII_1, XII_3
Salmonella reading	IV, XII_1, XII_2
Proteus (Gwatkin)	XII_2.....
Br. abortus #2308	
Br. melitensis #2500	
Br. suis #1255	

S. paratyphi A, var. durazzo, S. reading, and Proteus (Gwatkin) cultures were supplied by Dr. W. W. Fergeson of the Michigan Department of Health.

b. Groups of animals

Group VII:	2 birds	Injected with S. paratyphi A, var. durazzo
Group VIII:	2 birds	Injected with S. reading
Group IX:	3 birds	Injected with Proteus (Gwatkin)

Birds from Groups 1, 4, 5, and 6 (Experiment I) were also used in this experiment. The birds in Groups VII, VIII, and IX consisted of White Leghorn roosters approximately 8 months old. All the birds used were negative for Brucella and S. pullorum agglutinins.

c. Preparation of somatic antigens

1. Somatic antigen preparation of S. paratyphi A, var. durazzo, S. reading, and Proteus (Gwatkin).

a. A saline suspension of the organisms was seeded over tryptose agar in 16-oz bottles.

b. The bottles were incubated at 37° C for 24 to 36 hours.

c. A small amount of sterile saline was added to each bottle and the growth was suspended by gentle rocking.

d. The suspensions were pooled and 3 volumes of 95 percent ethyl alcohol were added. The suspensions were incubated in a 37° C waterbath for 1 to 2 hours when precipitation should be complete.

e. The supernatant was decanted and discarded. The remaining precipitate was centrifuged at high speed for 30 minutes.

f. The supernatant was decanted and discarded. The sediment was resuspended in phenolized saline (0.3 percent phenol).

g. About 7 ml of phenolized saline was added to 1 ml of packed cells. This was the stock somatic antigen.

2. Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255 antigens were prepared according to the directions of Huddleson (30).

3. The S. pullorum antigens were prepared as described in Experiment I.

d. Preparation of antisera

The preparation of S. pullorum and Brucella antisera has been described in Experiment I.

Somatic agglutinating sera for S. paratyphi A, var. durazzo, S. reading, and Proteus (Gwatkin), were prepared by injecting boiled saline suspensions of the above organisms into White Leghorn roosters (Groups VII, VIII, and IX). A series of intramuscular injections was given until a suitable titer was obtained. Approximately 5 days after the last injection the birds were bled by cardiac puncture. The clotted blood was centrifuged and the serum removed.

e. Preparation of mono-specific sera

Mono-specific serum	Antisera	Absorbing antigen
IX	S. pullorum #89817 IX, XII_1, XII_3	S. paratyphi A, var. durazzo II, XII_1, XII_3
XII_1	S. reading IV, XII_1, XII_2	Proteus (Gwatkin) XII_2.....
XII_2	S. reading IV, XII_1, XII_2	S. pullorum #89817 IX, XII_1, XII_3
XII_3	S. paratyphi A, var. durazzo II, XII_1, XII_3	S. reading IV, XII_1, XII_2

A heavy suspension of the antigen was placed in a test tube and centrifuged. The supernatant fluid was discarded. A 1:10 dilution of the antiserum was added to the packed cell suspension and thoroughly mixed. The mixture was incubated at 37° C for 2 hours. It was then centrifuged and the partially absorbed serum removed. The absorptions were repeated two more times which was sufficient to produce the mono-specific serum desired.

f. Agglutination tests

Reciprocal agglutination tests were carried out with S. paratyphi A, var. durazzo, S. reading, and Proteus (Gwatkin). Agglutination tests were also carried out using the above sera against the S. pullorum and Brucella antigens. Agglutination tests were then carried out with the mono-specific sera and the above organisms.

Agglutination tests using mono-specific sera (diluted 1:10) were set up as follows: 0.5 ml of antigen (diluted to a reading corresponding to 1 on the McFarland nephelometer scale) was added to each tube. To the initial tube 0.5 ml of the mono-specific serum was added. This produced a dilution of 1:20. From the first tube 0.5 ml of the 1:20 dilution was transferred to the second tube. Serial dilutions were carried out to 1:5120. Suitable antigen and serum controls were prepared.

Experiment III

Agglutinin absorption studies. Mirror absorption tests were carried out between S. pullorum #BAI and the Brucella species in order to determine the amount of cross reacting antigen or antigens that were present.

a. Cultures

The Brucella, S. pullorum #BAI, S. reading, and Proteus (Gwatkin) cultures were described in Experiments I and II.

b. Antisera preparation

The preparation of Brucella and S. pullorum #BAI antisera was described in Experiment I.

c. Preparation of antigens

Brucella, S. pullorum #BAI, S. reading, and Proteus (Gwatkin) antigens were prepared as described in Experiments I and II.

d. Absorption studies

Br. abortus #2308, Br. melitensis #2500, Br. suis #1255, and S. pullorum #BAI, were absorbed with their corresponding homologous antigens as well as with each other.

The absorption techniques used in this experiment were the same as those used in the preparation of monospecific sera (Experiment II).

Preabsorption titers were first determined. Agglutination tests were then carried out between the absorbed sera and the antigens listed previously.

Agglutination tests were carried out as described in Experiment II.

