THE DURATION OF VACCINAL IMMUNITY IN GUINEA-PIGS AGAINST BACTERIUM ABORTUS INFECTION

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THESIS

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INTRODUCTION

The pathogenicity of *Bacterium abortus* for guinea-pigs has been recognised for about fifteen years. During this time these animals have been used for both diagnostic and experimental work. The lesions caused in guinea-pigs by infection from this organism are very outstanding and clear, so that it is not difficult to diagnose. Schroeder and Cotton (1) were the first experimenters to describe the lesions in the organs of guinea-pigs due to *Bact. abortus* infection. The guinea-pigs had been fed milk containing this organism. The gross anatomical lesions were described as extreme enlargement and edema of the lymph glands generally; the appearance of small glistening nodules in the lungs which seem to be caused by the enlargement of minute lymph glands that were ordinarily too small to be visible; the conversion of the minute nodules in the lungs into larger, necrotic areas; an enormous enlargement of the spleen, often to thirty or forty times its normal volume; an irregular thickening of the capsule of the spleen, through which its surface becomes marked with white areas varying in size from mere points to several centimeters in diameter; an enlargement and degeneration of the liver, which organ becomes thickly beset on surface and section with irregular, pale yellow or

dirty white areas that seem to be due to an enormous proliferation of connective tissue and a consequent crowding out and obliteration of the liver cells proper; a diffuse, parenchymatous nephritis that reaches stages in which dense, fibrous nodules are formed in the cortex of the kidneys; and, in male guinea-pigs, a degeneration of the testicles, commonly beginning in the epididymis and often in the conversion of one or both testicles into structureless cysts filled with creamy pus.

The lesions in Bact. abortus infected guinea-pigs have been described microscopically by Seyfarth (8). The nodules in the lungs were scattered throughout the entire organ, and consisted of accumulations of lymphoid cells and groups of epitheloid cells. The nodules were just beneath the pleura or about the blood vessels or bronchi. The interstitial tissue was extensively involved with small nodules. The structure of the nodules was similar to if not identical with, the tuberculous nodules.

The spleen was found to contain greatly dilated blood sinuses, and there was marked hyperplasia of the endothelial cells in and around the malpighian corpuscles, with an active cellular proliferation of the splenic pulp. The epithelioid cells were observed in groups chiefly in relation to the malphighian bodies and directly beneath the capsule. Polymorphonuclear leukocytes were numerous. An occasional giant cell was distinguished.

Bact. abortus which has been grown on artificial media does not always produce lesions when injected into guinea-pigs. Some of the virulence seems to be lost when grown under laboratory conditions. Organisms of low virulence have been used to produce immunity but the results are not always certain as there is some danger of an increase in the virulence of organisms which have been passed through animals. Huddleson (2) has developed a non-virulent strain of Bact. abortus which seems to produce a fairly high degree of immunity. The use of this organism in a vaccine to protect animals against infection from virulent cultures of Bact. abortus has made it necessary to determine how long this immunity will last. The necessity for knowing the duration of this immunity has prompted the carrying out of this experiment.

It is the purpose of this experiment to show how long immunity to Bact. abortus infection will last in guinea-pigs by injecting them with a non-virulent strain of Bact. abortus then feeding these guinea-pigs a virulent strain of bovine Bact. abortus. The presence or absence of characteristic lesions in the spleen, liver, lungs, or testes of other organs and the isolation of Bact. abortus therefrom determines whether or not the animal is immuned.

REVIEW OF LITERATURE

The literature reveals very little research pertaining to the duration of immunity in animals which have been treated by a vaccine or other means of immunization. This is not only true of Bact. abortus but of other vaccines as well. The duration of immunity in animals vaccinated with Bact abortus has been stated in some instances but it has not been proved by experimental work. Certain investigators have drawn conclusions concerning the duration of immunity from a few experiments which had been planned for other purposes.

There has been no work done to show the duration of immunity acquired by injecting guinea-pigs with a non-virulent strain of Bact. abortus.

The only other experimental work of this nature with guinea-pigs has been done by Calmette, Negre, and Boguet (4). They injected bacilli of a non-virulent strain of Bacterium tuberculosum into the blood circulation of guinea-pigs and rabbits and obtained only temporary immunity as was shown by subsequent infection with virulent organisms. Immunity seemed to last for about five months in guinea-pigs and about six months in the rabbit. The immunity gradually became weaker and seemed to disappear as soon as the tubercle bacilli were entirely eliminated from the body or reabsorbed. These workers drew the conclusion that the immunity may be prolonged by renewing periodically the vaccinal injection

of the lymphatic organs.

