



THE GENERIC RELATIONSHIP OF
BRUCELLA, PASTEURELLA
PFEIFFERELLA AND HEMOPHILUS

THESIS FOR THE DEGREE OF M. S.

Ina Maxson

1934

THESIS



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Brucella
Pasteurella
Pfeifferella
Hemophilus

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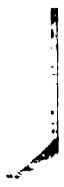
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Thesis

Submitted to the Faculty of Michigan State College
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Ina Maxson

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THESIS

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I. INTRODUCTION

In the literature of the past 15 years, fragmentary observations have appeared suggesting a serological relationship between various species of *Brucella* and *Pasteurella*, *Brucella* and *Hemophilus*, *Brucella* and *Pfeifferella**, and other combinations.

In 1930 Mallmann published a preliminary report of the interagglutinability of the *Brucella*, *Pasteurella*, and *Pfeifferella* genera. He observed quite marked cross agglutination. Using *Brucella abortus* and *Br. suis* immune rabbit serums, he obtained as high titres with the heterologous antigens as with the homologous ones. No attention was paid to the state of dissociation of the cultures used.

It is well known that when a smooth strain of most genera shifts to its rough form, profound antigenic changes occur. Very frequently the rough strain is serologically different from its smooth prototype. White (193) has demonstrated that the rough forms of the species of the genus *Salmonella* have a common rough antigen that is demonstrated serologically by marked cross agglutination. These same species in the smooth phase are distinctly different serologically.

* Although the genus *Pfeifferella* has been merged with *Actinobacillus* in the latest edition of Bergey's "Determinative Bacteriology," the name is retained in this thesis so as not to confuse with other members of the *Actinobacillus* group.



This antigenic relationship of the rough phase of other genera may also be true of the *Brucella*, *Pasteurella*, *Hemophilus*, and *Pfeifferella* groups.

A review of the literature of these genera would indicate that such might be the case. Furthermore, a close examination of the properties and characteristics of these genera shows a very close similarity.

For these reasons, a study of the *Brucella*, *Pasteurella*, *Hemophilus*, and *Pfeifferella* groups has been made. The work consists of morphological studies of both smooth and rough phases of representative species of these genera.

II. HISTORICAL RESUME

A serological and physiological correlation of *Brucella* and *Alkaligenes bronchisepticus* was reported by Evans in (1918). In the same year Povitzky and Worth (1918) showed a cross agglutination between *Alk. bronchisepticus* antigen. They report no cross agglutination between *H. pertussis* and *H. influenzae*.

Ferry and Noble (1918) found the same serological relationship between *Alk. bronchisepticus* and *H. pertussis*; also a cultural, morphological, and physiological similarity which is more marked after growth for some time on an artificial medium.

Francis and Evans (1926) found that 37 per cent of positive tularemia serums showed cross agglutination with



Br. abortus and Br. melitensis. In 3 cases the titres were equal for all 3 of these organisms, although as a rule the heterologous titres were lower. Burnet (1927) also reported interagglutinability of P. tularensis and Brucella in tularemia and Mediterranean fever.

Huddleson found positive cross agglutination of Pfeifferella mallei and Br. abortus.

In contradiction to the results obtained by Mallmann, Priestly (1933) reported no serological relationship between the Brucella and the Pasteurella genera. Thompson (1933) tested Pf. mallei serologically with strains of Br. abortus and P. tularensis and found no evidence of cross agglutination.

III. HISTORY OF CULTURES

All of the following Brucella organisms used in these studies are from the culture collection of Dr. I. F. Huddleson.

Br. abortus 966 was isolated in 1930 from a cow from a Michigan herd.

Br. suis 436 was isolated in 1928 and obtained from Crawford from Washington D. C.

Br. melitensis 316 is of human origin. It was obtained from Dr. Burnet, Pasteur Institute, Tunis, Algeria, and dissociated by Mallmann and Gallo (1933).

Br. melitensis 384 was isolated in 1929 from a mammary lymph node of a goat.

Br. abortus 4a was isolated in 1924 from an aborted



fetus from a cow from the Michigan State College experimental herd. It was dissociated by Mallmann and Gallo (1933).

Br. suis 404 was isolated in 1922 from a premature fetal pig from a naturally infected sow. It was received from J. W. Connaway, University of Missouri. This was also dissociated by Mallmann and Gallo (1933).

Br. suis 401 was isolated in 1925. The source of the culture is not known. It was obtained from Purdue University.

Br. melitensis 319 was obtained from Dr. K. F. Meyer, Hooper Foundation, San Francisco, May 10, 1921.

Br. abortus 5a was in the laboratory prior to 1916.

Pasteurella avicida was obtained from the Michigan State College stock culture collection.

Pasteurella tularensis 38 was a non pathogenic strain from Dr. Francis, obtained from the Michigan State Department of Health culture collection.

P. bovisepcticus was isolated from a buffalo with hemorrhagic septicemia. It was obtained from the Bureau of Animal Industry, Washington, D. C.

H. pertussis was obtained from the Michigan State Department of Health culture collection.

H. influenzae 1601 was obtained from the Michigan State Department of Health culture collection. It was rough when obtained.

Pf. mallei was obtained from the Michigan State College stock culture collection. It was rough when obtained.

1. The first part of the document is a list of names and dates, which appears to be a record of some kind. The names are written in a cursive script, and the dates are in a standard font. The list is organized into two columns, with names on the left and dates on the right. The names are: John Smith, James Brown, William Jones, Thomas White, and Robert Black. The dates are: 1810, 1811, 1812, 1813, and 1814. The list is followed by a section of text that is mostly illegible due to the quality of the scan. The text appears to be a description of the events that took place during the period covered by the list. The text is written in a cursive script, and the words are often difficult to read. The text is organized into paragraphs, with the first paragraph starting with the word "The" and the second paragraph starting with the word "The". The text is followed by a section of text that is also mostly illegible. The text appears to be a continuation of the description of the events that took place during the period covered by the list. The text is written in a cursive script, and the words are often difficult to read. The text is organized into paragraphs, with the first paragraph starting with the word "The" and the second paragraph starting with the word "The".

Pf. mallei F87 was obtained from the Bureau of Animal Industry, Washington, D. C.

IV. EXPERIMENTAL

1. Bacteriological Studies of Smooth Strains.

Bergey (1930) classifies the organisms of the *Brucella*, *Pasteurella*, *Hemophilus*, and *Pfeifferella* groups as minute Gram negative bacteria with a tendency to bipolar staining in the *Hemophilus* and *Pasteurella* genera. All the organisms used in the following studies are non-motile. With the exception of P. avicida all are inactive in carbohydrate media, none of them producing acid or gas.

H. influenzae, H. pertussis, P. tularensis, P. equisepticus, and the *Brucella* group are aerobes. P. avicida and Pf. mallei are facultative aerobes.

All organisms used produce small grayish colonies. Pf. mallei liquefies gelatin very slowly. No other organisms of these groups liquefy gelatin.

There is, thus among all of these genera a close morphological and physiological similarity of organisms.

Table I on the following page gives the characteristics of these bacteria as found in Bergey's manual of Determinative Bacteriology, (1933).

Table 1. - MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE ORGANISMS USED

IN THE FOLLOWING STUDIES. BERGEY (1933)

	Br.	Br.	P.	P. bovi-
: abortus	: melitensis	: avicida	: septicus	
: size of	: 0.4-0.5	: 0.3-0.4	: .25-0.4	: 0.5
: rods-microns	: by 0.6-1.5	: in length	: by 1.5-0.3	: by 0.6-1.0
: motility	: non-motile	: non-motile	: non-motile	: non-motile
: Staining	: Gram neg.	: Gram neg.	: Gram neg.	: Gram neg.
: growth	: singly	: singly and:	: bipolar	: bipolar
: gelatin	: pairs	: pairs	: singly	: singly
: stab	: slow growth	: no liq.	: no liq.	: no liq.
: blood agar	: transparent:			
: colonies	: very small			
: sterilized				
: potato slant				
: unheated				
: potato slant				
: indol	: not	: not	: -	: not
: formed			: -	: not
: nitrates	: not	: not	: -	: not
: reduced			: -	
: dextrose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: levulose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: galactose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: sucrose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: mannitol	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: aerobic		: facultative	: aerobic	
: agar slant		: ground glass:	: drop like	: gray white
: 66		: dark center:	: colony	: sl. raised
: litmus	: slightly	: unchanged	: no	: acid litmus
: milk	: alkaline	: or alk.	: change	: reduced

*a. acid g. gas



Table 1. (Continued)

	P.	H.	H.	Pf.
: tularensis	: pertussis	: influenzae	: mallei	:
: size of	: 0.2	: 0.2 - 0.3	: 0.2 - 0.3	: .25 - 0.4
: rods-microns	: by 0.3 - 0.7	: by 0.5 - 2.0	: by 0.5 - 2.0	: by 1.5 - 3.0
: motility	: non-motile	: non-motile	: non-motile	: non-motile
:	:	:	:	:
: staining	: Gram neg.	: Gram neg.	: Gram neg.	: Gram neg.
:	: bipolar	: bipolar	: bipolar	:
: growth	: singly	: singly and	: singly and	: usually
:	:	: pairs	: pairs	: singly
:	:	:	: rarely chains	:
: gelatin	:	: no growth	: no growth	:
: stab	:	:	:	:
: blood agar	:	: transparent	: transparent	:
: colonies	:	: very small	: very small	:
: sterilized	:	:	:	:
: potato slant	:	:	:	:
: unheated	:	:	:	:
: potato slant	:	:	:	:
: indol	:	: not	: some	: not
: formed	:	:	: strains	:
: nitrated	:	: not	: to	: not
: reduced	:	:	: nitrites	:
: dextrose	: no a. or g.*	: no a. or g.	: no a. or g.	: no a. or g.
: levulose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: galactose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: sucrose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: mannitol	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.
: aerobic	:	: aerobic	: aerobic	: facultative
: agar slant	: small visc.	: filiform	:	:
: 66	: viscous	: translucent	:	:
: litmus	: no growth	: alk. decolor-	:	: acid coag.
: milk	: in milk	: ized 10-20 da	:	: 10-12 days

* a. acid g. gas

At the beginning of these studies all cultures were plated and S colonies fished. A chicken infusion medium was used for culturing all organisms throughout this work. For about 3 months stock cultures of H. pertussis and H. influenzae were held upon blood chicken infusion agar, and P. tularensis upon chicken infusion agar containing 0.1 percent cystine. However, the colonial appearance of the cultures remained the same upon chicken infusion agar without the blood or cystine and these ingredients were omitted from the stock medium.

All organisms were checked morphologically and physiologically with Bergey's classification of these genera. The size of the rods of the same culture varied. Sometimes they appeared as cocci and at other examinations the organisms were definitely bacilli. However, they were never long rods when in the S colonial state.

Bipolar staining was not noted in the organisms with an S colonial appearance. All of the colonies were small, gray, glistening and moist with smooth edges and surface.

P. avicida although tested several times, at no time produced acid in 0.5 percent sucrose chicken infusion broth with Andrade's indicator.

P. tularensis grew in milk. This is contrary to Bergey. This may have been due to the fact that this was an old culture accustomed to growing upon artificial media. The reactions of all of the other cultures in litmus milk were the same as Bergey's

Pf. mallei S was lost before it was checked for its ability to liquefy gelatin in a stab culture. Pf. mallei R did not liquefy gelatin. No other organism of these groups attacked gelatin which was also true according to Bergey.

The following table II shows the morphological and physiological characteristics of the cultures used.

Table 2 - MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS AS FOUND IN IDENTIFYING

THE 8 STRAINS OF CULTURES USED.

	:Br. abortus	: Br. suis	:Br.melitensis
	: 966	: 436	: 384
: size of	: 0.4	: 0.4	: 0.4
: rods-microns	: by 0.5 - 1.5	: by 0.6 - 1.5	: by 0.6 - 1.0
: motility	: non-motile	: non-motile	: non-motile
: staining	: Gram neg.	: Gram neg.	: Gram neg.
: growth	: singly	: singly	: singly
: gelatin	: no	: no	: no
: stab	: liquefaction	: liquefaction	: liquefaction
: agar	: small, gray,	: small, gray,	: small, gray,
: colonies	: glistening	: glistening	: glistening
: litmus	: alkaline	: alkaline	: alkaline
: milk	:	:	:
: dextrose	: no a. or g.*	: no a. or gl.	: no a. or g.
: levulose	: no a. or g.	: no a. or g.	: no a. or g.
: lactose	: no a. or g.	: no a. or g.	: no a. or g.
: sucrose	: no a. or g.	: no a. or g.	: no a. or g.
: mannite	: no a. or g.	: no a. or g.	: no a. or g.
: maltose	: no a. or g.	: no a. or g.	: no a. or g.

* a. acid g. gas

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Table 2 - (continued)

	:P.	:P.	:H.	:Pf.	:
: avicida	: tularensis	: pertussis	: mallei	:	:
: size of	: 0.3	: 0.2	: 0.2	: 0.3	:
: rods-microns	: by 0.5 - 1.5	: by 0.4 - 1.0	: by 0.5 - 1.5	: by 1.0 - 1.5	:
: motility	: non-motile	: non-motile	: non-motile	: non-motile	:
: staining	: Gram neg.	: Gram neg.	: Gram neg.	: Gram neg.	:
: growth	: singly	: singly	: singly	: singly	:
: gelatin	: no	: no	: no	: no	:
: stab	: liquefaction	: liquefaction	: liquefaction	: liquefaction	:
: agar	: small, gray,	: small, gray	: small, gray,	: small, gray,	:
: colonies	: glistening	: glistening	: glistening	: glistening	:
: litmus	: no change	: alkaline	: alkaline	: acid after	:
: milk	:	:	:	: 10 days	:
: dextrose	: acid, no g.*	: no a. or g.	: no a. or g.	: no a. or g.	:
: levulose	: acid, no g.	: no a. or g.	: no a. or g.	: no a. or g.	:
: lactose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.	:
: sucrose	: no a. or g.	: no a. or g.	: no a. or gl.	: no a. or g.	:
: mannite	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.	:
: maltose	: no a. or g.	: no a. or g.	: no a. or g.	: no a. or g.	:

* a. acid g. gas

2. Methods of Dissociation Used.

Br. melitensis 384, Br. abortus 4a, Br. suis 404 and Br. melitensis were dissociated to their R strains by repeated 48 hour transfer in 10 percent homologous serum broth at 37°C. Br. suis 404 R and Br. melitensis 316 R were dissociated back to their S prototypes by Mallmann and Gallo, (1933). None of the other dissociants have been reverted.