Experiment IV

Cross agglutination studies of naturally infected turkeys with S. pullorum. Blood samples from Turkeys were sent to the Poultry Pathology Laboratory at Michigan State College for the serological diagnosis of pullorum disease. Sera that were positive or suspicious to the tube agglutination test were tested against Br. abortus #2308 and Br. melitensis #2500 antigens in dilutions of 1:25, 1:50, and 1:100. To rule out the possibility of any natural Brucella infections in turkeys, (which would give erroneous cross agglutination results) a modification of Castaneda's (7) 'filter paper surface fixation test' (Plate I) was used. This test was found to be specific for Brucella agglutinins and will not produce a positive reaction unless they are present.

a. Preparation of Brucella surface fixation antigen

Stock antigens of Br. abortus #2308 and Br. melitensis #2500 were diluted with 0.5 percent phenolized saline to an equal volume. A 1 percent aqueous solution of crystal violet was added to the suspension to produce a final concentration of 3 ml of dye to 100 ml of the diluted antigen.

b. Procedure of filter paper surface fixation test

1. Place a sheet of filter paper, Eaton-Dikeman No. 609 or Schleicher & Schuell No. 589, or other comparable paper,

on a test tube rack.

2. Place a drop of the test serum on the filter paper using a 4-4.5 mm loop.

3. Using a 2 mm loop immediately place one loopful of the stained antigen in the center of the serum drop.

4. Immediately place two loopfuls of saline on top of the antigen using the 2 mm loop.

5. If the serum contains Brucella agglutinins the antigen will remain in the center of the drop, if no Brucella agglutinins are present in the serum the antigen will spread through the drop to color the whole area (Plate I).

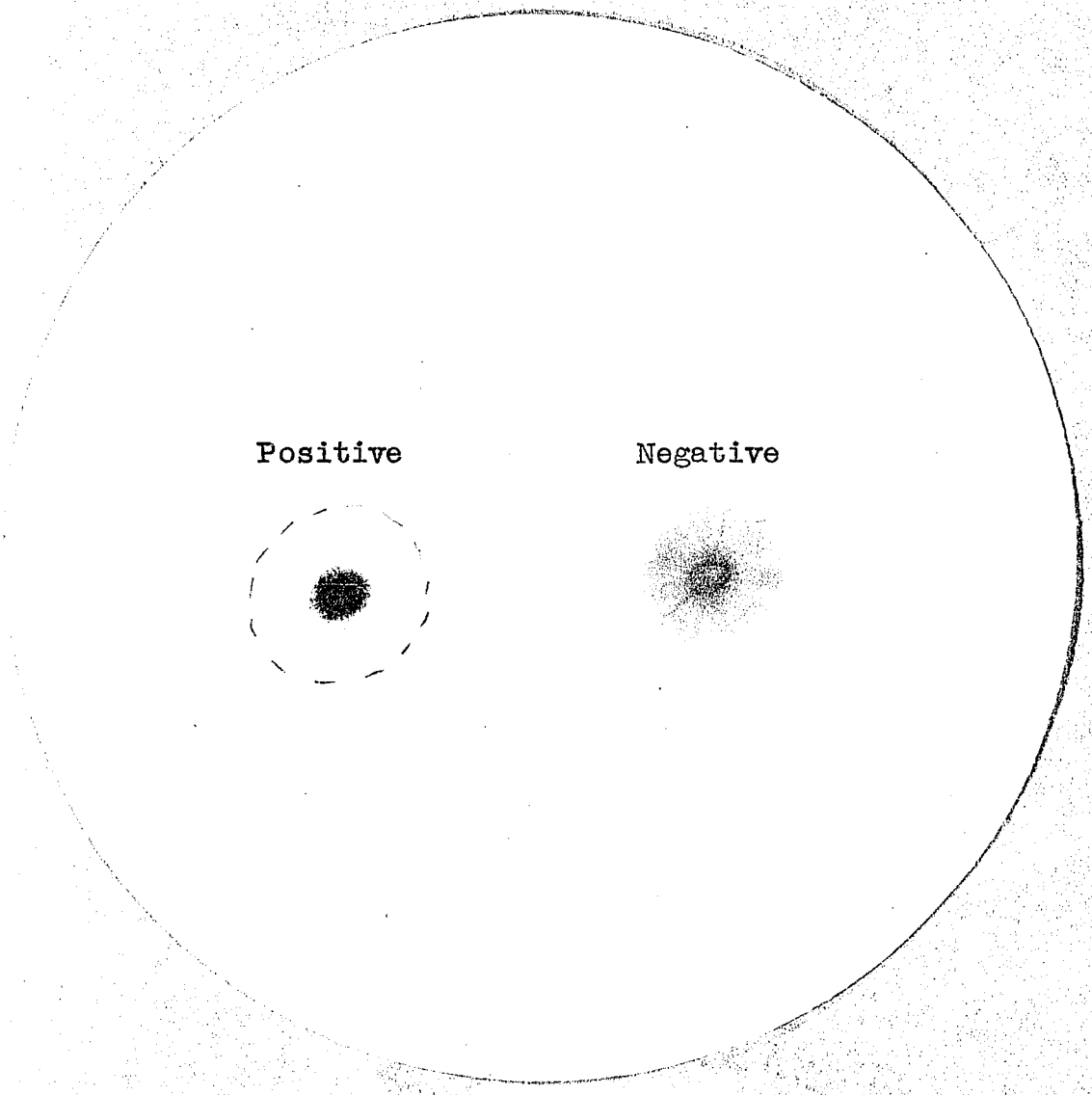

Plate I. Surface fixation test

RESULTS

In Experiment I reciprocal agglutination tests were carried out between S. pullorum #89817, #671, #BAI, and Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255.

S. pullorum and Brucella agglutinated their homologous antigens, Tables 1-6. No reciprocal cross agglutination reactions were observed between S. pullorum #89817 (standard strain) and Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255, Tables 10-12.

A cross agglutination was observed, in a few cases, when S. pullorum #671 (intermediate strain) was tested against the Brucella antigens, Table 8. In a few instances, cross reactions were observed when Brucella antisera were crossed with S. pullorum #671 and S. pullorum polyvalent antigens, Tables 10-12.