Schroeder and Cotton (5) have immunized guinea-pigs with a strain of Bact. abortus which had lost much of its original virulence for guinea-pigs and which caused lesions in them only when injected in very large doses. Thirty-three guinea-pigs were injected with the organism. Eighty-one days later 12 of them with twelve controls were injected subcutaneously with a virulent bovine strain of Bact. abortus. Nine of the treated pigs showed no microscopic lesions, two showed slight lesions, and one showed marked lesions. Of the 12 check, nine showed marked lesions and 3 slight lesions.

145 days after treatment 10 of the immunized pigs with 12 checks were exposed to a virulent strain of Bact. abortion. Five of the treated pigs showed no lesions, four showed slight lesions and 1 more marked lesions. Seven of the twelve check showed extensive lesions, four showed slight lesions, and one showed no lesions.

The author concluded from his experiment that a high degree of resistance against virulent abortion germs is obtained by injecting them with attenuated abortion germs, and that this immunity begins to decline sometime after the third month following the immunizing injection.

PREPARATION AND USE OF VACCINE

The vaccine was prepared by growing the non-virulent strain of Bact. abortus for forty-eight hours on liver agar. The liver agar used was prepared as described by Huddleson (6). The growth was then washed off with sterile physiological salt solution and the suspension was standardized so that the turbidity corresponded to turbidity V of McFarland's Nephelometer. Five tenths of one cubic centimeter of this suspension was injected subcutaneously with a small syringe.

After a period of one month, and each month thereafter, four pigs from the vaccinated group were placed in a cage with two normal control pigs. The cages were separated by a metal partition so that there was no possibility of transmission of infection from one cage to another. The pigs were then exposed to infection by sprinkling the feed with virulent organisms each morning for six days.

PREPARATION OF INFECTIVE MATERIAL

The virulent organisms were grown on liver agar in test tubes for forty-eight hours, then washed off with tap water and sprinkled over the feed of the pigs. At the beginning of the experiment three slants were fed daily for eight days. Some of the vaccinated pigs seemed to show signs of infection or rather enlarged organs, so the dosage was cut down to one agar slant a day for six days. It was

thought that the first amount was more than could be resisted with the degree of immunity which had been obtained.

Virulent organisms from four different sources were used in this experiment. All of these cultures were isolated from aborted fetuses sent in to this laboratory for bacteriological examination. Material from these fetuses was injected into guinea-pigs and six weeks later these guinea-pigs were autopsied and the organs smeared on agar plates. These plates were placed in ten per cent carbon dioxide for forty-eight hours. All of these pigs were heavily infected when they were autopsied and good growths were obtained on the plates.

Culture D 1 was isolated December 1, 1923. Culture B 54 was isolated October 1924. The cow from which this fetus came was bred March 9,1924 and aborted October 24,1924. Culture 44 was isolated from an aborted fetus, the dam of which was bred May 14, 1924 and aborted November 28, 1924. The dam of the fetus from which culture C 111 was isolated was bred July 16, 1924 and aborted February 11, 1925.

Method of Determining Infection

The six exposed pigs were kept in the cage for six weeks. This was considered a sufficient time for lesions to develop after exposure. At the end of this period of time all pigs were bled from the heart in the following manner: The animal was anesthetised and firmly fastened on its back to an animal board, a small space over the left side of the thorax was clipped free from hair,

the space was disinfected with phenolized saline solution and a small hypodermic needle was inserted between the second and third ribs into the left ventricle of the heart. If the needle punctured the right ventricle the blood flowed very slowly or in drops. Two or three cubic centimeters of blood were drawn from each pig in this manner and slanted before a clot was formed. The samples were placed in the ice box until the next day. When taken out of the ice box sufficient serum had formed above the clot to make agglutination tests. In some instances where serum did not form the clot had to be broken and centrifuged. The antigen for the agglutination tests was prepared by growing a culture of Bact. abortus for forty-eight Hours upon liver agar. The growth was washed off with carbol-saline solution (.5 per cent carbolic acid in .85 per cent solution of sodium chloride in water) and the suspensionwas standardized so that the turbidity corresponded to about .7 of tube one of McFarland's Nephelometer. Two cubic centimeters of this suspension was placed in each of five Wassermann tubes and the following quantities of blood serum added; .08, .04, .02, .01, and .005. Dilutions of approximately 1:25, 1:50, 1:100, 1:200, and 1:400 were obtained. These tubes were placed in the incubater and read at the end of 24 and 48 hours. The results shown in the tables were taken from the forty-eight hour reading.