P. avicida R was dissociated by holding the culture in the ice-box for several months. During this time the culture became a mixed R and S form. Typical S colonies were fished several times, but after being held for sometime as stock, during which time the culture was transferred only occasionally, the R type appeared when plates were streaked.

Attempts were made to dissociate P. tularensis and H. pertussis by repeated 48 hour transfer at 37°C., but each time the organisms died before typical R colonies were obtained. It had been noted that when the S organisms were grown upon 1 per cent dextrose chicken infusion agar, which had been used for holding stock strains, the cultures tended toward the R type. When grown upon chicken agar without dextrose, the culture remained more typically S. Concluding from this that it was perhaps the carbohydrate producing this change from S toward R, these organisms were grown in 1 per cent sucrose broth and dissociated to their R types by repeated 48 hour transfers at 37°C.

H. influenzae was an R form when received. Attempts to revert this organism to the S type by animal passage were unsuccessful. A mouse was given an intraperitoneal injection of 1 cc. of H. influenzae culture, containing the organisms from 1 slant. Within 24 hours the mouse died. The culture was not recovered. In second and third attempts the mice did not die. At 72 hours they were killed, but the injected culture was not recovered.

Pf. mallei was also an R strain when received. It had been held as a stock culture for many years.

3. Bacteriological Studies of R strains.

As with the S forms of these organisms, morphological and physiological studies were made of the R types. At some time during the period in which they were being observed for the following studies all R forms were Gram positive. This was not contamination for the same culture later after several transfers decolorized and took the counter stain, then again returned to Gram positive. Also Gram positive and Gram negative organisms were often present in the same slide preparation. All R cultures at some time during their use in agglutinin absorption studies showed barred and bipolar staining with methylene blue.

The R colonies were more opaque, larger, duller and flatter than the S type, with a granular and wrinkled appearance.

An exception was found in the P. avicida colonies, in which case the S and R colonies were much the same in size. The R forms were not flattened, but possessed the typical R surface and opaqueness.

Some of the organisms acquired motility in their change to the R type. P. avicida did not change its reaction toward litmus milk. H. pertussis lost its ability to produce an alkaline reaction in litmus milk.

The R forms of the Brucella genus retained their inactivity toward carbohydrates. P. avicida in its R form still did not produce acid in 0.5 per cent sucrose chicken broth and the reaction of this organism for the other sugars remained the same as in the S form. H. pertussis R by dissociation had gained the ability to produce acid in all the sugar media used, although in its S form no acid was produced in any sugar medium.

The ability to liquefy gelatin was not acquired by any organism by dissociation.

Table III, on the following page gives the morphological and physiological properties of the R forms of these groups of organisms.

TABLE 3 - MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE R
STRAINS OF THE CULTURES USED.

	:Br. abortus : 4a R	: Br. suis : 404 R	:Br. melitensis: : 316 R
: size of	: 0.4 - 0.6	: 0.4 - 0.6	: 0.4 - 0.6
: rods-microns	: by 2.0	: by 2.0	: by 2.0 - 4.0
: motility	: non-motile	: motile	: non-motile
:	:	:	:
: staining	: Gram - or -	: Gram - or -	: Gram - or -
:	: bipolar	: bipolar	: bipolar
: growth	: singly	: singly	: singly
:	:	:	:
: gelatin stab:	: no	: no	: no
:	: liquefaction	: liquefaction	: liquefaction
: agar	: opaque, rough:	: opaque, rough:	: opaque, rough:
: colonies	: dull	: dull	: dull
: litmus	: alk., reduced	: alk., reduced:	: alk., reduced
: milk	: at base	: at base	: at base
: dextrose	: - acid -gas	: -acid -gas	: -acid -gas
: levulose	: - acid -gas	: -acid -gas	: -acid -gas
: lactose	: - acid -gas	: -acid -gas	: -acid -gas
: sucrose	: - acid -gas	: -acid -gas	: -acid -gas
: mannite	: - acid -gas	: -acid -gas	: -acid -gas
: maltose	: - acid -gas	: -acid -gas	: -acid -gas

Table 3 - (Continued)

	P.	H.	H.	Pf.
: avicida R	: pertussis R	: H.	: H.	: mallei R
: size of	: 0.5	: 0.3	: 0.2 - 0.3	: 0.5
: rods-microns	: by 2.0	: by 1.5 - 2.0	: by 0.8 - 1.0	: by 1.0 - 2.0
: motility	: non-motile	: motile	: non-motile	: motile
: staining	: Gram - or -	: Gram - or -	: Gram - or -	: Gram - or -
: growth	: bipolar	: bipolar	: bipolar	: bipolar
: growth	: singly	: singly	: singly	: singly
: gelatin stab:	: no	: no	: no	: no
: liquefaction	: liquefaction	: liquefaction	: liquefaction	: liquefaction
: agar	: opaque, rough	: opaque, rough	: opaque, rough	: opaque, rough
: colonies	: dull	: dull	: dull	: dull
: litmus	: no change	: no change	: no change	: no change
: milk	:	:	:	:
: dextrose	: -acid -gas	: -acid -gas	: -acid -gas	: -acid -gas
: levulose	: -acid -gas	: -acid -gas	: -acid -gas	: -acid -gas
: lactose	: -acid -gas	: -acid -gas	: -acid -gas	: -acid -gas
: sucrose	: -acid -gas	: -acid -gas	: -acid -gas	: -acid -gas
: mannite	: -acid -gas	: -acid -gas	: -acid -gas	: -acid -gas
: maltose	: -acid -gas	: -acid -gas	: -acid -gas	: -acid -gas

4. Bacteriological Comparison of the S and R Forms.

From the studies of the morphological and the cultural properties of the S and R types, a varying degree of change in the different organisms is seen. The R type in each case was determined only by colonial appearance. From the data shown in the preceding tables there appeared to be an increase in the ability of all R organisms to become Gram positive. All R organisms had increased in size, also there was an increased tendency to barred or bipolar staining. Motility in several cases was found in the R form of the bacteria.

H. pertussis, R, an organism non-active in carbohydrate media, had acquired by dissociation the ability to produce acid in all the sugars used, dextrose, sucrose, mannite, levulose, and maltose. No other organism showed any change in activity toward the sugars used.

There appeared to be no correlation between the R and S types in their reaction in litmus milk. Some had become more active, others less active. Br. abortus 966 S, Br. suis 436 S and Br. melitensis 384 S gave an alkaline reaction in litmus milk. Br. suis 404 R, Br. melitensis 316 R and Br. melitensis 384 R had become more active toward litmus milk by dissociation, reducing the litmus as well as first showing the alkaline production characteristic of the S prototypes.

5. Rabbit Immunization with the S and R Forms of All Organisms Studied.

Rabbits were immunized with both the smooth and rough dissociants of each organism in which both forms were available. The immune serums for Br. abortus 966 S, Br. suis 436 S and Br. melitensis 384 S were produced by 1 intravenous injection of the unkilld cultures. One cc of a suspension with a density corresponding to tube No. 3, McFarland's nephelometer, was used. About 3 weeks later the animals were bled aseptically from the heart.

Antigens of all other cultures were killed by heating at 60°C for 1 hour and with 1 exception all were injected intravenously. An intraperitoneal injection of H. influenzae R was made because spontaneous agglutination produced clumps in the bacterin too large for safe intravenous injection.

The bacterins were given every 3 to 4 days in 1 cc amounts of a suspension, with a density corresponding to tube No. 3, McFarland's nephelometer. At this density about 1,000,000,000 organisms per cc were present. An average of about 7 injections were made before the titre was sufficiently high to bleed the rabbits. The blood was collected under sterile conditions from the heart and all serums were preserved with 0.5 per cent phenol.

6. Methods Used for Agglutination Tests and the Reading of Titres.

All antigen suspensions used in the agglutination tests were made in 0.1 per cent salt solution containing 0.1 per cent phenol. A density comparable to tube number 2 McFarland's nephelometer standards was used.

The serum serial dilutions were also made in 0.1 per cent salt solution containing 0.1 per cent phenol. Each tube contained 0.5 cc. of the serum dilution. To each was added from a dropper 1 drop of the antigen suspension. This gave a density corresponding to about $\frac{3}{4}$ the density of McFarland's standard tube number 1. A control tube was made for each test by omitting the serum, using only the saline solution plus antigen. After adding the antigen suspension the tubes were shaken and incubated for 48 hours at 37°C. At the end of this period the titres were read over a student reading lamp, held against a dark back ground. The shade was turned in such a way that the bottom edge was perpendicular to the table, and the shade between the reader and the light. The tube containing the agglutinated suspension was held against this perpendicular edge above the light. All readings were made with a hand lens, for the clumps in many cases were too small to be seen with the unaided eye. After this initial reading the tests were placed in the ice box over night and read again.

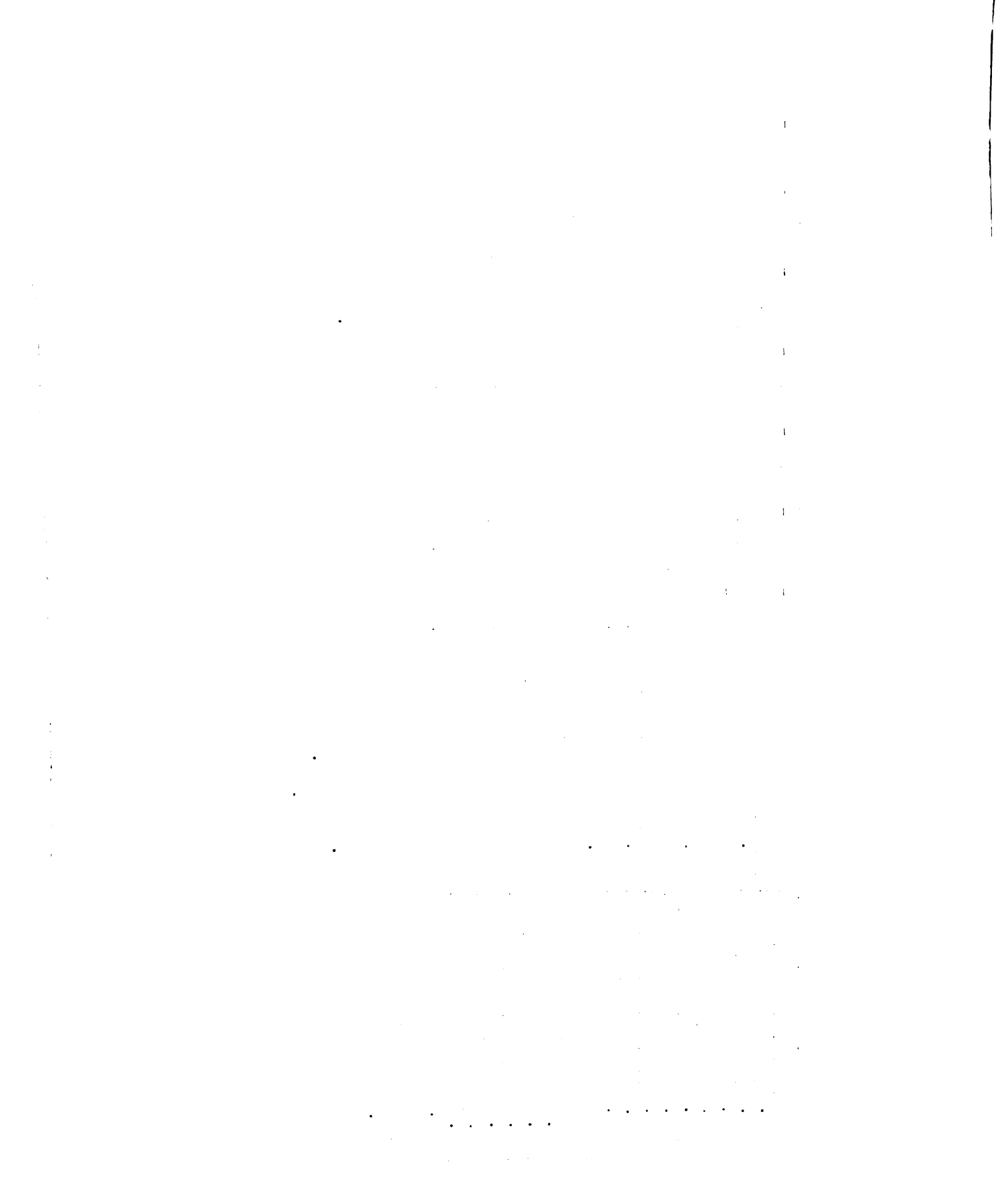
The titres of the immune serums for their homologous antigens are given in the following table 4.

Table 4 - TITRES OF IMMUNE RABBIT SERUMS FOR THEIR HOMOLOGOUS ANTIGENS

Antiserums	Antigens	1-20	1-40	1-80	1-60	1-320	1-640	1-380	1-2560
: Br. abortus 966 S		: ++	: ++	: ++	: +++	: +++	: +++	: +++	: +++
: Br. abortus 5a S	: Br. abortus 966 S	: +++	: +++	: +++	: ++	: +	: :	: :	: :
: Br. abortus 4a R		: -	: +++	: +++	: +++	: +++	: ++	: ++	: ++
: Br. suis 436 S		: ++	: +++	: +++	: +++	: +++	: +++	: +++	: +++
: Br. suis 401 S	: Br. suis 436 S	: +++	: +++	: +++	: +++	: ++	: ++	: ++	: ++
: Br. suis 404 R		: +++	: ++	: ++	: ++	: +	: :	: :	: :
: Br. melitensis 334 S		: ++	: ++	: +++	: +++	: +++	: +++	: +++	: +++
: Br. melitensis 519 S	: Br. melitensis 334 S	: +++	: ++	: ++	: ++	: ++	: :	: :	: :
: Br. melitensis 316 R		: ++	: ++	: +++	: +++	: +++	: ++	: ++	: ++
: Positive abortus *	: Br. abortus 966 S	: +++	: +++	: +++	: +++	: +++	: ++	: ++	: ++
: P. avicida S		: +++	: ++	: ++	: ++	: ++	: ++	: ++	: ++
: P. avicida R		: +++	: ++	: ++	: +	: +	: :	: :	: :
: P. bovissepticus S		: +++	: ++	: ++	: ++	: +	: :	: :	: :
: P. equisepticus S		: *	: :	: :	: :	: :	: :	: :	: :
: P. tularensis S		: +	: +	: ++	: ++	: ++	: ++	: ++	: ++
: H. pertussis S		: +++	: +++	: ++	: ++	: ++	: ++	: ++	: ++
: H. influenzae R		: *	: :	: :	: :	: :	: :	: :	: :
: Pf.mallei R		: -	: +++	: +++	: ++	: ++	: ++	: ++	: ++

*P. equisepticus antigen was lost before the agglutinating titre of its homologous serum could be made.