Cross agglutinations were observed when S. pullorum #BAI (variant strain) was crossed with the Brucella antigens. Cross reactions were also observed when Brucella antisera were tested against S. pullorum #BAI antigen, Tables 9-12. In a number of cases, however, no cross agglutinations or only atypical agglutinations were observed between some of the Brucella antisera and the S. pullorum polyvalent antigens. Why the results were not uniform is not known, Tables 10-12.

To get a better understanding of the above results the antigenic structure of S. pullorum should be recalled. See pages 6 and 7.

It was pointed out previously that no cross agglutination was observed between the standard strain S. pullorum #89817 and Brucella. Reciprocal cross agglutinations were observed between the variant strain S. pullorum #BAI and Brucella, while in a few cases the intermediate strain of S. pullorum #671 cross reacted with Brucella. From the above results it appears that the antigen common to S. pullorum and Brucella is the XII_2 antigen or a part thereof.

The cross reactions between S. pullorum #671 (intermediate strain) and Brucella are interesting. When S. pullorum #671 was first typed (single colony typing) approximately 18 percent (12 of the 75 colonies tested showed XII_2 activity) of the colonies showed evidence of the XII_2 antigen. Over a period of six months the number of colonies showing XII_2 activity has diminished to the point where now less than 1 percent of the colonies tested show any XII_2 activity. This could explain why cross reactions between this organism and Brucella were often erratic. It also indicates that S. pullorum #671 was undergoing form variation.

Form variation in the Salmonella was first described by Kauffmann in 1940 (36). He described a variation in antigen I (i.e. "I-Formenwechsel"). In 1941 Kauffmann (37) described "a variation in antigen XII fundamentally similar to that of I variation. Within the same strain there occur

colonies with a well-developed antigen XII (form ++), moderately well developed antigen XII (form +) and a weakly developed antigen XII (form ±) which forms dissociate each other."

In determining the antigenic structure of S. pullorum Edwards and Bruner (14) found that S. pullorum underwent antigen XII form variation. They observed, as did Kauffmann (36), that it was the XII_2 antigen that was responsible for the form variation in antigen XII. Antigens XII_1 and XII_3 did not undergo form variation. As Edwards and Bruner pointed out "It seems, then, that S. pullorum is subject to form variation which involves antigen XII_2. In normal cultures this variation occurs continuously, so that the XII_2 ++ and XII ± colonies can be found. However, it is possible for the organisms to become stabilized in the ± or ++ form, thus giving rise to "standard" and "variant" strains. According to this view, the variant strains are not a special strain of S. pullorum but can arise from any culture of the type by stabilization in the ++ form."

Experiment II was carried out further to test the hypothesis that the XII_2 antigen, or a part thereof, was responsible for the cross agglutination between S. pullorum and Brucella. Cross agglutination studies were also carried out between Brucella and other organisms, S. reading, and Proteus (Gwatkin), which were rich in XII_2 antigen.

A cross agglutination was observed between S. reading, Proteus (Gwatkin), and Br. abortus #2308, Br. melitensis #2500,

and Br. suis #1255. Although the organisms were rich in XII_2 antigen the cross reactions observed were not as pronounced as those between S. pullorum #BAI and Brucella, Tables 13 and 15. It is not known if this is a strain characteristic of the organisms involved.

Mono-specific antisera containing IX, XII_1, XII_2, and XII_3 agglutinins were prepared to rule out the possibility of the other antigenic components being responsible for the cross agglutination.

No agglutinations were observed when mono-specific sera containing agglutinins IX, XII_1, and XII_3 were tested against Br. abortus #2308, Br. melitensis #2500 and Br. suis #1255 antigens. However, a cross agglutination was observed between the XII_2 sera and Brucella, Table 14.

In this experiment the XII_2 mono-specific serum was prepared from S. reading antiserum. Although the XII agglutinins were present in abundance, only a small fraction of this agglutinin cross agglutinated with the Brucella antigens.

From the above results one can assume that a portion of the XII_2 antigen is responsible for the cross agglutination reactions studied. In all probability, the amount of the common antigen (for Brucella) present in S. reading, Proteus (Gwatkin), S. pullorum variant and intermediate strains, will vary with each culture. When other bacteria are examined, it may be found that organisms which contain a great deal of the XII_2 antigen will not cross agglutinate

with Brucella, and, on the other hand, organisms may be found with a limited amount of XII_2 antigen which will cross react to a high degree.

In Experiment III reciprocal agglutination tests were carried out between S. pullorum #BAI, Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255. Antisera of the above organisms were absorbed with their homologous and heterologous antigens. Agglutination tests were carried out using the absorbed sera against the above organisms and also against S. reading, and Proteus (Gwatkin) antigens. This was done in an attempt to determine the amount of cross reacting antigens which were present in the above organisms.

The results of the reciprocal agglutination tests (preabsorption titers) between Brucella, S. pullorum #BAI, S. reading, and Proteus (Gwatkin) can be found in Table 15.

Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255 antisera were absorbed with their homologous and heterologous antigens. Agglutination tests were carried out using the absorbed sera against their homologous and heterologous antigens. No significant agglutinations were observed. When the above absorbed sera were tested against S. pullorum #BAI, S. reading, and Proteus (Gwatkin) antigens no cross agglutination was observed, Tables 16-18, 20-22, and 24-26. When Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255 antisera were absorbed with S. pullorum #BAI

antigen, a uniform lowering of the antibody titer was noted. The lowering of the antibody titer was less noticeable when tested against S. reading and Proteus (Gwatkin) antigens, Tables 19, 23, and 27. This indicates that the antigen in Brucella, common to S. pullorum #BAI, S. reading, and Proteus (Gwatkin), exists in equal amounts in all three species of Brucella tested and it can be removed from each by its homologous and/or heterologous antigen.