As soon as the agglutination tests were completed the animals were killed and autopsied, and search was made for any anatomical changes. An attempt was made to culture

Bact abortus from the liver, spleen, lungs, kidneys, testes, urinary bladder, and gall bladder. These organs were smeared on gentian violet liver agar plates as described by Huddleson (6). These plates were placed in jars containing ten per cent carbon dioxide, sealed and incubated at thirty seven degrees centigrade for about six days. All colonies resembling Bact. abortus colonies, which appeared on the plates were transferred to liver agar slants and incubated. Agglutination tests were run using each organism as an antigen. Positive cow's serum was used in the agglutination tests. Agglutinations in only the highest dilutions were recorded in the tables here shown.

EXPERIMENTAL DATA

The data recorded give the results obtained each month for ten months. In order to make clear some of the results recorded it is necessary to state that about a month after this experiment was started the pigs became infected with an organism which caused the death of several animals. Some of those which recovered held their heads to one side and when they were autopsied it was found that they carried an infection in their ears. When cultured on liver agar the organism produced a very profuse, slimy growth. Pigs dying from this infection showed large amounts of exudate in the thoracic cavity and sometimes in the abdominal cavity. The atypical lesions here recorded were attributed to this cause. This interpretation was based on the fact that this same type of lesions was found in normal-guinea pigs which had never been exposed to Bact. abortus and which did not react to the agglutination test. The lesions in the liver were quite different from those caused by Bact. abortus. They consisted of irregular, dirty brown foci, sometimes five millimeters in diameter. Some were longer than wide and others were about the same dimensions each way. The edges were irregular and the surfaces was flat or not elevated above the surface of the liver. The spleen was sometimes enlarged but did not show this type of lesion.

The lesions which were considered typical of Bact. abortus lesions corresponded in some respects to those

described by Schroeder and Cotton (7) and in other respects to those described by Seyfarth (8). The external appearance of the body was normal in the majority of cases, this being particularly true with immunized pigs. Controls which were heavily infected were emaciated, the hair was rough, and the pig felt soft and flabby when handled. The spleen appeared to be the organ most constantly infected, as was shown by both anatomical changes and cultural findings. The spleen was usually enlarged, and directly under the capsule were minute nodules or foci which were grayish white in color and raised. The surface of the spleen appeared rough due to these raised areas. The spleen was not always enlarged, for in some pigs which showed lesions in other organs the spleen was normal. The liver was the next most commonly infected. It usually contained grayish white or pearly white foci, varying in size from that of a pin point to a millimeter in diameter. These foci were usually raised and glistening, showing very clearly through the capsule of the liver. Enlargment of the liver was never noted. One or both testicles sometimes contained lesions or abscesses. The epididymus was more often involved. Sometimes the testicles were atrophied. No lesions were observed in the kidney, however, despite the fact that the organism was cultured from this organ many times. In a few animals the lungs contained foci from which Bact. abortus was obtained occasionally.

Since the time between injection and the first appearance of infection of immunized pigs is the important

factor in this experiment it will be necessary to pay particular attention to the dates recorded.

TABLE I, GROUP I

Pigs Exposed to Infection 35 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic findings B. *abortus* Positive in
2	370	-	9/29/24	473	11/3/24	556	12/30/24	Atypical white foci in liver Other organs normal	*B. abortus* Negative	1:50
11	340	-	"	485	"	571	"	White foci in liver, other organs normal	No cultural findings	1:500
28	400	-	"	433	"	464	"	Atypical foci in liver, spleen enlarged, lungs partially collapsed	Frei-lander's pneumo bacillus from lungs	1:200
31	390	-	"	500	"	590	"	Few atypical foci in liver spleen enlarged	No cultural findings	1:500
1			Control	370	"	540	"	Spleen twice normal size pearly white foci in liver	*B. abortus* cultured from fetus	1:500
2			Control	245	"	322	"	Few pearly white foci in liver other organs normal	No cultural findings	1:200