* The homologous titre of H. influenzae R immune serum was not determined. The clumped antigen made agglutination tests impossible. The antigen was also anticomplementary, therefore complement fixation tests could not be made.



After determining the titre of each antiserum with its homologous antigen, the titres of all immune serums with all heterologous antigens were tested with the following results. Table V.

TABLE V.

CROSS AGGLUTINATION OF ALL R AND S ANTISERUMS WITH ALL
R AND S HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

Antigens	Immune Serums															
	Br. abortus 966 S	Br. abortus 5a S	Br. suis 436 S	Br. suis 401 S	Br. melitensis 384 S	Br. melitensis 319 S	Positive abortus *	Br. abortus 4a R	Br. suis 404 R	Br. melitensis 316 R	P. avicida S	P. avicida R	P. bovissepticus S	P. equisepticus S	P. tularensis S	H. pertussis S
Br. abortus 966 S	8	4	4	4	8	5	6	1	4	3	5	8	5	5	7	4
Br. suis 436 S	8	6	8	7	8	3	8	2	1	2	1	4	3	4	5	1
Br. melitensis 384 S	8	5	6	3	6	5	8	2	1	1	4	3	2	3	4	2
Br. abortus 4a R		4	5	5		4	5	6	6	4	3	6	6	6	3	1
Br. suis 404 R		5	3	5		4	5		4	6	1	2	4	2	3	2
Br. melitensis 316 R		4		3		5	5		5	7	3	3	6	3	5	3
Br. melitensis 384 R	2	2	2	2		2	4	4	4	3	1	1	4	1	2	1
P. avicida S	4	4	2	5	1	3	4	4	3	4	8	5	4	3	4	8
P. avicida R	2	4	1	3	4	1	8	5	5	5	6	7	5	5	8	8
P. bovissepticus S	4		3		5		6				3	4	3	1	3	3
P. tularensis S		5	6	6		5			4	5	2	4	5	2	5	1
P. tularensis R		4	4	4		5	6		4	3	6	3	5	4	3	3
H. pertussis S	6	4	4	4	5	6	7		4	4	6	4	8	5	5	7
H. pertussis R	2	4	3	4	4	4	3	4	4	2	3	4	6	3	4	6
Pf. mallei R	2	6	3	6	3	4	8		6	6	2	4	7	3	4	2
Pf. mallei S	3	1	2	2	3	2	7	5	4	2	3	4	3	4	3	1

The figures represent the number of the tube of greatest dilution showing an agglutination of a turbidity of 2 or greater.

The dilutions are designated as (1) 1-20, (2) 1-40, (3) 1-80, (4) 1-160, (5) 1-320, (6) 1-640, (7) 1-1280, (8) 1-2560.

* Positive serum from a naturally infected cow.

There is marked cross agglutination between the *Brucella*, *Pasteurella*, *Hemophilus* and *Pfeifferella* genera as shown in the preceding table. The *Brucella* immune serums agglutinated all antigens, usually giving a slightly higher titre for their specific antigens.

Pf. Mallei S antigen did not give a positive titre in a dilution greater than 1 to 80 with smooth immune serum. With rough immune serums a slightly higher titre was produced and positive abortus serum from naturally infected cow agglutinated Pf. mallei S in as high dilutions as it did its homologous antigens.

Some of the species antiserum of each of the other genera used agglutinated some species of all genera in dilutions as high as 1 to 1,280.

The agglutinating titres of Br. abortus and H. pertussis S antigens, with all serums were more nearly parallel, than either antigen showed with other members of its own group, although Br. abortus and H. pertussis S antisera did not agglutinate equally with all antigens. H. pertussis S serum agglutinated P. avicida S and R strains in dilutions equal to its homologous antigens. P. avicida S serum showed only slightly less agglutination for H. pertussis S but only one half this titre for H. pertussis R.

Pf. mallei R serum gave a low titre for P. avicida S and R, the titre being approximately equal for both strains. P. avicida S serum gave a low titre for Pf. mallei S and R

antigens. P. avicida R serum agglutinated both in a slightly higher dilution.

Tables VI and VII contain the agglutination data for the rough immune serums. The R serums gave a higher titre for the specific R antigens than for the homologous S antigens. However for the heterologous agglutination, sometimes the titre was higher for an R serum and an R antigen, sometimes for an R serum and S antigen, and in some cases the same titre for both S and R was shown. In general the Brucella R serums gave a rather higher titre for heterologous S antigens than homologous S antigens. Likewise the S Brucella antigens seemed to show more agglutination for the heterologous R serums than those of their own group.

TABLE VI.

CROSS AGGUTINATION OF R IMMUNE SERUMS WITH HOMOLOGOUS
AND HETEROLOGOUS R ANTIGENS.

Antigens	R serums					
	Br. abortus 4a R	Br. suis 404 R	Br. melitensis 316 R	P. avicida R	H. influenzae R	Pf. mallei R
Br. abortus 4a R	6	6	4	6	3	5
Br. suis 404 R		4	6	2	8	3
Br. melitensis 316 R		5	7	3	-	4
Br. melitensis 384 R	4	4	3	1	2	5
P. avicida R	5	5	5	7	7	2
H. pertussis R	4	4	2	4	6	4
Pf. mallei R		6	6	4	3	7

TABLE VII.

CROSS AGGUTINATION OF R IMMUNE SERUMS WITH HOMOLOGOUS
AND HETEROLOGOUS S ANTIGENS.

Antigens	R serums					
	Br. abortus 4a R	Br. suis 404 R	Br. melitensis 316R	P. avicida R	H. influenzae R	Pf. mallei R
Br. abortus 966 S	1	4	3	8	6	6
Br. suis 436 S	2	1	2	4	1	4
Br. melitensis 384 S	2	1	1	3	4	5
P. avicida S	4	3	4	5	3	2
P. bovissepticus S				4	4	4
P. tularensis S		4	5	4	6	7
H. pertussis S		4	4	4	5	7
Pf. mallei S	5	4	2	4	1	3

The picture for the interactions of the S antiserums with homologous and heterologous antigens was much the same as for the R antiserums. Tables VIII and IX show these data. Higher titres for specific antigens and antiserums were shown with a varying relationship of the heterologous serums and antigens. The H. pertussis S immune serum with Bq. abortus 966 S antigen gave a higher titre than with the Br. abortus 4a R antigen. H. pertussis S immune serum with P. tularensis S and R antigen gave a higher titre for the R antigen, practically none being shown for the S strain, P. avicida S and R cultures have a large and equal amount of agglutination for their specific antiserums and for H. pertussis S immune serum.

The data for positive abortus serum from a naturally infected cow were interesting. For some cultures demonstrating twice as high a titre for the S strain as for the R and for others twice as high a titre for the R as the S strain.

In the Pasteurella genera, aside from the specific agglutination of P. avicida S serum and P. tularensis S serum with their specific antigens, the agglutination titres of the S Pasteurella serums were higher for the R antigens.

TABLE VIII.

CROSS AGGLUTINATION OF S IMMUNE SERUMS WITH HOMOLOGOUS AND HETEROLOGOUS R ANTIGENS.

Antigens	S serums									
	Br. abortus 966 S	Br. abortus 5a S	Br. suis 436 S	Br. suis 401 S	Br. melitensis 384 S	Br. melitensis 319 S	Positive abortus	P. avicida S	P. bovissepticus S	P. equisepticus S
Br. abortus 4a R	4	4	5	5		4	5	3	6	6
Br. suis 404 R		5	3	5		4	5	1	4	2
Br. melitensis 316 R		4		3		5	5	3	6	3
Br. melitensis 384 R	2	2	2	2	-	2	4	1	4	1
P. avicida R	2	4	1	3	4	1	8	6	5	5
P. tularensis R		4	4	4		5	6	6	5	4
H. pertussis R	2	4	3	4	4	4	3	3	6	3
Pf. mallei R	2	6	3	6	3	4	8	2	7	3

TABLE IX.

CROSS AGGLUTINATION OF S IMMUNE SERUMS WITH HOMOLOGOUS AND HETEROLOGOUS S ANTIGENS

Antigens	S serums									
	Br. abortus 966 S	Br. abortus 5a S	Br. suis 436 S	Br. suis 401 S	Br. melitensis 384 S	Br. melitensis 319 S	Positive abortus	P. avicida S	P. bovissepticus S	P. equisepticus S
Br. abortus 966 S	8	4	4	4	8	5	6	5	5	5
Br. suis 436 S	8	6	8	8	8	3	8	1	3	4
Br. melitensis 384 S	8	5	6	3	6	5	8	4	2	3
P. avicida S	4	4	2	5	1	3	4	8	4	3
P. bovissepticus S	4		3		5		6	3	3	1
P. tularensis S		5	6	6		5		2	5	2
H. pertussis S	6	4	4	4	5	6	7	6	8	5
Pf. mallei S	3	1	2	2	3	2	7	3	3	4

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All the antigens of each organism studied have been made with cultures with the same colonial appearance, either typical S or R colonies. But these antigens made up on different dates, although apparently in the same stage of dissociation, will give varying titres with the same serum. Not only is the agglutinating antigen a cause of varied results, but the state of dissociation of the cultures used for immunization will, of course, control the amount of S or R agglutinin in the antiserum produced. Some of the cultures used in these studies have been held in stock for several years as evidenced by the culture histories. Thus they are likely of different serological composition than recently isolated organisms. McKenzie, Fitzgerald and Irons (1933) in studies of S and R variants of S schotmulleri, S morgani and Shigella paradysenteriae (Flexner), state that their "results appear to demonstrate that serological, morphological and physiological characters are capable of independent variation during the dissociative processes." A dissociation apparent only serologically may have taken place.

While there can be no doubt of a marked serological relation between these groups of organisms the state of dissociation influences this interagglutinability. This may explain the inability of some to find cross agglutination of these groups or organisms. For this reason agglutinin absorption studies have been made to determine whether the S agglutinogens of one organism are the same as the R agglutinogens of another or whether some apparently S organisms are more R than S or so called R strains are more S than R.

8. Agglutinin Absorption Studies.

Agglutinin absorption studies of each immune serum were made of all antigens showing a positive cross agglutination in dilutions of 1 to 40 or over. For agglutinin absorption all serums were diluted 1 to 50 with 0.1 per cent salt solution containing 0.1 per cent phenol. To avoid dilution the growth was washed from 1 slant culture with 3.5 cc. of diluted serum. This was absorbed at 37°C for 1.5 hours. This supernatant solution was decanted to another slant, absorbed the same length of time at 37°C., again centrifuged, and finally titrated against the absorbing antigen. This was sufficient absorption for some of the serums. Those which were not completely absorbed were incubated with another dose of antigen, for 1.5 hours and held over night in the ice box to completely remove the specific agglutinins.

For still other serums it was necessary to dilute the immune serum with physiological saline. This made the agglutinin absorption less specific. Otherwise it was impossible to completely absorb the agglutinins with one antigen, for another antigen of the same organism, but grown at a different time. That is, unless the absorbing antigen and the antigen used for testing complete absorption for specific agglutinins were not made up at the same time the result was the same as though two different organisms had been used.

For the absorption in physiological saline a dilution of 0.1 cc. of serum in 0.6 cc. of 0.86 per cent saline was used. After absorption sufficient distilled water was added (diluted 8 times) to make an approximately 1 to 50 dilution

which at the same time produced a 0.1 per cent salt solution for titrations, the same serum and salt dilution which had been used in the other titrations. The absorptions with the R Brucella antigens were made in this way.

After the immune serums had been absorbed the agglutination titre of each was tested again with each antigen which had previously shown a positive cross agglutination in dilutions of 1 to 40 or over.

The following tables show the results of agglutinin absorption and the cross agglutination titres after absorption compared with cross agglutination results before absorption.

The first column for each serum in these tables are the original titres of the unabsorbed serums with the homologous and heterologous R and S antigens. These titres are represented by the number of the tube of greatest dilution showing an agglutination of a turbidity of 2 or greater. The dilutions are designated as (1) 1-20, (2) 1-40, (3) 1-80, (4) 1-160, (5) 1-320, (6) 1-640, (7) 1-1280, (8) 1-2560, (9) 1-5120.

The second column for each serum presents the titres of the absorbed serums with the homologous and heterologous S and R antigens. The dilutions are designated as (1) 1-50, (2) 1-100, (3) 1-200, (4) 1-400, (5) 1-800, (6) 1-1600, (7) 1-3200.

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. ABORTUS
966 S ANTIGEN, WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S Immune Serums		966 S	Br. abortus	Br. abortus 5a S	Br. abortus 436 S	Br. suis 436 S	Br. suis 401 S	Br. melitensis 384 S	Br. melitensis 319 S	P. avicida S	R. bovis septi-	R. equi septi-	P. tularensis	H. pertussis
S. and R. antigens	Br. abortus 966 S	9	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. abortus 4a R	8	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. suis 436 S	8	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. suis 404 R	8	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. melitensis 384 S	8	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. melitensis 384 R	2	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. melitensis 384 S	2	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Br. melitensis 316 R	4	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	P. avicida S	2	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	P. avicida R	6	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	P. tularensis S	6	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	P. tularensis R	6	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	H. pertussis S	2	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	H. pertussis R	2	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Pf. mallei S	3	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed
	Pf. mallei R	2	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed	absorbed

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. ABORTUS
966 S ANTIGEN, WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

R Immune Serums									
	unabsorbed Br. abortus 4a R	absorbed unabsorbed Br. suis 404 R	Br. melitensis 316 R	P. avicida R	H. influenzae R	unabsorbed absorbed Pf. mallei R			
Br. abortus 966 S	1	4	3	8	6	6	6	5	
Br. abortus 4a R	6	6	4	6	4	2	6	5	
Br. suis 436 S		1	2	4	1	-	4	2	
Br. suis 404 R		4	5	2	4	-	3	2	
Br. melitensis 384 S	3	1	1	3	4	-	5	2	
Br. melitensis 384 R	4	4	3	1	2	-	5	2	
Br. melitensis 384 S		1	1	3	4	-	5	2	
Br. melitensis 316 R		5	6	3	-	-	4	2	
P. avicida S	5	3	3	5	3	-	2	2	
P. avicida R	5	6	5	7	7	-	7	4	
P. tularensis S		4	5	4	6	-	4	4	
P. tularensis R		4	3	3	4	-	7	4	
H. pertussis S		4	4	4	5	-	4	4	
H. pertussis R		4	2	4	6	-	3	1	
Pf. mallei S	4	4	5	4	1	2	3	7	6
Pf. mallei R		4	3	4	3	3	7	6	

CROSS AGGUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. SUI S
436 S ANTIGEN, WITH HOMOLOGOUS S AND R ANTIGENS.