S. pullorum #BAI antiserum was absorbed with its homologous antigen. No agglutination was observed when the absorbed serum was tested against its homologous and various Brucella antigens, Table 28. However, when S. pullorum #BAI antiserum was absorbed with Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255 cells a uniform lowering of the antibody titer was observed, Tables 29-31.

It would appear from the above results that the antigen common between S. pullorum #BAI and Br. abortus #2308, Br. melitensis #2500, and Br. suis #1255 is a part of the XII_2 antigen. There appears to be an equal amount of the common antigen in the species of Brucella tested. There is an indication, however, that the amount of the cross reacting antigen in S. reading and Proteus (Gwatkin) differs from that which appears in S. pullorum #BAI. Whether this is a strain characteristic of the organism used was not determined.

An interesting sidelight in the work of McCullough, Eisele, and Beal (40) in their study of the antigenic relationship between Vibrio comma and Brucella may be mentioned.

They found that the H antigen of V. comma produced the most pronounced cross agglutination reactions with Br. abortus, while insignificant reactions were noted with Br. suis, and no cross reactions were observed when tested against Br. melitensis.

Felsenfeld et al. (21) reported that V. cholerae can cross agglutinate with S. pullorum. However, it seems unlikely that the common antigen found in the XII_2 antigen is responsible for the cross agglutination reactions between V. cholerae and S. pullorum.

In Experiment IV an attempt was made to determine the number of birds naturally infected with S. pullorum that would produce a cross agglutination with Brucella antigens.

One hundred and seventy-six S. pullorum suspicious and/or reactor turkeys were tested against Br. abortus #2308 and Br. melitensis #2500 antigens in dilutions of 1:25, 1:50, and 1:100. Of this total 29 (seventeen percent) showed a cross reaction with the Brucella antigens. In all cases the sera cross agglutinated both Brucella antigens. There was no evidence of Brucella agglutinins, as shown by a negative filter paper surface fixation test, in any of the birds tested. The reason why this figure appears small may be explained as follows: there may have been only a few reactor birds which harbored the XII_2 antigen; the titers of the birds may have been sufficiently high to produce a positive S. pullorum agglutination response (1:20), but not

high enough to produce a cross agglutination with the *Brucella* antigens; it should also be borne in mind that these cross reactions may not have been due to the presence of *S. pullorum* agglutinins. It is known, as Foshey pointed out (22), that a great many organisms, unrelated to *Brucella*, may cross react with *Brucella*. It is entirely possible that many of the positive agglutination reactions, on the basis of which diagnoses of brucellosis were made, were not caused by homologous brucella agglutinins but by group agglutinins.

TABLE 1

EXPERIMENT I

AGGLUTINATION RESPONSE OF S. PULLORUM #89817 (STANDARD STRAIN) ANTISERUM WHEN TESTED AGAINST VARIOUS S. PULLORUM ANTIGENS

S. pullorum #89817 Antisera

Antigens	852	896	810	813	800	888
S. pullorum #89817	320	160	1280	640	2560	1280
S. pullorum #671	320	160	1280	640	1280	1280
New Hampshire std. antigen	320	160	1280	640	2560	2560
K antigen regular	+	+	+	+	+	+
K antigen polyvalent	+	+	+	+	+	+
Redigen regular	+	+	+	+	+	+
Redigen polyvalent	+	+	+	+	+	+

Figures indicate highest dilution at which agglutination occurred.

TABLE 2

EXPERIMENT I

AGGLUTINATION RESPONSE OF S. PULLORUM #671 (INTERMEDIATE STRAIN) ANTISERUM WHEN TESTED AGAINST VARIOUS S. PULLORUM ANTIGENS

S. pullorum #671 Antisera

Antigens	898	812	849	883	884	828	830	834
S. pullorum #671	640	1280	640	1280	1280	640	640	320
S. pullorum #89817	640	1280	640	1280	1280	640	320	320
New Hampshire antigen	640	1280	640	1280	1280	640	640	320
K antigen regular	+	+	+	+	+	+	+	+
K antigen polyvalent	+	+	+	+	+	+	+	+
Redigen regular	+	+	+	+	+	+	+	+
Redigen polyvalent	+	+	+	+	+	+	+	+

Figures indicate highest dilution at which agglutination occurred.

TABLE 3

EXPERIMENT I

AGGLUTINATION RESPONSE OF S. PULLORUM #BAI (VARIANT STRAIN) ANTISERUM WHEN TESTED AGAINST VARIOUS S. PULLORUM ANTIGENS

S. pullorum #BAI Antisera

Antigens	205	289	225	193	236	240	241
S. pullorum #BAI	320	1280	640	2580	2580	640	640
S. pullorum #89817	80	320	80	160	160	40	80
S. pullorum #671	80	160	40	160	160	20	40
K antigen regular	+	+	+	+	+	+	+
K antigen polyvalent	+	+	+	+	+	+	+
Redigen regular	+	+	+	+	+	+	+
Redigen polyvalent	+	+	+	+	+	+	+

Figures indicate highest dilution at which agglutination occurred.

TABLE 4

EXPERIMENT I

AGGLUTINATION RESPONSE OF BRUCELLA ABORTUS #2308 ANTISERUM WHEN TESTED AGAINST VARIOUS BRUCELLA ANTIGENS

Antisera	Antigens		
	Br. abortus #2308	Br. melitensis #2500	Br. suis #1255
827	2560	2560	2560
800	1280	1280	1280
231	2560	2560	2560
216	1280	1280	1280
831	640	640	640
844	1280	1280	1280
869	160	160	160
867	160	80	80

Figures indicate highest dilution at which agglutination occurred.