The data in table 1 shows the results obtained the first thirty five days after vaccination. Blood tests were made at the end of 30 days, but five days elapsed before time of autopsy in order to perform an intradermal test on each pig. This group was fed three slants a day for eight days. Culture DI was the organism used. The vaccinated pigs of this group made an average gain of 70 grams and the controls made an average gain of 74.5 grams. Atypical lesions were found in the vaccinated pigs but Bact. abortus was not cultured from them. Number 28 showed an enlarged spleen, but since an organism producing a slimy growth was cultured from this organ, the enlargment was attributed to this organism. Number 31 also showed an enlarged spleen which was adherent to the wall of the body cavity. It was an old lesion which had healed and since nothing was cultured from it the cause was not attributed to Bact. abortus.

Both of the controls showed typical Bact. abortus lesions but Bact. abortus was cultured from only one pig. The failure to culture Bact. abortus from the other pig and the agglutination reaction of 1:200 cannot be accounted for1

TABLE II, GROUP II.

Pigs Exposed to Infection 65 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic findings B.abortus Positive in
32	360	-	9/29/24	450	12/3/24	412	1/30/25	All organs normal head turned to one side due to infection in ear	No cultural findings	1:100
33	345	-	"	600	"	749	1/23/25	Atypical lesions in liver, other organs normal	No cultural findings	1:200
47	365	-	"	525	"	600	"	All organs normal	No cultural findings	1:500
48	295	-	"	510	"	442	1/30/25	All organs normal	One atypical colony	1:200
3			Control	580	"	710	"	Typical pearly white foci in liver, spleen 2 x normal	B.abortus from spleen	1:500
4			Control	540	"	679	"	Large abscess in testicles	Heavy growth of B.abortus from testicle, spleen and liver	1:200

The second group shown in table number II was autopsied at the end of sixty five days. They were fed three agar slants a day for eight days of culture B.54. The average gain for the vaccinated pigs was 29.5 grams and the average gain for the controls was 134.5 grams. None of the vaccinated pigs showed typical lesions and Bact. abortus was not cultured from any of them. Pig number 32 carried its head to one side and when autopsied an abscess was found in one ear. Pig number 33 showed a few small irregular shaped lesions in its liver. There was a wide variation in the agglutination reaction of the vaccinated pigs, ranging from 1:100 to 1:500.

Both of the controls showed typical lesions and Bact. abortus was cultured from both. Pig number 4 reacted only 1:200 to the agglutination test but heavy growths of Bact. abortus were obtained from the testicles spleen and liver. Bact. abortus was cultured from the spleen of number 3.

TABLE III, GROUP III

Pigs Exposed to Infection 92 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacterio-logic Results	Serologic findings B. abortus Positive in
1	415	-	9/29/24	634	12/30/24	626	2/24/25	All organs normal	Few clear colonies cultured from spleen	1:500
3	370	-	"	579	"	649	"	All organs normal	No cultural findings	Neg.
5	395	-	"	548	"	574	"	Small abscess on testicle other organs normal	Clear colonies from testicle	1:500
6	400	-	"	576	"	604	"	One testicle smaller, other organs normal	No cultural findings	1:500
5			Control	476	"	583	"	Typical lesions in liver, spleen 4 x normal size	B. abortus from spleen and liver	1:500
6			Control	486	"	575	"	Typical foci in liver, spleen 2 x normal	B. abortus from spleen	1:500

Table III contains the data from the group of pigs autopsied the third month or at the end of 92 days. They were fed three agar slants per day of culture Number 44. The vaccinated pigs of this group made an average gain of 29.2 grams, and the controls made an average gain of 98 grams. One of the vaccinated pigs, number 5, showed a small abscess in one testicle. The organism cultured from this lesion was agglutinated by positive cow's serum only in a dilution of 1:25 while the organism in the controls was agglutinated in a dilution of 1:500 by the same serum. It was, therefore, not considered a Bact. abortus lesion. None of the other vaccinated pigs showed any lesions or was Bact. abortus cultured from any of them. The blood from pig number 3 was negative to the agglutination test. Both controls showed an enlarged spleen and typical foci in the liver. Bact. abortus was cultured from all of these organs.