	R Immune Serums									
	unabsorbed Br. abortus 4a R	unabsorbed Br. suis 404 R	absorbed Br. melitensis 316 R	unabsorbed P. avicida R	absorbed H. influenzae R	unabsorbed Pf. mallei R	absorbed	unabsorbed	absorbed	unabsorbed
S. and R. Antigens										
Br. abortus 966 S	1	4	3	8	6	9	absorbed	unabsorbed	absorbed	unabsorbed
Br. abortus 4a R	6	9	4	9	4	9	absorbed	unabsorbed	absorbed	unabsorbed
Br. suis 436 S		1	2	4	1	4				
Br. suis 404 R		4	5	2	4	3				
Br. melitensis 384 S	3	1	1	3	4	5				
Br. melitensis 384 R	4	4	3	1	2	5				
Br. melitensis 384 S		1	1	3	4	4				
Br. melitensis 316 R		5	6	3	-	4				
P. avicida S	5	3	3	5	3	2				
P. avicida R	5	6	5	7	7	2				
P. tularensis S		4	5	4	6	7				
P. tularensis R		4	3	3	4	4				
H. pertussis S		4	4	4	5	7				
H. pertussis R	4	4	2	4	6	4				
Pf. mallei S		4	5	4	1	2				
Pf. mallei R		4	3	4	3	7				

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. MELITENSIS
384 S ANTIGEN, WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S. and R. Antigens	S Immune Serums														
	Br. abortus 966 S	Br. abortus 4a R	Br. suis 436 S	Br. suis 404 R	Br. melitensis 384 S	Br. melitensis 384 R	Br. melitensis 384 S	Br. melitensis 316 R	P. avicida S	P. avicida R	P. tularensis S	P. tularensis R	H. pertussis S	H. pertussis R	Pf. mallei S
Br. abortus 966 S	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. abortus 4a R	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. suis 436 S	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. suis 404 R	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. melitensis 384 S	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. melitensis 384 R	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. melitensis 384 S	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Br. melitensis 316 R	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
P. avicida S	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
P. avicida R	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
P. tularensis S	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
P. tularensis R	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
H. pertussis S	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
H. pertussis R	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Pf. mallei S	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Pf. mallei R	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. MELITENSIS
384 S ANTIGEN, WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	R Immune Serums										Y
	Br. abortus 4a R	Br. suis 404 R	Br. melitensis 316 R	P. aviciida R	H. influenzae R	Pf. mallei R	unabsorbed	absorbed	unabsorbed	absorbed	
S. and R. Antigens											
Br. abortus 966 S	1	4	3	8	6	6	unabsorbed	absorbed	unabsorbed	absorbed	2
Br. abortus 4a R	6	9	4	9	4	6	unabsorbed	absorbed	unabsorbed	absorbed	2
Br. suis 436 S		1	2	4	1	4	unabsorbed	absorbed	unabsorbed	absorbed	5
Br. suis 404 R		4	5	2	4	3	unabsorbed	absorbed	unabsorbed	absorbed	5
Br. melitensis 384 S	3	1	1	3	4	5	unabsorbed	absorbed	unabsorbed	absorbed	3
Br. melitensis 384 R	4	4	3	1	2	4	unabsorbed	absorbed	unabsorbed	absorbed	3
Br. melitensis 384 S		1	1	3	4	5	unabsorbed	absorbed	unabsorbed	absorbed	3
Br. melitensis 316 R		5	6	3	3	4	unabsorbed	absorbed	unabsorbed	absorbed	3
P. aviciida S	5	3	3	5	3	2	unabsorbed	absorbed	unabsorbed	absorbed	2
P. aviciida R	5	6	5	7	1	3	unabsorbed	absorbed	unabsorbed	absorbed	2
P. tularensis S		4	5	4	6	7	unabsorbed	absorbed	unabsorbed	absorbed	7
P. tularensis R		4	3	3	4	4	unabsorbed	absorbed	unabsorbed	absorbed	4
H. pertussis S		4	4	4	5	7	unabsorbed	absorbed	unabsorbed	absorbed	7
H. pertussis R		4	2	4	6	4	unabsorbed	absorbed	unabsorbed	absorbed	4
Pf. mallei S	4	4	5	4	1	3	unabsorbed	absorbed	unabsorbed	absorbed	3
Pf. mallei R		4	3	4	3	7	unabsorbed	absorbed	unabsorbed	absorbed	2

1. The first part of the report is a general introduction to the subject of the study. It discusses the importance of the study and the objectives of the research. It also provides a brief overview of the methodology used in the study.

2. The second part of the report is a detailed description of the study area. It includes information about the location of the study area, the population of the study area, and the characteristics of the study area. It also discusses the data sources used in the study.

3. The third part of the report is a detailed description of the study results. It includes information about the findings of the study, the conclusions drawn from the findings, and the implications of the findings. It also discusses the limitations of the study and the need for further research.

4. The fourth part of the report is a conclusion and recommendations section. It summarizes the findings of the study and provides recommendations for future research and policy. It also discusses the importance of the study and the need for further research.

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. AVICIDA S ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S. and R. Antigens	S Immune Serums													
	unabsorbed Br. abortus 966 S	absorbed Br. abortus 5a S	unabsorbed Pos. abortus	absorbed Br. suis 436 S	unabsorbed Br. suis 401 S	unabsorbed Br. melitensis 384 S	absorbed Br. melitensis 319 S	unabsorbed P. avicida S	absorbed P. bovisepticus S	unabsorbed P. Equi-septicus	absorbed P. tularensis S	unabsorbed H. pertussis S	absorbed Pf. mallei S	
Br. abortus 966 S	8	4	6	1	4	8	5	3	5	6	1	4	1	
Br. abortus 4a R	8	6	3	6	7	8	4	1	3	4	3	1	1	
Br. suis 436 S	8	5	5	3	5	6	5	4	4	3	7	6	2	
Br. suis 404 R	8	5	8	6	3	6	5	1	2	4	6	3	3	
Br. melitensis 384 S	2	2	2	1	2	2	2	4	1	2	1	2	2	
Br. melitensis 384 R	8	5	4	3	3	6	5	1	3	3	4	3	1	
Br. melitensis 384 S	8	4	8	6	4	6	5	3	5	6	5	4	4	
Br. melitensis 316 R	4	6	5	2	5	1	3	8	4	5	4	8	3	
P. avicida S	2	4	8	1	3	8	1	6	2	5	8	7	6	
P. avicida R	6	5	3	6	4	5	5	2	2	3	3	5	3	
P. tularensis S	6	4	6	4	4	5	5	6	5	5	4	3	2	
P. tularensis R	6	4	5	4	4	5	6	7	7	6	5	6	2	
H. pertussis S	3	1	7	2	1	3	2	5	2	3	3	3	3	
H. pertussis R	2	4	5	4	4	4	4	2	6	5	5	6	2	
Pf. mallei S	3	4	8	1	2	3	2	5	5	3	3	3	3	
Pf. mallei R	2	4	4	3	4	3	3	2	7	4	4	2	2	

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	R Immune Serum					
	Br. abortus 4a R	Br. suis 404 R	Br. mellitensis 316 R	P. avicola R	H. influenzae R	Pf. mallei R
Br. abortus 966 S	1	4	3	3	1	6
Br. abortus 4a R	6	6	4	6	1	6
Br. suis 436 S		1	2	4	1	4
Br. suis 404 R		4	5	2	4	4
Br. mellitensis 384 S	3	1	1	3	1	5
Br. mellitensis 384 R	4	4	3	1	2	5
Br. mellitensis 384 S		1	1	3	1	5
Br. mellitensis 316 R		5	6	3	1	4
P. avicola S	5	3	3	5	3	2
P. avicola R	5	6	5	7	7	2
P. tularensis S		4	5	4	6	7
P. tularensis R		4	3	3	4	4
H. pertussis S		44	4	4	5	7
H. pertussis R		4	2	4	6	4
Pf. mallei S	4	4	5	4	1	3
Pf. mallei R		4	3	3	1	7

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. TULARENSIS S ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R Antigens													
S and R Antigens	Immune Serums												
	Br. abortus 966 S	Br. abortus 5a S	Pos. abortus	unabsorbed Br. suis 436 S	absorbed Br. suis 436 S	unabsorbed Br. suis 401 S	Br. meliten- sis 284 S	unabsorbed Br. meliten- sis 219 S	absorbed P. avicida S	unabsorbed P. bovis- septicus	absorbed P. equi- septicus	unabsorbed P. tulare- nsis S	absorbed H. pert- ussis S
Br. abortus 966 S	8	4:5	6	4	4	4	8	5	5	5	5	7	4
Br. abortus 4a R		4	8	4	-	5	4	-	3	6	3	3	1
Br. suis 436 S	8	6:3	8	8	6	7	8	3	1	3	4	6	1
Br. suis 404 R		5	5	5	-	5	1	4	1	4	2	3	2
Br. melitensis 384 S	8	5:4	8	6	-	3	6	5	4	2	3	4	2
Br. melitensis 384 R	2	2	4	1	2	2	2	2	1	4	1	3	1
Br. melitensis 384 S		5:4	8	1	-	3	6	4	4	2	3	4	2
Br. melitensis 316 R		4	5	6	-	4	1	5	3	6	3	5	3
P. avicida S	4	6	5	2	-	5	1	3	8	4	1	4	3
P. avicida R	2	4	8	1	1	3	4	1	6	5	1	3	3
P. tularensis S	6	5	3	6	-	6		5	2	5	2	4	1
P. tularensis R		4	6	4	-	4		5	6	5	3	3	3
H. pertussis S	6	4	5	4	-	4	5	6	7	7	5	5	7
H. pertussis R	2	4	5		-	4		4	2	6	3	5	6
Pf. mallei S	3	1	7	2	-	2	3	2	3	3	3	3	3
Pf. mallei R	2	4	8	3	-	4	3	2	2	7	1	4	2

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CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. TULARENSIS S ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R Antigens	R Immune Serums									
	Br. abortus 4a R	Br. suis 404 R	Br. melitensis 316 R	P. avicida R	H. influenzae R	Pf. mallei R	absorbed	unabsorbed	absorbed	unabsorbed
Br. abortus 966 S	1:1	4:4	3:3	8:8	6:6	6:6	absorbed	unabsorbed	absorbed	unabsorbed
Br. abortus 4a R	6:6	6:6	3:3	6:6	4:4	6:6	absorbed	unabsorbed	absorbed	unabsorbed
Br. suis 436 S	.	1:1	2:2	4:4	1:1	4:4	absorbed	unabsorbed	absorbed	unabsorbed
Br. suis 404 R	.	4:4	5:5	2:2	4:4	3:3	absorbed	unabsorbed	absorbed	unabsorbed
Br. melitensis 384 S	3:3	1:1	1:1	3:3	4:4	5:5	absorbed	unabsorbed	absorbed	unabsorbed
Br. melitensis 384 R	4:4	4:4	3:3	1:1	2:2	5:5	absorbed	unabsorbed	absorbed	unabsorbed
Br. melitensis 384 S	.	1:1	1:1	3:3	4:4	5:5	absorbed	unabsorbed	absorbed	unabsorbed
Br. melitensis 316 R	.	5:5	6:6	3:3	7:7	2:2	absorbed	unabsorbed	absorbed	unabsorbed
P. avicida S	5:5	6:6	3:3	5:5	7:7	2:2	absorbed	unabsorbed	absorbed	unabsorbed
P. avicida R	5:5	6:6	3:3	5:5	7:7	2:2	absorbed	unabsorbed	absorbed	unabsorbed
P. tularensis S	.	4:4	5:5	4:4	6:6	7:7	absorbed	unabsorbed	absorbed	unabsorbed
P. tularensis R	.	4:4	3:3	4:4	5:5	7:7	absorbed	unabsorbed	absorbed	unabsorbed
H. pertussis S	.	4:4	6:6	4:4	5:5	7:7	absorbed	unabsorbed	absorbed	unabsorbed
H. pertussis R	.	4:4	6:6	4:4	5:5	7:7	absorbed	unabsorbed	absorbed	unabsorbed
Pf. mallei S	4:4	4:4	5:5	4:4	1:1	3:3	absorbed	unabsorbed	absorbed	unabsorbed
Pf. mallei R	.	4:4	3:3	4:4	2:2	7:7	absorbed	unabsorbed	absorbed	unabsorbed