TABLE 5

EXPERIMENT I

AGGLUTINATION RESPONSE OF BR. MELITENSIS #2500 ANTISERUM WHEN TESTED AGAINST VARIOUS BRUCELLA ANTIGENS

Antisera	Antigens		
	Br. abortus #2308	Br. melitensis #2500	Br. suis #1255
835	1280	1280	1280
868	160	320	160
866	80	80	80
848	640	1280	640
239	2560	2560	2560
277	2560	2560	2560
821	640	640	640
875	640	640	
882	160	160	
815	80	80	

Figures indicate highest dilution at which agglutination occurred.

TABLE 6

EXPERIMENT I

AGGLUTINATION RESPONSE OF BR. SUIS #1255 ANTISERUM WHEN TESTED AGAINST VARIOUS BRUCELLA ANTIGENS

Antisera	Antigens		
	Br. abortus #2308	Br. melitensis #2500	Br. suis #1255
838	1280	1280	640
873	2560	2560	2560
876	1280	1280	1280
865	640	640	640
230	2560	2560	2560
263	1280	1280	1280

Figures indicate highest dilution at which agglutination occurred.

TABLE 7

EXPERIMENT I

AGGLUTINATION RESPONSE OF S. PULLORUM #89817 (STANDARD STRAIN) ANTISERUM WHEN TESTED AGAINST VARIOUS BRUCELLA ANTIGENS

	Antigens		
S. pullorum #89817 antisera	Br. abortus #2308	Br. melitensis #2500	Br. suis #1255
852	neg.	neg.	neg.
896	neg.	neg.	neg.
810	neg.	neg.	neg.
813	neg.	neg.	neg.
800	neg.	neg.	neg.
888	neg.	neg.	neg.

TABLE 8

EXPERIMENT I

AGGLUTINATION RESPONSE OF S. PULLORUM #671 (INTERMEDIATE STRAIN) ANTISERUM WHEN TESTED AGAINST VARIOUS BRUCELLA ANTIGENS

S. pullorum #671 antisera	Antigens: Br. abortus #2308	Br. melitensis #2500	Br. suis #1255
898	neg.	40	neg.
812	neg.	20	neg.
849	neg.	20	neg.
883	neg.	40	neg.
884	neg.	neg.	neg.
828	neg.	neg.	neg.
830	80	neg.	
834	20	neg.	

Figures indicate highest dilution at which agglutination occured.

Neg. - no agglutination observed in dilutions lower than 1:20.

TABLE 9

EXPERIMENT I

AGGLUTINATION RESPONSE OF S. PULLORUM #BAI (VARIANT STRAIN) ANTISERUM WHEN TESTED AGAINST VARIOUS BRUCELLA ANTIGENS

	Antigens		
S. pullorum #BAI antisera	Br. abortus #2308	Br. melitensis #2500	Br. suis #1255
205	80	80	80
289	320	160	320
225	160	80	80
193	640	320	640
236	320	320	320
240	80	80	80
241	80	80	160

Figures indicate the highest dilution at which agglutination occurred.

TABLE 10

EXPERIMENT I

AGGLUTINATION RESPONSE OF BR. ABORTUS #2308 ANTISERUM WHEN TESTED AGAINST VARIOUS S. PULLORUM ANTIGENS

Antisera

S. pullorum antigens	827	800	231	216	831	844	869	867
S. pullorum #89817	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
S. pullorum #671	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
S. pullorum #BAI			640	320				
K antigen regular	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
K antigen polyvalent	neg.	neg.	+	+	+	neg.	±	neg.
Redigen regular	±	±	±	neg.	neg.	neg.	neg.	±
Redigen polyvalent	neg.	+	+	+	±	±	neg.	neg.

Figures indicate highest dilution at which agglutination occurred.

± - slow or suspicious reactions

TABLE 11

EXPERIMENT I

AGGLUTINATION RESPONSE OF BR. MELITENSIS #2500 ANTISERUM WHEN TESTED AGAINST VARIOUS S. PULLORUM ANTIGENS

	Antisera								
S. pullorum antigens	835	868	866	848	239	277	821	875	882
S. pullorum #89817	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
S. pullorum #671	neg.	neg.	20	20	neg.	neg.	neg.	neg.	neg.
S. pullorum #BAI					320	320			
K antigen regular	neg.	neg.	neg.	±	neg.	neg.	neg.	±	neg.
K antigen polyvalent	neg.	neg.	neg.	+	+	+	neg.	±	neg.
Redigan regular	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Redigan polyvalent	neg.	neg.	neg.	neg.	+	+	neg.	+	neg.

Figures indicate highest dilution at which agglutination occurred.
± slow or suspicious reactions

TABLE 12

EXPERIMENT I

AGGLUTINATION RESPONSE OF BR. SUIS #1255 ANTISERUM WHEN TESTED AGAINST VARIOUS S. PULLORUM ANTIGENS

	Antisera					
S. pullorum antigens	838	873	876	865	230	263
S. pullorum #89817	neg.	neg.	neg.	neg.	neg.	neg.
S. pullorum #671	neg.	neg.	neg.	neg.	neg.	neg.
S. pullorum #BAI					640	320
K antigen regular	neg.	neg.	neg.	neg.	neg.	neg.
K antigen polyvalent	±	±	±	±	+	+
Redigen regular	neg.	neg.	neg.	neg.	neg.	neg.
Redigen polyvalent	±	±	±	±	+	+

Figures indicate highest dilution at which agglutination occurred.

± - slow or suspicious reactions

TABLE 13

EXPERIMENT II

AGGLUTINATION RESPONSE OF S. PARATYPHI A, VAR. DURAZZO, S. READING, AND PROTEUS (GWATKIN) WHEN TESTED AGAINST THEIR HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

Antisera (Preabsorption titers)

Antigens	S. paratyphi A, var. durazzo		S. reading		Proteus (Gwatkin)		
	850	802	155	839	208	895	893
S. paratyphi A, var. durazzo 'O'	2560	5012	320	320	0	0	0
S. reading 'O'	320	640	2560	2560	320	320	320
Proteus (G) 'O'	0	0	640	640	640	1280	1280
S. pullorum #89817	1280	1280	40	40	0	0	0
S. pullorum #671	1280	1280	160	160	40	40	80
S. pullorum #BAI	80	320	1280	1280	320	180	180
Br. abortus #2308	0	0	160	160	40	40	40
Br. melitensis #2500	0	0	80	160	40	40	40
Br. suis #1255	0		160		40	40	

Figures indicate highest dilution at which agglutination occurred.