TABLE IV, GROUP IV

Pigs Exposed to Infection 112 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacterio-logic Results	Serologic findings B. abortus Positive in
50	474	-	12/17/24	837	4/8/25	837	5/30/25	All organs normal	No cultural findings	1:25
51	397	-	"	744	"	686	"	Atypical foci in liver, other organs normal	B. abortus from spleen	1:500
52	449	-	"	812	"	790	"	Typical foci in liver, other organs normal	B. abortus from spleen	1:500
53	462	-	"	654	"	615	"	All organs normal	No cultural findings	1:50
11			Control	639	"	595	"	Small abscess in testicle	B. abortus cultured from spleen testicle and kidney	1:500
12			Control	513	"	490	"	Spleen 2 x normal	B. abortus from spleen and urine	1:200
72	427	-	1/14/25	Not exposed		799	"	All organs normal	No cultural findings	1:50
77	557	-	"	Not exposed		805	"	All organs normal	No cultural findings	1:50

The data in table IV were obtained from the pigs autopsied the fourth month or at the end of 112 days. They were fed one slant a day of culture CIII for six days. The vaccinated pigs of this group showed an average loss of 30.6 grams. The controls showed an average loss of 33.5 grams. It will be seen that Bact. abortus was cultured from two of the vaccinated pigs while only one of them showed typical Bact. abortus lesions. The other vaccinated pigs showed no lesions nor was Bact. abortus cultured from the organs. The antibody index of these two pigs was also low. Both controls showed lesions and Bact. abortus was cultured from the spleen, testicles, and kidney of number 11 and from the spleen and urinary bladder of number 12 in very large quantities.

It is difficult to explain why two of the vaccinated pigs showed no lesions, no cultural findings and a low agglutination titre while the other two gave a high agglutination titre, Bact. abortus was cultured from both and one showed lesions. The control from which large numbers of Bact. abortus organisms were cultured reacted only 1:200 to the agglutination test. It would seem that pig number 12 had become a carrier of Bact. abortus with the infection in the urinary bladder. This would correspond very closely to the typhoid carrier in the human.

Under these conditions it may be seen that the vaccinated pigs were not exposed to infection for a period of six days but were being constantly exposed for six weeks to enormous quantities of virulent Bact. abortus. It seemed that the other two vaccinated pigs were able to resist this infection since no lesions were found and Bact. abortus was not cultured. The agglutinins in both of these vaccinated pigs had practically disappeared but some degree of immunity remained. It is evident, therefore, that the presence of agglutinins is not an indication of immunity.

This was the first group in which vaccinated non-exposed pigs were used. This gives a check on the vaccinated pigs before they were exposed to virulent organisms. It also gives a check on the virulence of the organism being used as a vaccine. Neither lesions nor Bact. abortus organisms were present in these pigs. At the end of 145 days the agglutinins had dropped to 1:50. It should also be noted that these pigs were vaccinated 20 days after those exposed the fourth month and 106 days after those used during the other months.

TABLE V, GROUP V

Pigs Exposed to Infection 150 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic Findings B. abortus Positive in
4	365	-	9/29/24	890	2/26/25	901	4/25/25	All organs normal	No cultural findings	1:200
12	400	-	"	865	"	950	"	All organs normal	No cultural findings	1:200
13	360	-	"	669	"	527	"	All organs normal	No cultural findings	1:100
16	335	-	"	827	"	755	"	Head carried on one side. All organs normal	No cultural findings	1:500
7			Control	362	"	485	"	Typical foci on liver, abscess in one testicle	B. abortus cultured from spleen and testicle	1:500
8			Control	459	"	562	"	Typical foci in liver, spleen 2 x normal, large abscess in both testes	B. abortus cultured from urine and testes	1:500

Table V shows the results for the fifth month or at the end of 150 days. This group of pigs was fed one agar slant a day of culture C111 for six days. The vaccinated pigs of this group showed a n average loss of 29.5 grams between time of exposure and date of autopsy. The controls showed an average gain of 113 grams during this same period of time. The difference in the age of the pigs probably accounts for the difference in gain or loss. The vaccinated pigs had practically reached thier maximun weight before time of exposure, while the controls were about half grown. They would, therefore, increase in weight much more rapidly. There were no lesions present in the vaccinated pigs and Bact. abortus was not cultured from any of them. The agglutination titre had dropped considerably in three of the vaccinated pigs. Both controls showed lesions in the testes and liver and number 8 showed enlarged spleen. Bact. abortus was cultured from the testes, spleen, and urinary bladder of these pigs.