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY H. PERTUSSIS S ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	S Immune Serums													
	unabsorbed Br.abortus 966 S	absorbed unabsorbed Br.abortus 5a S	unabsorbed Pos. abortus	unabsorbed Br.suis 436 S	absorbed unabsorbed Br.suis 401 S	Br. melitensis 384 S	absorbed unabsorbed Br. melitensis 319 S	Br. melitensis 319 S	absorbed unabsorbed P. avicida S	Br. melitensis 319 S	absorbed unabsorbed P. bovis septicus	unabsorbed P. equisepti- cus S	absorbed unabsorbed P. tularen- sis S	absorbed unabsorbed H. pertussis S
Br. abortus 966 S	8	4	6	4	5	8	5	5	5	5	5	5	7	4
Br. abortus 4a R	8	4	8	4	5	8	5	5	5	5	5	5	7	4
Br. suis 436 S	8	6	8	8	5	8	5	5	5	5	5	5	7	4
Br. suis 404 R	8	5	8	5	5	8	5	5	5	5	5	5	7	4
Br. melitensis 384 S	8	5	8	6	5	8	5	5	5	5	5	5	7	4
Br. melitensis 384 R	2	2	8	2	5	6	5	5	5	5	5	5	7	4
Br. melitensis 384 S	2	5	8	6	5	8	5	5	5	5	5	5	7	4
Br. melitensis 316 R	4	4	5	2	5	8	5	5	5	5	5	5	7	4
P. avicida S	4	6	5	2	5	8	5	5	5	5	5	5	7	4
P. avicida R	2	4	8	1	5	8	5	5	5	5	5	5	7	4
P. tularensis S	6	5	6	6	5	8	5	5	5	5	5	5	7	4
P. tularensis R	6	4	6	4	5	8	5	5	5	5	5	5	7	4
H. pertussis S	6	4	5	4	5	8	5	5	5	5	5	5	7	4
H. pertussis R	2	4	5	4	5	8	5	5	5	5	5	5	7	4
Pf. mallei S	3	1	7	2	4	8	5	5	5	5	5	5	7	4
Pf. mallei R	2	4	8	3	5	8	5	5	5	5	5	5	7	4

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY H. PERTUSSIS S ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	R Immune Serums									
	unabsorbed Br. abortus 4a R	unabsorbed Br. suis 404 R	absorbed Br.	unabsorbed Br. melitensis 316 R	unabsorbed P. avicida R	unabsorbed H. influenzae R	unabsorbed Pt. mallei R	absorbed Pt. mallei R	absorbed H. influenzae R	absorbed Pt. mallei R
Br. abortus 966 S	1	4	3	8	8	6	6	1	6	1
Br. abortus 4a R	6	6	4	6	6	4	4	6	4	1
Br. suis 436 S		1	2	4	4	1	1	4	1	
Br. suis 404 R		4	5	2	2	4	4	3	4	
Br. melitensis 384 S	3	1	1	3	3	4	5	1	4	1
Br. melitensis 384 R	4	4	3	1	1	2	5	2	2	2
Br. melitensis 384 S		1	1	3	3	4	5	1	4	1
Br. melitensis 316 R		5	6	3	3	6	4	5	4	
P. avicida S	5	3	3	5	5	3	2	2	3	
P. avicida R	5	6	5	7	7	7	2	2	7	
P. tularensis S		4	5	4	4	6	4	7	4	
P. tularensis R		4	3	3	3	4	4	4	4	
H. pertussis S		4	4	4	4	5	7	7	5	
H. Pertussis R		4	2	4	4	5	4	4	6	
Pf. mallei S	4	4	5	4	4	1	3	3	1	1
Pf. mallei R		4	3	4	4	3	7	7	3	1

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. ABORTUS 4a R ANTIGEN WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S Immune Serums		unabsorbed Br. abortus 966 S	unabsorbed Br. abortus 5a S	absorbed	Pos. absorbed	unabsorbed Br. suis 436 S	absorbed	unabsorbed Br. suis 401 S	absorbed	Br. melitensis 384 S	absorbed	Br. melitensis 319 S	unabsorbed P. avicida S	absorbed	unabsorbed R. bovis septi	absorbed	unabsorbed R. equi septi	absorbed	unabsorbed P. tularensis	absorbed	unabsorbed H. pertussis S
Br. abortus 966 S	8	4	4	1	6	4	3	4	5	3	4	5	5	1	5	1	5	2	7	1	4
Br. abortus 4a R	8	4	4	1	8	4	3	5	7	3	4	5	3	1	4	1	3	2	3	1	1
Br. suis 436 S	8	5	5	1	5	5	6	1	1	1	3	2	1	1	1	2	4	4	6	2	1
Br. suis 404 R	8	5	5	1	8	6	1	5	3	6	4	2	1	1	4	3	2	3	3	1	2
Br. melitensis 384 S	8	2	2	1	4	1	1	2	3	6	5	2	4	1	2	3	1	3	4	3	2
Br. melitensis 384 R	2	5	5	1	8	6	1	2	3	6	5	2	4	1	2	3	2	4	3	1	1
Br. melitensis 316 R	4	6	4	1	5	2	2	4	3	5	5	2	3	3	6	1	1	5	4	5	3
P. avicida S	2	6	4	1	5	2	1	3	5	1	3	1	8	4	4	1	1	2	4	8	3
P. avicida R	6	5	4	1	3	6	2	4	6	5	5	5	2	2	5	5	2	3	4	3	1
P. tularensis S	6	4	4	1	6	4	3	4	4	5	6	5	6	2	5	5	1	3	5	3	7
P. tularensis R	6	4	4	1	5	3	3	4	4	5	6	6	7	4	7	6	1	5	5	6	6
H. pertussis S	3	1	1	1	7	2	2	2	2	3	2	2	3	2	3	5	1	3	3	2	3
H. pertussis R	2	4	4	1	5	3	3	4	4	5	6	6	2	4	7	6	1	5	5	6	6
Pf. mallei S	3	1	1	1	7	2	2	2	2	3	2	2	3	2	3	5	1	3	3	2	3
Pf. mallei R	2	4	4	1	8	1	1	4	4	3	3	3	2	1	7	6	1	3	4	4	2

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. ABORTUS 4a R ANTIGEN WITH HOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	R Immune Serum									
	Br. abortus 4a R	unabsorbed Br. suis 404 R	absorbed	Br. suis 316 R	P. avida R	H. influenzae R	Pf. mallei R			
Br. abortus 966 S	9	4	1	unabsorbed	absorbed	absorbed	absorbed	6	4	1
Br. abortus 4a R	6	6	1	unabsorbed	absorbed	absorbed	absorbed	4	4	1
Br. suis 436 S	1	4	1	unabsorbed	absorbed	absorbed	absorbed	1	4	1
Br. suis 404 R	4	4	1	unabsorbed	absorbed	absorbed	absorbed	7	4	1
Br. melitensis 384 S	3	1	1	unabsorbed	absorbed	absorbed	absorbed	4	5	2
Br. melitensis 384 R	4	4	1	unabsorbed	absorbed	absorbed	absorbed	2	5	2
Br. melitensis 384 S	1	1	1	unabsorbed	absorbed	absorbed	absorbed	4	5	1
Br. melitensis R, 316	5	5	1	unabsorbed	absorbed	absorbed	absorbed	3	1	4
P. avida S	5	3	1	unabsorbed	absorbed	absorbed	absorbed	3	2	3
P. avida R	5	6	1	unabsorbed	absorbed	absorbed	absorbed	7	2	2
P. tularensis S		4	1	unabsorbed	absorbed	absorbed	absorbed	6	4	4
P. tularensis R		4	1	unabsorbed	absorbed	absorbed	absorbed	4	4	1
H. pertussis S		4	1	unabsorbed	absorbed	absorbed	absorbed	5	7	5
H. pertussis R		4	1	unabsorbed	absorbed	absorbed	absorbed	6	4	5
Pf. mallei S	4	4	1	unabsorbed	absorbed	absorbed	absorbed	1	3	1
Pf. mallei R		4	1	unabsorbed	absorbed	absorbed	absorbed	3	7	1

2. The second part of the document focuses on the implementation of the proposed changes. It details the steps involved in the process, from initial planning to final execution. This section highlights the challenges faced during the implementation phase and provides strategies to overcome them. It also discusses the role of different departments in ensuring a smooth transition.

3. The third part of the document addresses the financial aspects of the project. It provides a detailed breakdown of the costs involved, including personnel, materials, and overheads. This section also includes a comparison of the expected costs with the actual expenses, allowing for a clear assessment of the project's financial performance. The importance of budgeting and cost control is stressed throughout this section.

4. The fourth part of the document discusses the impact of the project on the organization. It evaluates the benefits realized, such as improved efficiency, reduced costs, and enhanced customer satisfaction. This section also identifies areas for further improvement and suggests ways to sustain the gains achieved. The overall conclusion is that the project has been successful in achieving its objectives and that the organization is well-positioned for future growth.

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. SUI S 404 R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens		S Immune Serums											
		unabsorbed Br. abortus 966 S	unabsorbed Br. abortus 5a S	unabsorbed Pos. abortus	unabsorbed Br. suis 436 S	unabsorbed Br. suis 401 S	Br. melitensis 384 S	unabsorbed Br. melitensis 319 S	P. avicida S	P. bovisep-ticus S	unabsorbed P. tularensis S	absorbed H. pertussis S	
Br. abortus 966 S	8	4	3	6	4	4	5	5	5	6	7	4	
Br. abortus 4a R		4	1	8	4	5	4	4	3	6	3	1	
Br. suis 436 S	8	6	2	8	8	7	3	3	1	3	6	1	
Br. suis 404 R		5		5	5	5	4	4	1	4	3	2	
Br. melitensis 384 S	8	5		8	6	3	5	5	4	2	4	2	
Br. melitensis 384 R	2	2		4	2	2	2	2	1	3	3	1	
Br. melitensis 384 S	8	5	1	8	6	3	5	5	4	2	4	2	
Br. melitensis 316 R		4	1	5	2	4	6	5	3	6	5	3	
P. avicida S	4	6	-	5	2	5	1	3	6	4	4	8	
P. avicida R	2	4	-	8	1	3	4	1	6	5	8	8	
P. tularensis S	6	5	-	3	6	6		5	2	2	4	1	
P. tularensis R		4	4	6	4	4		5	6	3	3	3	
H. pertussis S	6	4	1	5	4	4	5	6	8	7	5	7	
H. pertussis R	2	4	-	5	4	4		4	2	3	5	6	
Pf. mallei S	3	1	-	7	2	2	3	2	3	3	3	3	
Pf. mallei R	2	4	-	8	3	4	3	3	2	7	4	2	

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. SUIIS 404 R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	R Immune Serums							
	unabsorbed Br. abortus 4a R	absorbed 4a R	unabsorbed Br. suis 404 R	absorbed 404 R	Br. melitensis 316 R	P. avicida R	H. influenzae R	absorbed Pf. mallei R
S and R antigens								
Br. abortus 966 S	unabsorbed	absorbed	4	1	5	8	6	6
Br. abortus 4a R	3	3	9	4	4	4	4	9
Br. suis 436 S			1	1	1	1	1	4
Br. suis 404 R			4	4	2	2	7	5
Br. melitensis 384 S	3	3	1	1	3	3	4	5
Br. melitensis 384 R	4	4	4	4	1	1	2	5
Br. melitensis 384 S	8	8	1	1	3	3	4	5
Br. melitensis 316 R	5	5	5	5	6	6	4	4
P. avicida S	5	5	3	3	4	5	3	2
P. avicida R	5	5	9	1	4	7	7	2
P. tularensis S	3	3	4	4	4	4	6	7
P. tularensis R	6	6	4	4	3	3	4	4
H. pertussis S	5	5	4	4	4	4	5	7
H. pertussis R	5	5	4	4	4	4	6	4
Pf. mallei S	4	4	4	4	5	4	1	3
Pf. mallei R	8	8	4	4	4	4	3	7

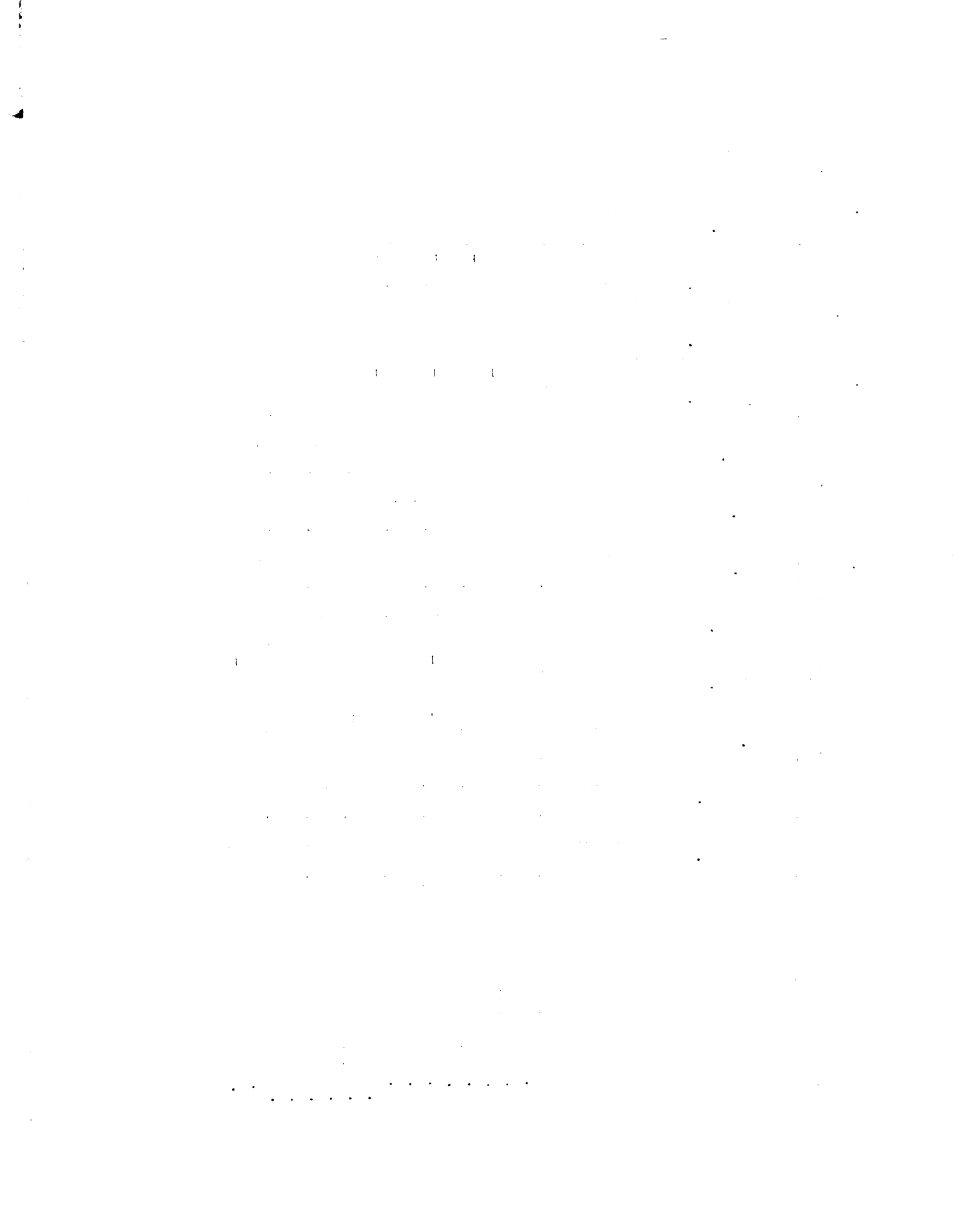
1. The first part of the report is a general introduction to the project, which includes a brief history of the project and a description of the objectives. This section is followed by a detailed description of the methodology used in the study, including the data sources and the statistical methods employed. The results of the study are then presented in a series of tables and figures, which are accompanied by a detailed discussion of the findings. Finally, the report concludes with a summary of the main findings and a list of recommendations for future research.