TABLE 14

EXPERIMENT II

AGGLUTINATION RESPONSE OF MONO-SPECIFIC SERA WHEN TESTED AGAINST VARIOUS ANTIGENS

Mono-specific Sera

Antigens	IX	XII_1	XII_2	XII_3
S. pullorum #89817	0	80	0	320
S. paratyphi A, var. durazzo	0	80	0	1280
S. reading	0	320	640	0
Proteus (Gwatkin)	0	0	640	0
Br. abortus #2308	0	0	80	0
Br. melitensis #2500	0	0	80	0
Br. suis #1255	0	0	80	0

Figures indicate highest dilution at which agglutination occurred.

TABLE 15

EXPERIMENT III

PREABSORPTION TITERS

Antisera

Antigen	S. pullorum #BAI (193)	Br. abortus #2308 (216)	Br. melitensis #2500 (277)	Br. suis #1255 (230)
S. pullorum #BAI	2560	640	320	640
S. reading	1280	160	80	160
Proteus (Gwatkin)	640	80	160	80
Br. abortus #2308	640	1280	2560	2560
Br. melitensis #2500	320	1280	2560	2560
Br. suis #1255	640	1280	2560	2560

Figures indicate highest dilution at which agglutination occurred.

TABLE 16

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. ABORTUS #2308
ANTISERUM ABSORBED WITH BR. ABORTUS #2308 ANTIGEN

Antigen	Slide agg.	Serum dilutions					
		20	40	80	160	320	640
Br. abortus #2308	-	-	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 17

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. ABORTUS #2308
ANTISERUM ABSORBED WITH BR. MELITENSIS #2500 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. abortus #2308	-	-	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 18

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. ABORTUS #2308
ANTISERUM ABSORBED WITH BR. SUIS #1255 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. abortus #2308	-	-	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 19

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. ABORTUS #2308 ANTISERUM ABSORBED WITH S. PULLORUM #BAI ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640	1280	2560
Br. abortus #2308	+	3+	3+	3+	3+	3+	3+	±	-
Br. melitensis #2500	+	3+	3+	4+	4+	4+	3+	±	-
Br. suis #1255	+	4+	4+	4+	4+	4+	3+	+	-
S. pullorum #BAI	-	-	-	-	-	-	-		
S. reading	-	-	-	-	-	-	-		
Proteus (Gwatkin)	-	-	-	-	-	-	-		

± incomplete agglutination

- no agglutination observed

TABLE 20

AGGLUTINATION RESPONSE OF BR. MELITENSIS #2500 ANTISERUM ABSORBED WITH BR. MELITENSIS #2500 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. melitensis #2500	-	-	-	-	-	-	-
Br. abortus #2308	-	-	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 21

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. MELITENSIS #2500 ANTISERUM ABSORBED WITH BR. ABORTUS #2308 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. melitensis #2500	-	-	-	-	-	-	-
Br. abortus #2308	-	±	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

± incomplete agglutination
- no agglutination observed

TABLE 22

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. MELITENSIS #2500 ANTISERUM ABSORBED WITH BR. SUIS #1255 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. melitensis #2500	-	-	-	-	-	-	-
Br. abortus #2308	-	-	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 23

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. MELITENSIS #2500 ANTISERUM ABSORBED WITH S. PULLORUM #BAI ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640	1280	2560
Br. melitensis #2500	+	4+	4+	4+	4+	4+	4+	3+	±
Br. abortus #2308	+	4+	4+	4+	4+	3+	3+	3+	±
Br. suis #1255	+	4+	4+	4+	4+	4+	3+	3+	±
S. pullorum #BAI	-	-	-	-	-	-	-		
S. reading	-	-	-	-	-	-	-		
Proteus (Gwatkin)	-	-	-	-	-	-	-		

± incomplete agglutination
- no agglutination observed

TABLE 24

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. SUIS #1255 ANTISERUM ABSORBED WITH BR. SUIS #1255 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. suis #1255	-	-	-	-	-	-	-
Br. abortus #1255	-	-	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 25

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. SUIS #1255 ANTISERUM ABSORBED WITH BR. ABORTUS #2308 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. suis #1255	-	-	-	-	-	-	-
Br. abortus #2308	-	±	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

± incomplete agglutination
- no agglutination observed

TABLE 26

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. SUIS #1255 ANTISERUM ABSORBED WITH BR. MELITENSIS #2500 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
Br. suis #1255	-	-	-	-	-	-	-
Br. abortus #2308	-	-	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-

- no agglutination observed

TABLE 27

EXPERIMENT III

AGGLUTINATION RESPONSE OF BR. SUIS #1255 ANTISERUM ABSORBED WITH S. PULLORUM #BAI ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640	1280	2560
Br. suis #1255	+	4+	4+	4+	4+	3+	3+	2+	-
Br. abortus #2308	+	4+	4+	4+	3+	3+	3+	3+	-
Br. melitensis #2500	+	4+	4+	4+	4+	4+	3+	3+	±
S. pullorum #BAI	-	-	-	-	-	-	-		
S. reading	-	-	-	-	-	-	-		
Proteus (Gwatkin)	-	-	-	-	-	-	-		