TABLE VI, GROUP VI

Pigs Exposed to Infection 180 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacterio-logic Results	Serologic Findings B. abortus Positive in
7	400	-	9/29/24	985	3/28/25	1031	5/20/25	Typical foci in liver, other organs normal	No cultural findings	1:500
17	330	-	"	784	"	647	5/16/25	Typical foci in liver. Spleen 5x normal, other organs normal	No cultural findings	1:500
18	325	-	"	903	"	532	"	Lungs partially solidified. Head carried to one side, other organs normal	Organism producing slimy growth cultured	1:500
20	265	-	"	682	"	630	5/20/25	All organs normal	No cultural findings	1:500

TABLE VI, GROUP VI (continued)

Pigs Exposed to Infection 180 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 Wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic Findings B. abortus Positive in
9			Control	487	4/3/25	Died	4/25/25	Liver covered with fibrinous exudate, thoracic cavity full of fibrinous exudate spleen enlarged	Slimy organism cultured from all parts	
10			Control	501	"	582	5/20/25	Typical foci in liver, spleen 2x normal, abscess in both testes	B. abortus cultured from kidney and testes	1:500
60	467	-	1/14/25	Not	exposed	890	"	Spleen twice normal, small hemorrhages on kidney	B. abortus like organism cultured	1:500
61	468	-	"	Not	exposed.	970	"	All organs normal	No cultural findings	1:50

Table VI shows the results obtained for the sixth month or at the end of 180 days. These pigs were fed one agar slant a day of culture C111 for six days. The vaccinated pigs showed an average loss of 78.5 grams between time of exposure to virulent culture and autopsy. The controls made an average gain of 81 grams. Bact. abortus was not cultured from any of the vaccinated pigs however, one of them, Number 17, showed typical pearly foci in the liver and an enlarged spleen. All of these pigs have a high agglutination titre. An organism producing a slimy growth was cultured from the lungs of Number 18. The lungs were partially solidified and adherent to the body wall. It also carried an infection in its ear.

Control Number 9 died about a month before this group was to be autopsied. Death was due to an infection with an organism previously described. The growth of the organism on the plates was so heavy that Bact. abortus could not be detected. Control Number 10 showed lesions in the liver and both testes, also an enlarged spleen. Bact. abortus was cultured from these organs.

One of the vaccinated non-exposed pigs of this group showed no lesions or cultural findings and gave an agglutination titre of 1:50. The other pig showed lesion but an organism resembling Bact. abortus in some respects was cultured from the spleen. When agglutination tests were made on this organism it did not agglutinate. The blood of this pig reacted 1:500 to the agglutination test.

TABLE VII, GROUP VII

Pigs Were Exposed to Infection 211 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic Findings B. abortus Positive in
9	390	-	9/29/24	662	4/28/25	606	6/27/25	Typical foci in liver, spleen 2 x normal	B. abortus like organism cultured from urine	1:500
10	350	-	"	835	"	852	6/22/25	Typical foci in liver, spleen 2 x normal	B. abortus like organism cultured from lung	1:500
24	420	-	"	854	"	912	6/27/25	All organs normal	No cultural findings	1:50
25	440	-	"	807	"	637	6/22/25	Typical foci in liver	B. abortus cultured from spleen	1:100

TABLE VII, GROUP VII (continued)

Pigs Exposed to Infection 211 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic Findings B. abortus Positive in
13			Control	470	4/28/25	499	6/22/25	Typical foci in liver, spleen ex normal	Heavy growth of B. abortus from testes and urine also spleen	1:500
14			Control	479	"	544	"	Typical foci in liver, spleen 5 x normal	B. abortus cultured from lungs and spleen	1:500
62	493	-	1/14/25	Not exposed		820	6/27/25	All organs normal	No cultural findings	1:200
68	467	-	"	Not exposed		637	"	Atypical foci in liver	No cultural findings	1:50

Table VII shows the results for the seventh month or 211 days. This group was fed one agar slant a day of culture C111 for six days. The vaccinated pigs showed an average loss of 38 grams from time of exposure to virulent culture to date of autopsy. The controls made an average gain of 47 grams. Three of the vaccinated pigs showed typical foci in the liver and two of these showed enlarged spleens. An organism resembling Bact. abortus was cultured from the urine of Number 9 and from the lungs of Number 10, but it would not agglutinate with positive Cow's serum. Bact. abortus was cultured from the spleen of Number 25. The blood of Number 25 reacted only 1:100 while the blood of Number 8 and 10 reacted 1:500. This cannot be accounted for unless Number 25 had just recently become infected from the controls. The other vaccinated pig Number 24, showed no lesions nor was Bact. abortus cultured from it. Both controls showed typical foci in the liver and very large spleens. Bact. abortus was cultured from the spleen of both pigs and in large numbers from the testes and urine of Number 13. Bact abortus was cultured from the lungs of Number 14. This was uncommon in this experiment. Both vaccinated controls were free from lesions and the presence of Bact. abortus. The agglutinations titre of Number 62 was 1:50, thus showing that at the end of six months the agglutinins had become greatly reduced.