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CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. 384 R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	S Immune Serums											
	unabsorbed Br. abortus 966 S	absorbed Dr. abortus 5a S	unabsorbed Pos. abortus	unabsorbed Br. suis 436 S	unabsorbed Br. suis 401 S	unabsorbed Br. melitensis 384 S	unabsorbed Br. melitensis 219 S	unabsorbed P. avicida S	unabsorbed P. bovi- septicus S	unabsorbed P. equi- septicus S	unabsorbed P. tularen- sis S	unabsorbed H. pertussis
Br. abortus 966 S	8	4	6	2	4	8	5	5	5	5	7	4
Br. abortus 4a R	8	4	8	4	5	8	4	3	6	3	3	1
Br. suis 436 S			8	5	7			1	3	4	6	1
Br. suis 404 R			5	8	5			1	4	2	3	2
Br. melitensis 384 S	8	5	8	6	3	6	5	4	2	3	4	3
Br. melitensis 384 R	2	2	4	2	2		2	1	4	1	3	1
Br. melitensis 384 S			8	6	3	6	5	4	2	3	4	2
Br. melitensis 316 R			5		4		5	3	6	3	5	3
P. avicida S	4	6	5		5	1	3	8	4	3	4	8
P. avicida R	2	4	8	1	3	4	1	6	5	5	8	8
P. tularensis S	6	5	3	6	6		5	2	5	2	4	1
P. tularensis R			6	4	4		5	6	5	3	3	3
H. pertussis S	6	4	5	4	4	5	6	7	7	5	5	7
H. pertussis R	2	4	5		4		4	2	6	3	3	6
Pf. mallei S	3	1	7	2	2	5	2	5	5	3	3	3
Pf. mallei R	2	4	8	3	4	3	2	2	7	3	4	2

S and R antigens



CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. 384 R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	R Immune Serums									
	unabsorbed Br. abortus 4a R	absorbed Br. suis 404 R	unabsorbed Br. melitensis 316 R	absorbed P. avicida R	unabsorbed H. influenzae R	absorbed Pt. mallei R	unabsorbed Br. abortus 4a R	absorbed Br. suis 404 R	unabsorbed Br. melitensis 316 R	absorbed P. avicida R
Br. abortus 966 S	1	4	3	8	6	1	1	4	3	8
Br. abortus 4a R	6	6	4	6	4	6	6	6	4	6
Br. suis 436 S		1	2	4	1	4		1	1	4
Br. suis 404 R		4	5	2	4	4		4	4	4
Br. melitensis 384 S	3	1	1	3	4	5	3	1	1	3
Br. melitensis 384 R	4	4	3	1	2	5	4	3	2	1
Br. melitensis 384 S		1	1	3	4	5		1	1	3
Br. melitensis 316 R		5	6	3	7	4		5	4	3
P. avicida S	5	3	3	5	3	3	5	3	3	5
P. avicida R	5	6	5	7	7	3	5	6	5	7
P. tularensis S		4	5	4	6	7		4	4	7
P. tularensis R		4	3	3	4	4		4	3	4
H. pertussis S		4	4	4	5	7		4	4	7
H. pertussis R		4	2	4	6	4		4	3	4
Pt. mallei S	4	4	5	4	1	3	4	4	1	3
Pt. mallei R		4	3	4	3	7		4	7	3

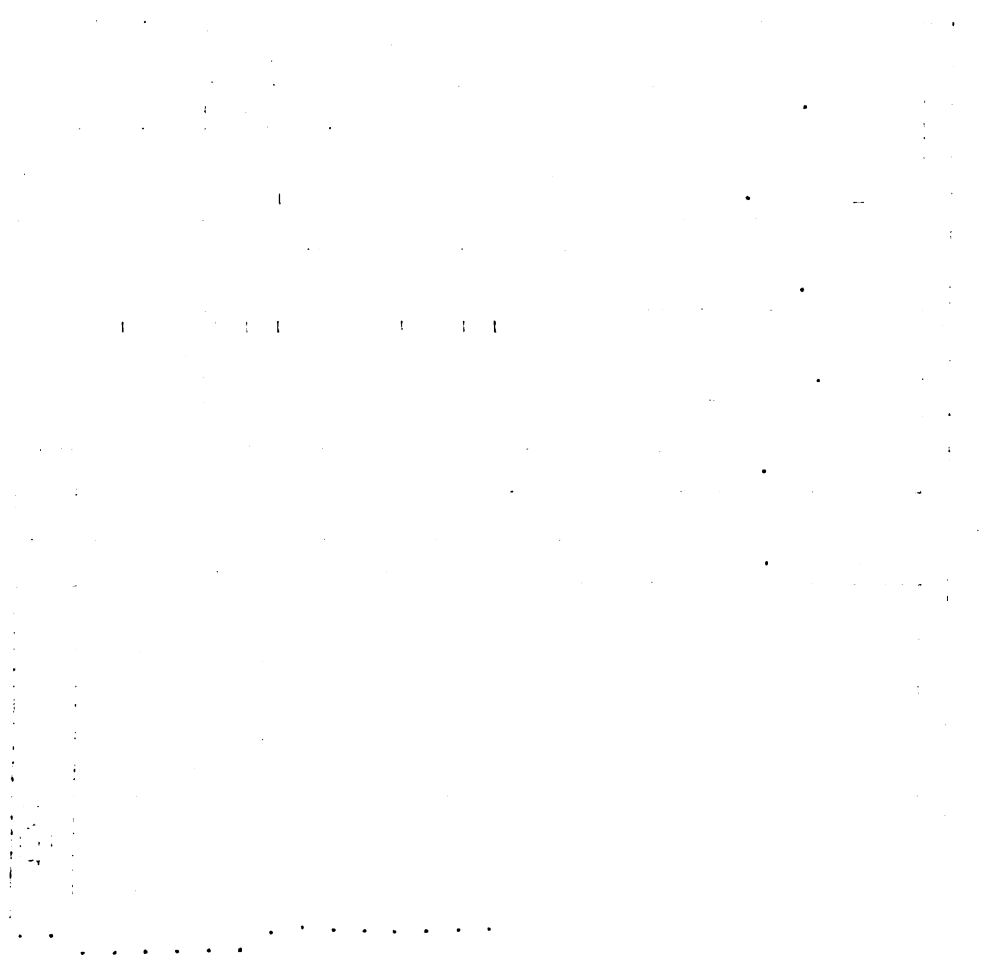
S and R antigens

CROSS AGGUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. MELITENSIS 316 R ANTIGEN, WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

		S Immune Serums																								
		unabsorbed Br. abortus 966 S	absorbed	unabsorbed Br. abortus 5a S	absorbed	Pos. abortus	unabsorbed Br. suis 426 S	absorbed	unabsorbed Br. suis 401 S	absorbed	Br. melitensis 384 S	unabsorbed	Br. melitensis 319 S	absorbed	P. avicida S	unabsorbed	P. avicida S	absorbed	unabsorbed P. bovisapticus S	absorbed	unabsorbed P. equisetus S	absorbed	unabsorbed P. tularensis S	absorbed	H. pertussis S	absorbed
Br. abortus 966 S		8	4	4	2	6	4	4	4	1	8	5	4	4	5	3	3	5	5	5	7	3	4	4	4	4
Br. abortus 4a R		8	6	4	1	8	3	4	7	6	8	3	4	4	5	3	3	6	6	6	3	3	6	3	1	1
Br. suis 426 S			5	5	1	5	3	3	5	1	1	4	6	6	3	1	1	3	3	4	2	3	3	3	2	2
Br. suis 404 R			5	5	1	8	3	3	3	1	6	5	4	4	4	1	4	6	2	4	3	1	3	3	1	1
Br. melitensis 384 S		8	5	2	1	3	6	2	2	1	6	2	5	5	5	4	6	2	2	2	3	3	4	5	2	2
Br. melitensis 384 R		2	5	5	1	4	1	3	3	1	6	5	4	4	4	1	6	2	4	3	1	3	3	4	5	2
Br. melitensis 384 S		8	5	5	1	8	6	3	3	1	6	5	5	5	5	4	6	2	2	2	3	3	4	5	2	2
Br. melitensis 316 R			4	4	1	5	2	6	4	1	4	3	5	5	3	5	4	4	3	3	3	3	4	5	3	3
P. avicida S		4	6	6	1	5	1	2	5	1	1	3	5	5	3	8	6	4	4	3	3	3	4	5	8	8
P. avicida R		2	4	4	1	8	1	6	3	1	4	1	3	3	6	5	5	5	5	5	5	5	8	5	5	5
P. tularensis S		6	5	5	6	3	6	6	6	1	4	5	4	4	2	4	2	5	2	2	3	3	3	4	1	1
P. tularensis R			4	4	6	6	4	4	4	1	5	5	5	5	6	6	5	4	5	3	3	3	3	3	3	3
H. pertussis S		6	4	4	6	5	4	4	4	1	5	6	6	6	7	4	4	7	7	5	5	5	5	5	7	6
H. pertussis R		2	4	4	6	5	3	3	4	1	5	4	4	4	2	2	2	6	6	3	3	3	3	3	3	3
Pf. mallei S		3	1	1	6	7	2	2	2	1	3	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3
Pf. mallei R		2	4	4	1	8	3	3	4	1	3	3	5	5	2	2	2	7	7	7	3	3	4	4	2	2

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY BR. MELITENSIS 316 R ANTIGEN, WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	R Immune Serums									
	unabsorbed Br. abortus 4a R	absorbed Br. suis 404 R	unabsorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R	unabsorbed avicida R	absorbed H. influenzae R	unabsorbed Pf. mallei R	absorbed	unabsorbed	absorbed
Br. abortus 966 S	1	4	3	3	8	6	6	6	9	9
Br. abortus 4a R	6	6	4	4	6	4	4	4	9	9
Br. suis 436 S		1	2	2	4	1	1	4	4	4
Br. suis 404 R		4	5	5	2	4	4	3	3	3
Br. melitensis 384 S	3	1	1	1	3	4	4	5	5	5
Br. melitensis 384 R	4	4	3	3	1	2	2	5	5	5
Br. melitensis 384 S		1	1	1	3	4	4	4	4	4
Br. melitensis 316 R		5	6	6	3	-	-	4	4	4
P. a vicida S	5	3	3	3	5	3	3	2	2	2
P. avicida R	5	6	5	5	7	7	7	2	2	2
P. tularensis S		4	5	5	4	6	6	7	7	7
P. tularensis R		4	3	3	3	4	4	4	4	4
H. pertussis S		4	4	4	4	5	5	7	7	7
H. pertussis R		4	2	2	4	6	6	4	4	4
Pf. mallei S	4	4	5	5	4	1	1	3	3	3
Pf. mallei R		4	3	3	4	3	3	7	7	7



CROSS AGGLOUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. AVICIDA R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	S Immune Serums											
	unabsorbed Br. abortus 966 S	absorbed Br. abortus 966 S	unabsorbed Br. suis 436 S	absorbed Br. suis 436 S	unabsorbed Br. suis 401 S	absorbed Br. melitensis 384 S	unabsorbed Br. melitensis 384 S	absorbed Br. melitensis 319 S	unabsorbed P. avicida S	absorbed P. bovisep-ticus S	unabsorbed P. equisepticus S	absorbed P. tularen-sis S
Br. abortus 966 S	8	4	4	4	4	8	5	5	5	1	5	7
Br. abortus 4a R		4	4	4	5		4	4	3	1	3	1
Br. suis 436 S	8	8	8	6	7	8	3	3	1	3	4	6
Br. suis 404 R		5	5	5	5	1	4	4	1	2	2	3
Br. melitensis 384 S	8	8	5	5	3	6	5	5	4	3	1	4
Br. melitensis 384 R	2	4	6	6	2		2	2	1	1	3	1
Br. melitensis 384 S	8	8	5	5	3	6	5	5	4	3	3	4
Br. melitensis 316 R		4	6	5	4		5	5	3	3	5	5
P. avicida S	4	6	2	6	5	1	3	3	8	4	3	4
P. avicida R	2	4	1	1	3	4	6	6	6	5	8	8
P. tularensis S	6	5	6	6	6		5	5	2	2	3	4
P. tularensis R		4	4	4	4		6	6	6	5	3	3
H. pertussis S	6	4	4	5	4	5	6	6	7	7	5	5
H. pertussis R	2	4			4		2	2	2	6	3	3
Pf. mallei S	3	1		7	2	3	3	3	3	3	3	3
Pf. mallei R	2	4	2	3	4	3	2	2	2	7	4	4

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. AVICIDA R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	R Immune Serums									
	unabsorbed Br. abortus 4a R	absorbed Br. suis 404 R	absorbed Br. melitensis 316 R	unabsorbed P. avicida R	absorbed H. influenzae R	unabsorbed Pt. mallei R	absorbed	unabsorbed	absorbed	absorbed
Br. abortus 966 S	1	4	3	8	6	9	1	9	1	absorbed
Br. abortus 4a R	6	6	4	9	4	9	1	4	1	absorbed
Br. suis 436 S		1	2	4	1	4	-	4	4	
Br. suis 404 R		4	5	2	4	4	-	4	3	
Br. melitensis 384 S	3	1	1	3	1	4		4	5	
Br. melitensis 384 R	4	4	3	1	2	5		5	5	
Br. melitensis 384 S		1	1	3	4	4		4	5	
Br. melitensis 316 R		5	6	3	-	4		4	4	
P. avicida S	5	3	3	5	3	2		2	2	
P. avicida R	5	6	5	7	7	2		2	2	
P. tularensis S		4	5	4	6	7	1	4	7	
P. tularensis R		4	3	3	4	4		4	4	
H. pertussis S		4	4	4	5	7	-	7	4	
H. pertussis R		4	2	4	6	4		4	3	
Pf. mallei S	4	4	5	4	1	3		3	7	
Pf. mallei R		4	3	4	3	7		7		

S and R antigens

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. TULARENSIS R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS AND R ANTIGENS.