± incomplete agglutination
- no agglutination observed

TABLE 28

EXPERIMENT III

AGGLUTINATION RESPONSE OF S. PULLORUM #BAI ANTISERUM ABSORBED WITH S. PULLORUM #BAI ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640
S. pullorum #BAI	-	-	-	-	-	-	-
S. reading	-	-	-	-	-	-	-
Proteus (Gwatkin)	-	-	-	-	-	-	-
Br. abortus #2308	-	-	-	-	-	-	-
Br. melitensis #2500	-	-	-	-	-	-	-
Br. suis #1255	-	-	-	-	-	-	-

- no agglutination observed

TABLE 29

EXPERIMENT III

AGGLUTINATION RESPONSE OF S. PULLORUM #BAI ANTISERUM ABSORBED WITH BR. ABORTUS #2308 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640	1280	2560
S. pullorum #BAI	+	4+	4+	4+	3+	3+	3+	2+	±
S. reading	+	4+	4+	3+	3+	2+	+	±	-
Proteus (Gwatkin)	+	4+	4+	4+	2+	2+	+	±	-
Br. abortus #2308	-	-	-	-	-	-	-		
Br. melitensis #2500	-	-	-	-	-	-	-		
Br. suis #1255	-	-	-	-	-	-	-		

± incomplete agglutination
- no agglutination observed

TABLE 30

EXPERIMENT III

AGGLUTINATION RESPONSE OF S. PULLORUM #BAI ANTISERUM ABSORBED WITH BR. MELITENSIS #2500 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640	1280	2560
S. pullorum #BAI	+	4+	4+	4+	4+	4+	2+	2+	±
S. reading	+	4+	4+	3+	3+	3+	+	+	-
Proteus (Gwatkin)	+	4+	4+	4+	3+	2+	±	±	-
Br. abortus #2308	-	-	-	-	-	-	-		
Br. melitensis #2500	-	-	-	-	-	-	-		
Br. suis #1255	-	-	-	-	-	-	-		

± incomplete agglutination
- no agglutination observed

TABLE 31

EXPERIMENT III

AGGLUTINATION RESPONSE OF S. PULLORUM #BAI ANTISERUM ABSORBED WITH BR. SUIS #1255 ANTIGEN

Antigen	Slide agg.	Serum dilutions 20	40	80	160	320	640	1280	2560
S. pullorum #BAI	+	4+	4+	4+	4+	4+	3+	3+	-
S. reading	+	4+	4+	3+	3+	3+	2+	±	-
Proteus (Gwatkin)	+	4+	4+	3+	3+	3+	±	±	-
Br. abortus #2308	-	-	-	-	-	-	-		
Br. melitensis #2500	-	-	-	-	-	-	-		
Br. suis #1255	-	-	-	-	-	-	-		

± incomplete agglutination
- no agglutination observed

DISCUSSION

It has been pointed out by Foshey (22), Cioglia (8, 9), and others that a number of salmonellae can cross agglutinate with Brucella. However, no one as far as I have been able to determine, has made an attempt to determine what the common antigens in these groups of organisms are. Cioglia (8, 9), however, determined whether the H or O antigens were responsible for the cross agglutination between Br. melitensis and the Salmonella which he studied.

In this experiment it was found that a part of the XII_2 antigen can cross agglutinate with Brucella. A number of organisms in the genus Salmonella possess antigen XII_2. Some of these are: S. enteridites, S. paratyphi B, S. typhi-murium, S. derby, S. abortus-ovis, S. abony, S. brandenburg, S. typhosa, and S. essen 173. These organisms are known to cause infection in man and animals. One can speculate on the possibility that some of these organisms when found in cattle can produce agglutinins in sufficient quantity to cause a cross agglutination when being tested for Bangs disease.

This speculation can also be applied when carrying out agglutination tests for undulant fever. It is known that S. typhosa contains the XII_2 antigen(37). It would

be possible for one who has had typhoid fever, or if one is immunized against typhoid fever to develop cross reacting agglutinins for Brucella.

It should again be re-emphasized that it is not known, at present, whether all cultures which contain the XII_2 antigen also contain that portion of the antigen which can cross agglutinate with Brucella.

In discussing the problem of cross agglutination reactions between S. pullorum and Brucella the question can be raised as to the prevalence and importance of Brucella infection in fowl. The frequency and importance of brucellosis in birds is still under discussion.

Van Roekel, Bullis, Flint, and Clarke (56) examined 25,202 chickens; the area covered represented approximately every county in Massachusetts. No reactors to Brucella antigen were found in any of the birds when a dilution of 1:25 and 1:50 were used.

McNutt and Purwin (41) examined 69 flocks containing over 10,000 birds, and found less than 2 percent reactors. No one flock contained more than 12 percent reactors.

Emmel (18) found 16.5 percent reactors in one flock of 90 chickens.

Huddleson and Emmel (31) examined four flocks which they believed had brucellosis. In flock number one, 114 birds were tested. Seventeen of these birds' blood sera agglutinated Br. abortus in dilutions of 1:25 to 1:100. In flock number 3, 64 birds were examined. Thirteen of

these birds' blood sera were found to agglutinate Br. abortus in dilutions varying from 1:25 to 1:100. "Eleven of these birds were purchased, killed, autopsied, and organs cultured for the genus Brucella. . . . Cultures from all the organs remained sterile. Fifteen eggs were taken from these birds and cultured for Brucella and none were found to be infected." In the fourth flock "three birds were received for diagnosis at the laboratory. The flock consisted of 800 birds. Thirty were sick. Ten had died, all showing emaciation, paleness of the comb, wattles, and about the head, diarrhea, and extreme weakness. A few had shown paralysis just before death. Egg production had dropped 15 percent." The three birds examined had titers to Brucella ranging from 1:50 to 1:100. "A species of Brucella was isolated from the lungs, kidneys, and spleen of bird No. 1. Cultures made from the organs of the remaining birds remained sterile."