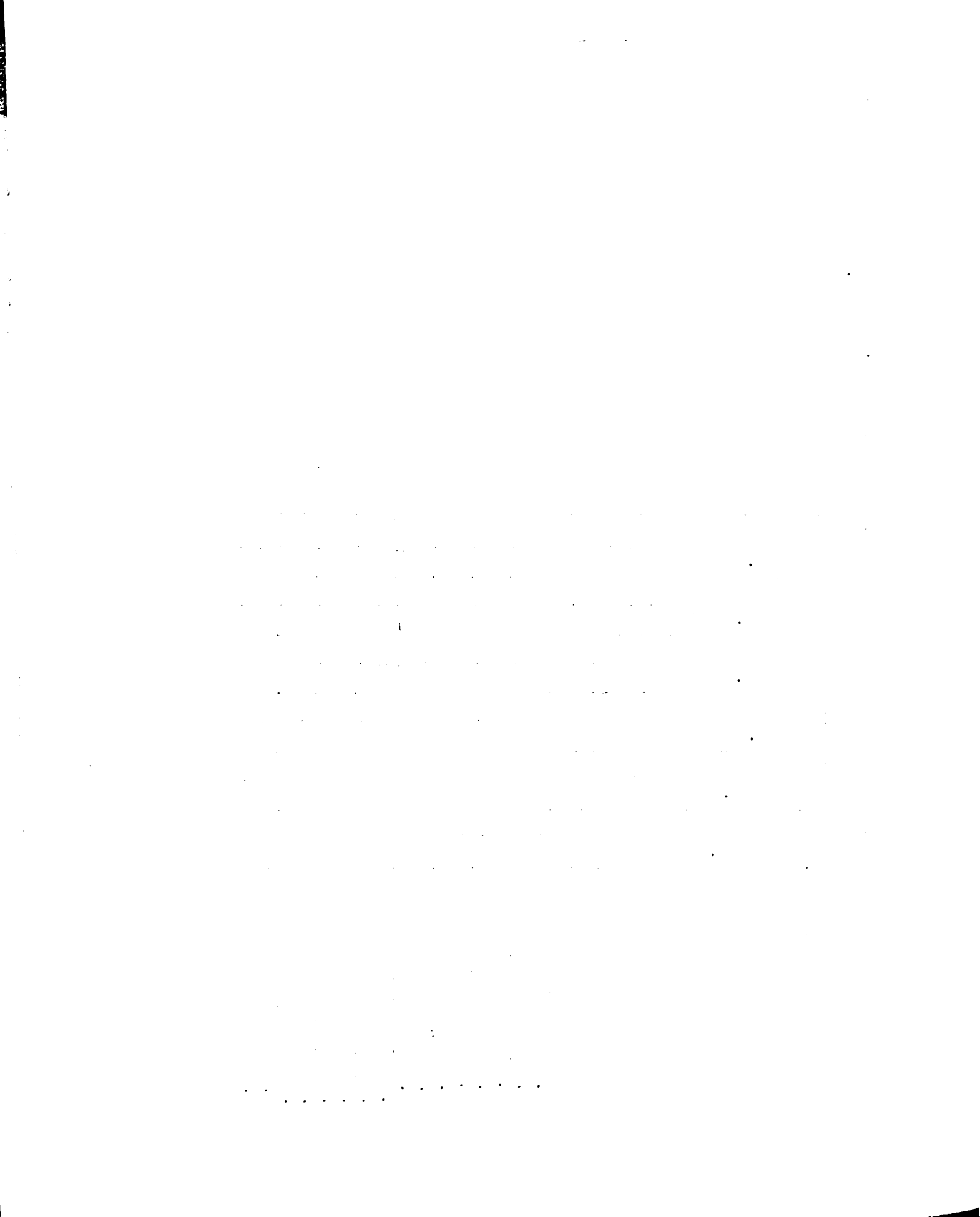
S and R antigens	S. Immune Serums													
	unabsorbed Br. abortus 966 S	absorbed Br. abortus 5a S	unabsorbed Pos. abortus	unabsorbed Br. suis 436 S	absorbed Br. suis 401 S	unabsorbed Br. melitensis 384 S	absorbed Br. melitensis 319 S	unabsorbed P. avicida S	unabsorbed P. bovi- septicus S	unabsorbed P. equise- pticus S	absorbed P. tularen- sis S	unabsorbed H. pertussis S	absorbed	
Br. abortus 966 S	8	4	6	4	4	8	5	5	5	5	7	4	..	
Br. abortus 4a R	8	4	8	4	5	8	4	3	6	3	5	1	..	
Br. suis 436 S		6	8	8	7		3	1	3	4	6	1	..	
Br. suis 404 R		5	5	5	5	1	4	1	4	2	3	2	..	
Br. melitensis 384 S	8	5	8	6	3	6	5	4	2	3	4	2	..	
Br. melitensis 384 R	2	2	4	2	2		2	1	4	1	3	1	..	
Br. melitensis 384 S	8	5	8	6	3	6	5	4	2	3	4	2	..	
Br. melitensis 316 R		4	5		4		3	3	6	3	5	3	..	
P. avicida S	4	6	5	2	5	1	3	8	4	3	4	8	..	
P. avicida R	2	4	8	1	3	4	1	6	5	5	8	8	..	
P. tularensis S	6	5	3	6	6		5	2	5	2	4	1	..	
P. tularensis R		4	6	4	4		5	6	5	3	3	3	..	
H. pertussis S	6	4	5	4	4	5	6	7	7	5	5	7	..	
H. pertussis R	2	4	5	4	4		4	2	6	3	5	6	..	
Pf. mallei S	3	1	7	2	2	3	2	3	3	3	3	3	..	
Pf. mallei R	2	4	8	3	4	3	3	2	7	3	4	2	..	

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CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY P. TULARENSIS R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	R Immune Serums									
	Br. abortus 966 S	Br. abortus 4a R	Br. suis 436 S	Br. suis 404 R	Br. melitensis 384 S	Br. melitensis 384 R	Br. melitensis 384 S	Br. melitensis 316 R	P. avicida S	P. avicida R
Br. abortus 966 S	1	4	4	4	3	3	3	3	8	6
Br. abortus 4a R	6	6	9	4	4	4	4	4	6	6
Br. suis 436 S		1	4	2	1	1	1	1	4	4
Br. suis 404 R		4	4	5	3	3	3	3	2	3
Br. melitensis 384 S	3	1	1	1	1	1	1	1	4	5
Br. melitensis 384 R	4	4	4	3	3	3	3	3	4	5
Br. melitensis 384 S		1	1	1	1	1	1	1	4	5
Br. melitensis 316 R		5	5	6	3	3	3	3	4	4
P. avicida S	5	3	3	3	3	3	3	3	5	2
P. avicida R	5	6	6	5	5	5	5	5	7	2
P. tularensis S		4	4	5	5	5	5	5	4	7
P. tularensis R		4	4	3	3	3	3	3	6	4
H. pertussis S		4	4	4	4	4	4	4	4	7
H. pertussis R		4	4	2	2	2	2	2	4	4
Pf. mallei S	4	4	4	5	5	5	5	5	4	3
Pf. mallei R		4	4	3	3	3	3	3	4	7

S and R antigens



CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY H. PERTUSSIS R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

S and R antigens	S Immune Serums													
	unabsorbed Br. abortus 966 S	absorbed Br. abortus 966 S	unabsorbed Br. abortus 5a S	absorbed Br. abortus 5a S	unabsorbed Pos.	absorbed Br. suis 436 S	unabsorbed Br. suis 436 S	absorbed Br. suis 401 S	unabsorbed Br. melitensis 384 S	absorbed Br. melitensis 384 S	unabsorbed Br. melitensis 319 S	absorbed P. avicida S	unabsorbed P. avicida S	absorbed P. septicus S
Br. abortus 966 S	8	8	4	3	6	4	4	4	8	5	3	5	5	5
Br. abortus 4a R			4	-	8	5	5	4	4	3	3	3	6	3
Br. suis 436 S	8	8	6	4	8	7	8	3	8	3	3	1	3	4
Br. suis 404 R			5	-	5	5	5	4	1	1	1	1	4	2
Br. melitensis 384 S	8	8	5	-	8	3	3	5	6	1	1	4	2	3
Br. melitensis 384 R	2	2	2	-	4	2	6	2	1	1	1	1	4	1
Br. melitensis 384 S	8	8	5	-	8	3	3	3	5	5	5	4	2	3
Br. melitensis 316 R			4	-	5	4	4	6	6	3	3	3	6	3
P. avicida S	4	4	6	-	5	5	5	1	3	3	3	8	4	3
P. avicida R	2	2	4	-	8	3	3	4	1	1	1	6	5	8
P. tularensis S	6	6	5	-	3	6	6	5	5	5	5	2	5	2
P. tularensis R			4	-	6	4	4	4	5	5	5	6	7	3
H. pertussis S	6	6	4	-	5	4	4	4	5	6	7	7	5	5
H. pertussis R	2	2	4	-	5	4	4	4	4	6	6	6	5	6
Pf. mallei S	3	3	1	-	7	2	2	2	3	2	1	3	3	3
Pf. mallei R	2	2	4	-	8	3	4	4	3	3	1	2	4	2

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY H. PERTUSSIS R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	R Immune Serums									
	unabsorbed Br. abortus 4a R	absorbed Br. suis 404 R	absorbed Br. melitensis 316 R	unabsorbed P. aviciida R	absorbed H. influenzae R	unabsorbed Pf. mallei R	absorbed	absorbed	absorbed	absorbed
S and R antigens										
Br. abortus 956 S	1	4	3	8	6	6	6	6	6	6
Br. abortus 4a R	6	6	4	6	4	6	6	6	6	6
Br. suis 436 S		1	2	4	1	4	4	4	4	4
Br. suis 404 R		4	5	2	4	3	3	3	3	3
Br. melitensis 384 S	3	1	1	3	4	5	5	5	5	5
Br. melitensis 384 R	4	4	3	1	2	5	5	5	5	5
Br. melitensis 384 S		1	1	3	4	5	5	5	5	5
Br. melitensis 316 R		5	6	3	-	4	4	4	4	4
P. aviciida S	5	3	3	5	3	2	2	2	2	2
P. aviciida R	5	6	5	7	7	2	2	2	2	2
P. tularensis S		4	5	4	6	7	7	7	7	7
P. tularensis R		4	3	3	4	4	4	4	4	4
H. pertussis S		4	4	4	5	7	7	7	7	7
H. pertussis R		4	2	4	6	4	4	4	4	4
Pf. mallei S	4	4	5	4	1	3	3	3	3	3
Pf. mallei R		4	3	4	3	7	7	7	7	7

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CROSS AGGLOUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY PF. MALLEI R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

	S Immune Serums											
	966 S unabsorbed Br.abortus	5a S unabsorbed Br.abortus	abortus Pos. unabsorbed	436 S unabsorbed Br.suis	401 S unabsorbed Br.suis	Br. melitensis 384 S unabsorbed	Br. melitensis 319 S unabsorbed	R. aviciida S unabsorbed +	P. bovisep- ticus S unabsorbed	P. equise- pticus S unabsorbed	P. tularen- sis S unabsorbed	H. pertussis S unabsorbed
Br. abortus 966 S	8	4	2	4	2	8	5	5	5	4	7	4
Br. abortus 4a R		4	8	4	1	2	4	3	6	3	3	1
Br. suis 436 S	8	6	2	5	7	8	3	1	3	4	6	1
Br. suis 404 R		5	5	2	5	1	4	1	4	2	3	2
Br. melitensis 384 S	8	5	8	6	3	6	5	4	2	3	4	2
Br. melitensis 384 R	2	2	4	2	2	6	2	1	4	1	3	1
Br. melitensis 384 R	8	5	8	6	3	6	5	4	2	3	4	2
Br. melitensis 316 R	8	4	5	6	4	6	5	3	6	3	5	3
P. eviciida S	4	6	8	2	5	1	3	8	4	5	4	8
P. eviciida R	2	4	8	1	3	4	1	6	5	5	8	8
P. tularensis S	6	5	3	6	6		5	2	5	2	4	1
P. tularensis R		4	6	4	4		5	6	5	3	3	3
H. pertussis S	6	4	5	4	4	5	6	7	7	5	5	7
H. pertussis R	2	4	5	4	4		4	2	6	3	5	6
Pf. mallei S	3	1	7	2	2	5	2	3	3	3	3	3
Pf. mallei R	2	4	8	3	4	3	2	2	7	3	4	2

CROSS AGGLUTINATION OF S AND R IMMUNE SERUMS, ABSORBED BY PF. MALLEI R ANTIGEN,
WITH HOMOLOGOUS AND HETEROLOGOUS S AND R ANTIGENS.

R and S antigens	R Immune Serums									
	unabsorbed Br. abortus 4a R	unabsorbed Br. suis 401 R	absorbed Br. melitensis 316 R	unabsorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R	absorbed Br. melitensis 316 R
Br. abortus 966 S	1	4	3	3	3	3	3	3	3	3
Br. abortus 4a R	6	9	4	4	4	4	4	4	4	4
Br. suis 436 S		1	2	2	2	2	2	2	2	2
Br. suis 404 R		4	5	5	5	5	5	5	5	5
Br. melitensis 384 S	3	1	1	1	1	1	1	1	1	1
Br. melitensis 384 R	4	4	3	3	3	3	3	3	3	3
Br. melitensis 384 R		1	1	1	1	1	1	1	1	1
Br. melitensis 316 R		5	6	6	6	6	6	6	6	6
P. avicida S	5	3	3	3	3	3	3	3	3	3
P. avicida R	5	6	5	5	5	5	5	5	5	5
P. tularensis S		4	5	5	5	5	5	5	5	5
P. tularensis R		4	3	3	3	3	3	3	3	3
H. pertussis S		4	4	4	4	4	4	4	4	4
H. pertussis R		4	4	4	4	4	4	4	4	4
Pf. mallei S	4	4	5	5	5	5	5	5	5	5
Pf. mallei R		4	3	3	3	3	3	3	3	3

The data for serums absorbed by Br. abortus 966 S (page), showed that positive abortus serum from a naturally infected cow still agglutinated Br. suis 436 S antigen in 1 to 800 dilution as compared to the original titre of 1 to 2,560. Br. melitensis 319 S absorbed by Br. abortus 966 S antigen, agglutinated Br. abortus 4a R antigen in a higher dilution than before absorption, but showed no removal of agglutinin for its specific antigen. Br. abortus 966 S antigen removed none of the agglutinin from Pf. mallei immune serum for Br. abortus 4a R antigen or Pf. mallei R antigen. However, Pf. mallei R immune serum, absorbed by Br. abortus 4a R antigen showed no agglutination for its specific antigen, Pf. mallei R.

Br. melitensis 316 R immune serum showed no absorption by Br. abortus 966 S antigen for its specific antigen. The agglutinin which P. avicida R immune serum contained for P. avicida S antigen was not removed by absorption with Br. abortus 966 S antigen, but much of the agglutinin for P. avicida R was removed. Br. abortus 966 S immune serum unabsorbed by any antigen gave a slightly higher titre for P. avicida S than R.

Br. suis 404 R immune serum agglutinin for P. tularensis S was not absorbed by Br. abortus 966 S antigen. H. pertussis S agglutinin was absorbed and H. pertussis R agglutinin was not. The H. pertussis R agglutinin was absorbed from Br. melitensis 319 S immune serum. Br. suis 404 R immune serum agglutinin for its specific antigen was removed. The agglutinins for P. tularensis S and H. pertussis R were removed

from Br. melitensis immune serum by absorption with Br. abortus 966 S antigen.

In the cross agglutination studies with unabsorbed serums, the agglutinating titres of Br. abortus and H. pertussis S antigens with all serums were more nearly parallel than either antigen showed with other members of its own group. After agglutinin absorption of all serums the titres with these two antigens are the same in the majority of cases, although not as nearly parallel as before absorption. Br. suis 401 S immune serum absorbed by H. pertussis S antigen showed no decrease in agglutination titre for Br. abortus 966 S antigen. Br. suis 404 R immune serum absorbed by P. tularensis S antigen showed no decrease in titre for H. pertussis S, but the titre of Br. suis 404 R immune serum absorbed by P. tularensis S antigen was negative for Br. abortus 966 S antigen (page).

The immune serums of the Brucella organisms, Br. abortus 5a S, Br. suis 401 S, Br. melitensis 319 S, and Br. melitensis 316 R, which were absorbed by Br. suis 404 R antigen, showed no removal of agglutinin for Br. abortus 966 S antigen. The absorbed serums agglutinated H. pertussis S antigen in a 1 to 50 dilution, with this exception, Br. melitensis 316 R absorbed immune serum, agglutinated H. pertussis S antigen with the same titre as the unabsorbed Br. melitensis R serum. Br. suis 404 R unabsorbed immune serum agglutinated both Br. abortus 966 S antigen and H. pertussis S antigen

in 1 to 160 dilution. Br. abortus 5a S absorbed by Br. melitensis 316 R antigen removed much of the agglutinin for Br. abortus 966 S. The titre of this serum for H. pertussis S antigen was higher than before absorption. P. tularensis S immune serum absorbed by H. pertussis R antigen did not agglutinate Br. abortus 966 S antigen. No removal of agglutinin for H. pertussis S was shown.

The H. pertussis S unabsorbed serum agglutinated P. avicida S and R antigens in dilutions equal to its homologous antigen. H. pertussis S immune serum absorbed by H. pertussis S antigen showed but slight removal of P. avicida S agglutinin. P. avicida R agglutinin was completely removed. H. pertussis S immune serum absorbed by P. avicida R antigen agglutinated P. avicida S antigen in a slightly lower titre than before absorption, but H. pertussis S antigen was agglutinated in 1 to 50 dilution as compared to 1 to 1280 dilution, the original titre of the unabsorbed serum.

Every attempt to show an absolute serological similarity between any two organisms has resulted in just such data as shown above, complete absorption or the absence of absorption when the opposite might be expected. Many of the tests which gave varying results which could be questioned as error, have been repeated with the same absorbed serum and the same antigen. The same titre was found each time.

The cross agglutination studies of these 4 genera have shown apparently inconsistent results. The results of the agglutinin absorption studies were doubly varied, for instead of one variable factor there were two, the agglutinating antigen and the absorbing antigen. The antigens for titration of absorbed serums were made up in quantities sufficient to last several weeks. The absorbing organisms were freshly transferred and cultured each time that they were used. This probably in some cases explains the difficulty found in completely absorbing the specific agglutinins. Although bacteriologically apparently the same antigens were used for absorbing and for the agglutinating check on sufficient absorption of the specific agglutinins, two serologically different organisms may have developed.

V. DISCUSSION

The cross agglutination studies have shown that there is interagglutinability of these genera and the agglutinin absorption results have emphasized this fact. It has also emphasized the fact that agglutination titres are influenced by the state of dissociation of the cultures used as antigens. In many instances the lack of correlation between members of the same genera in the quantity of agglutinin produced and the agglutininogen is as great as the variation between members of different genera.

Previous reports of dissociation and serological classification of each of these groups demonstrate this same irregularity of results. Evans (1918) found Br. abortus and Br. melitensis to be alike morphologically, culturally, biochemically and serologically. She found that Br. abortus antiserums with Br. abortus or Br. melitensis antigens gave the same titre, but Br. melitensis antigen with Br. abortus and Br. melitensis serums gave a different titre for each. She concludes that Br. abortus and Br. melitensis antiserums contain more than one agglutinin, that the agglutinin in the two antiserums are alike in kind but differ in proportion and that the corresponding agglutininogens are present in the antigens in corresponding variation.

Feusier and Meyer (1920) divided the abortus and

melitensis strains into 4 groups by agglutinin absorption tests. Evans (1923) divided the Brucella organisms into 7 groups by agglutinin absorption tests and Burnet (1925) also by agglutination and agglutinin absorption tests divided them into 2 types.

Orcutt (1926) working with 6 strains found no serological distinction. Two melitensis strains were found to be identical with the bovine and swine strains by means of agglutination and agglutinin absorption studies. Carpenter and Boak (1929) because of the serological difference shown between the R variants suggested a division of the Br. abortus strains into 2 groups.

The reason for this kaleidoscopic appearance of the cross agglutination and agglutinin absorption results of the Brucella group seems to be given by Zdrodowski, Brenn and Voskressinski (1930) who state that the R and S forms showed distinct serological differentiation. The organisms changed their serological and bacteriological positions according to their state of dissociation.

Also Plastring and McAlpine (1932) divided the abortus and melitensis organisms into 5 serological groups. Evidence of variation in serological properties of the genus Brucella, especially Br. melitensis under ordinary laboratory conditions has been noted and discussed from the standpoint of explaining the lack of agreement in results reported by various investigators. The strains are not correlated into the same groups serologically and biologically.

These reports would seem to show a serological difference when a cultural, morphological, and physiological variation was not visible. In the present attempt to correlate the *Brucella*, *Pasteurella*, *Hemophilus*, and *Pfeifferella* groups the only characteristic used to determine the R or S type of organism has been its colonial appearance. Mackenzie, Fitzgerald and Irons (1933) in a study of the independent variation of the biological characteristics of bacteria as a result of dissociation, state that the "agglutinative, agglutogenic absorptive qualities" vary independently of each other. In the reversion of the R cultures back to the S types not once was complete reversion of all characteristics observed. Thus an organism may be smooth according to more concepts, but rough to others. A serological examination of organisms will detect character changes not biochemically or morphologically noted, thus the irregularity in serological classification.

A review of work done upon the *Pasteurella* genera shows the same picture, varied serological titres from different states of dissociation, varied classification of apparently identical cultures.

DeKruif (1921) found the coexistence of organisms of different degrees of virulence in cultures of the bacillus of rabbit septicemia. He called the virulent bacteria the D form and the less virulent the G form. Agglutination tests showed a marked serological difference

in these two types of culture. They were morphologically indistinguishable in form and possessed like fermentation reactions. The G form flocculated rapidly in fluid media and formed translucent bluish colonies of low virulence.

Lal (1927) has summarized previous work in the correlation of the *Pasteurella* genera, in part as follows:

Cochenour (1924) was unable to distinguish the strain isolated from the tissues of a buffalo from other *Pasteurella* organisms by employing cultural, serological and animal protection methods. Tanaka (1926) stated that there is not sufficient difference between the members of the group in agglutination, fermentation, or complement fixation properties to be of diagnostic value. Some investigators divided the group into two or three distinct types, each consisting of a few organisms indistinguishable from each other, although derived from different animal hosts. Koske (1904) reports *P. avicida* indistinguishable from *Br. suis* by agglutination reactions. Roderick (1922) studied the morphological, cultural, biochemical and immunological properties of the *Pasteurella* genera and divided it into 2 groups, (1) a bovine swine type and (2) an ovine avian-rabbit-cavia type. Wassermann and Ostertag (1904), Hutyra and Marek (1912) found that the immune sera against one organism was protective against the homologous infection, but not against the heterologous bacteria of this group. Joest (1906) reports

that blood serum strongly immunized against one member of this group will be found protective. Lal concluded from his work that the Pasteurella organisms tested by complement fixation showed a certain amount of group specific reaction, an exception being P. lewisii. Homologous strains showed a high degree of specific fixation. Although not easily distinguishable the organisms were a distinct species.

Hughes (1930) by reciprocal agglutination tests with several strains of P. avicida showed a close serological relationship. Webster (1930) on the basis of colony formation divided P. avicida into three groups. A "fluorescent" colony of high virulence, stable in suspension found in severe epidemics, a "blue" colony unstable in suspensions associated with endemics and an "intermediate" type.

The Hemophilus genera, probably because of its greater instability on artificial media, more than any of the others showed varying serological results. Particularly was this true of the H. influenzae organisms, a culture which very readily becomes dissociated when held in stock and is very difficult to revert to its original form. Pittman (1931) reported that spontaneous conversion of H. influenzae from its S to R type readily took place in artificial culture media. It may be delayed by certain cultural procedures or hastened by growth in type specific antiserum. The artificial conversion of R strains to the S form has been observed, but this change is carried out only with difficulty.

Bordet and Sleeswyck (1910) found in freshly isolated H. pertussis organisms grown on chocolate agar and plain agar, a serological difference. Old stock strains did not show this difference. Krumwiede and Mishulow (1923) in studies of a series of strains of H. pertussis of typical morphological and cultural characteristics, divided them into two groups by agglutinin absorption tests.

Leslie and Gardner (1931) by cross agglutination and agglutinin absorption divided 32 strains of H. pertussis into four serological groups. All freshly isolated organisms fell into one group. By growth on various media all cultures have been changed to fall into all four types.

Roos (1919) concluded that the various strains of influenzae do not differ in kind as indicated by cross agglutination and agglutinin absorption tests. Valentine and Cooper (1919) reported that their results indicated that under the term "B. influenzae" they were dealing with a group of organisms which were heterogenous in character as determined by immunological reactions, cross agglutination, and agglutinin absorption tests.

Povitzky and Denny (1921) found 4 to 7 influenzal meningitis strains, isolated years apart, proved by agglutinin absorption, to be of one type. From the respiratory cases 5 strains from different individuals were obtained which were of the same type. No other groups of more 2 members were obtained. In general they found that strains of the same immunological characteristics had the same cultural

properties, but there were some exceptions.

Jordan and Sharp (1922) found that as a rule each strain of H. influenzae possessed a serological individuality. Occasionally strains from different sources were serologically identical. Repetition of agglutination tests with the same strain antigen and serum did not give identical results. At some time almost every serum agglutinated every antigen, although at other times the results were negative. They found cross agglutination between H. pertussis and H. influenzae. Five strains of H. pertussis agglutinated in dilutions of 1 to 50 or 1 to 100 with serums obtained from two different rabbits immunized against an influenzae strain. The serums of two other influenzae immunized rabbits did not show agglutination. These antisera against 6 strains of Alk. bronchisepticus gave negative titres. Pittman (1931) by means of cross precipitation and agglutination reactions divided 15 S strains into 2 serological groups.

Povitzky (1918) showed that Pf. mallei gave variable serological reactions under different conditions of growth. Only 1 strain out of 15 in the stock collection proved constant in serological reactions, and that only when great care was exercised in the preparation of the culture medium.

VI. SUMMARY.

To show the relationship between the *Brucella*, *Pasteurella*, *Hemophilus* and the *Pfeifferella* genera, bacteriological, morphological, cultural, physiological and serological (cross agglutination and agglutinin absorption) studies have been made of these organisms in their smooth and rough forms. The dissociation state of these bacteria has been determined by colonial appearance alone.

Cultures which have been held in stock for many years have been used as well as more recently isolated strains. These organisms are alike morphologically and physiologically.

Immune serum was produced by rabbit injection with both smooth and rough strains. Cross agglutination tests showed interagglutinability of all genera used. In almost every case immune serums showed the highest titres with their specific antigens. Rough serums of the *Brucella* genus have shown higher titres for a smooth heterologous antigens than the smooth types of their own genus.

No closer relation was found among the rough forms than among the smooth, either bacteriologically or serologically. An S immune serum often demonstrated higher agglutinability for some S antigens, some R antigens, and sometimes the same titre for both S and R heterologous antigens. The reciprocal agglutination and agglutinin absorption tests often varied. This has been explained by the intermediate states of dissociation of cultures used. Although the colonial appearance of the cultures in these tests were checked constantly for definite S or R types,

changes brought about by slight difference in medium composition, temperature of incubator and frequency of transfer over a period of several months, can be detected only through serological examination and have brought about the results shown. The organisms can not be divided into the same groups by biological and serological classification.

The literature shows a marked lack of agreement of serological classifications of all these groups individually. These studies have not only shown the irregularity of serological results within each group of organisms, but that the same dissimilarity exists between groups as within groups. This dissimilarity does not detract from the proof of relationship of these genera but rather adds to it.

VII. CONCLUSIONS

(1) The Brucella, Pasteurella, Hemophilus and Pfeifferella genera are closely related morphologically, physiologically and serologically.

(2) The degree of their interagglutinability depends upon the state of dissociation of the cultures used for the serological studies.

(3) Agglutinin absorption studies demonstrate the same irregular serological similarity between genera which is shown within genera.

(4) Cultures apparently identical by morphological and physiological properties are found to be variants by serological examination.

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