Anguelov, cited by Stafseth (53) reported that brucellosis is very prevalent in modern poultry plants in Bulgaria.

Felsenfeld (20) reported that brucellosis in fowl is a serious problem in Eastern Europe. Brandly (3) also reported that Russian workers have stated that brucellosis is a major poultry problem in Russia.

In most of the diagnoses of brucellosis in fowl the majority of investigators (13, 31, 39, 41, 42) have been unable to isolate the organism from the affected birds.

Most of the diagnoses of brucellosis have been based on the finding of agglutinins which agglutinate Brucella antigens and evidence of the disease in other animals on the farm.

"The vast majority of authors have failed to observe symptoms of brucellosis in birds, and it is not certain that the symptoms described by others have actually been due to the disease." (53).

Felsenfeld et al. (21) stated "Due to frequent lack of clinical symptoms in birds infected with Brucella and the possibility that the death of some birds may be misinterpreted as pullorum disease, because of the serologic cross reactions with pullorum antigens, the true cause of some diseases in poultry may be obscured."

The most important aspect of brucellosis in fowl is its relationship to public health. Brucellosis in fowl constitutes a definite public health hazard. This has been pointed out by Felsenfeld et al. (21), Brandly (2), Ingalls (32), Brandly (3), and Felsenfeld (20).

Felsenfeld et al. (21) remarked "one should keep in mind the possibility of human infection by eating Brucella-infected poultry meat. The meager pathologic signs make it difficult or even impossible, to detect all infected chickens during food inspection." In another paper Felsenfeld (20) further pointed out "Since blood cultures frequently become positive during such infections, chickens may serve not only as vectors of brucellosis on the farm but if slaughtered

during the bacteremia, may provide meat that is infected with Brucella."

This same thought was expressed by Brandly (3), "the carcasses of diseased birds often contain myriads of pathogenic organisms which are introduced into the kitchen with the carcasses; and knives, sinks, pans, hands, towels, etc, are contaminated by these disease germs. In preparing chicken salads, cold chicken sandwiches, etc. these organisms can be reintroduced into the edible product and cases of food poisoning or infection are the result."

"Since birds can become infected with Brucella and may thus serve as agents of transmission of this disease not only to other birds but to mammals as well, one should take steps to prevent fowl from being in contact with infected mammals. Good poultry hygiene demands that poultry should be confined within premises set aside for this type of livestock and not be allowed in barns, hog yards, etc. The practice of throwing dead chickens on a manure pile or elsewhere, where hogs or other birds may eat them, is to be condemned." (53).

At present birds are not routinely tested for brucellosis. If, however, Brucella testing of birds becomes necessary, S. pullorum agglutinins may interfere with an accurate serological diagnosis. To overcome this, the use of the filter paper fixation test may prove to be a valuable diagnostic test.

To test the hypothesis that the filter paper surface

fixation test is specific for Brucella agglutinins, the antisera of a number of organisms that are known to cross agglutinate with Brucella were tested using this method. These included Proteus OXK, Proteus OX2, Proteus OX19, Proteus (Gwatkin), S. reading, and S. pullorum #BAI. None of the above antisera produced a positive reaction, while Brucella antisera continued to give a positive test.

Although the filter paper surface fixation test may prove to be of value in the diagnosis of brucellosis, the isolation and identification of one of the members of the genus Brucella constitutes the only positive way of diagnosing this disease.

SUMMARY AND CONCLUSIONS

No cross agglutination reactions were observed between standard strain S. pullorum #89817 and Brucella. In a number of cases cross reactions were noted between intermediate strain S. pullorum #671 and Brucella. Cross agglutination reactions were observed between variant strain S. pullorum #BAI and Brucella. The antigenic structure of S. pullorum was determined by Edwards and Bruner (14) to be IX, XII_1, (XII_2), XII_3. From the above results it appears that the XII_2 antigen, (found in the intermediate and variant strains of S. pullorum) or a part thereof, was responsible for the observed cross reactions.

Reciprocal agglutination tests were also carried out with Brucella and other organisms, Salmonella reading and Proteus (Gwatkin) which contain the XII_2 antigen. The cross reactions observed between these organisms and Brucella were less pronounced than those noted between S. pullorum #BAI and Brucella. There appears to be a quantitative difference in the amount of the cross reacting antigen (for Brucella) in S. pullorum #BAI, S. reading, and Proteus (Gwatkin). Whether this is a strain characteristic of the organisms involved is not known.

Mono-specific sera containing agglutinins IX, XII_1, XII_2, and XII_3 were prepared. Only the XII_2 mono-specific

sera produced cross agglutinations with the Brucella antigens. It was noted that only a small fraction of the XII_2 antigen was involved in these cross reactions.

Agglutinin absorption studies carried out with S. pullorum #BAI and Brucella indicated that the antigen common to S. pullorum #BAI, S. reading, and Proteus (Gwatkin) existed in approximately equal amounts in all three species of Brucella tested.

One hundred and seventy-six S. pullorum suspicious and reactor turkeys were tested using Br. abortus #2308 and Br. melitensis #2500 antigens in dilutions of 1:25, 1:50, and 1:100. Of this total, 29 (seventeen percent) showed a cross reaction with the Brucella antigens.

A number of salmonellae, pathogenic for man and animals, contain antigen XII_2. These organisms, if they contain the antigenic factor common for Brucella, may produce agglutinins in sufficient quantity to cause false positive agglutination tests for brucellosis.

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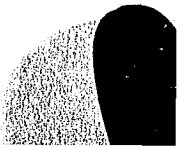

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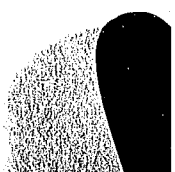

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