TABLE VIII, GROUP VIII

Pigs Exposed to Infection 243 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacterio-logic Results	Serologic Findings B. abortus Positive in
29	390	-	9/29/24	752	5/30/25	689	7/28/25	All organs normal	No cultural findings	Neg.
30	380	-	"	859	"	860	"	All organs normal	No cultural findings	1:500
36	395	-	"	772	"	567	7/17/25	All organs normal	B. abortus cultured from spleen kidneys testes and liver	1:500
37	430	-	"	1060	"	1039	7/28/25	Typical foci in liver, spleen 3 x normal	B. abortus cultured from spleen and testes	1:200

TABLE VIII, GROUP VIII (continued)

Pigs Exposed to Infection 243 Days After Immunization

o. ig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacterio-logic Results	Serologic Findings B. abortus Positive
15			Control	589	5/30/25	614	7/28/25	Typical foci in liver, spleen 2 x normal	B. abortus cultured from lungs and spleen	1:500
16			Control	559	"	410	"	Atypical foci in liver, testicle atrophied, lungs nearly collapsed		1:25
74	583	-	1/14/25	Not exposed		779	8/3/25	All organs normal	No cultural findings	-
78	567	-	"	Not exposed		692	"	All organs normal	No cultural findings	-

Table VIII shows the results obtained for the eighth month or 243 days. These pigs were fed one agar slant a day for six weeks. The vaccinated pigs showed an average loss of 67 grams and the controls showed an average loss of 62 grams between the time of exposure to virulent culture and time of autopsy. Only one of the vaccinated pigs of this group, Number 37, showed the presence of lesions, however, Bact. abortus was cultured from two, Numbers 36 and 37. Number 36 showed signs of heavy infection since the organism was cultured from the spleen, kidneys, testes, and liver. The absence of characteristic lesions cannot be accounted for. Bact. abortus was cultured from the spleen and testes of Number 37. The other two vaccinated pigs showed no lesions or cultural findings. Number 29 was negative to the agglutination test. It seems evident that this pig either failed to pick up enough organisms to cause an increase in the agglutinins or the agglutinins had entirely disappeared and did not reappear when the pig was exposed to virulent culture.

Control Number 15 showed typical lesions in the liver and spleen. Bact. abortus was cultured from the lungs and spleen. Control Number 16 did not show any characteristic Bact. abortus lesions. The liver showed a few typical lesions, the spleen was normal, the lungs were practically solidified and adherent to the thoracic wall, the testes were atrophied and the pig was emaciated generally. A slimy organism was cultured from all organs. Such a heavy growth was

produced that no other organism could be seen on the plates. The blood of this pig reacted 1:25 to the agglutination test. It would seem from this that Bact. abortus was not present.

Both vaccinated controls were negative to Bact. abortus culturally and anatomically. They reacted negatively to the agglutination test, thus showing that agglutinins will last about seven months after a single injection of living organisms.

TABLE IX, GROUP IX

Pigs Exposed to Infection 271 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of autopsy	Anatomic Lesions	Bacteriologic Results	Serologic Findings B. abortus Positive in
39	335	-	9/29/24	830	6/27/25	765	8/18/25	Typical foci in liver and spleen, spleen 2x normal	No cultural findings	1:500
40	350	-	"	884	"	807	"	All organs normal	No cultural findings	1:500
45	440	-	"	865	"	785	"	Typical foci in spleen, spleen 5 x normal testes atrophied	B. abortus cultured from liver	1:500
41	340	-	"	742	"	672	"	All organs normal	No cultural findings	1:500

TABLE IX GROUP IX (continued)

Pigs Exposed To Infection 271 Days After Immunization

No. Pigs	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos.	Date of Autopsy	Anatomic Lesions	Bacteriologic Results	Serologic Findings B. abortus Positive in
17			Control	545	6/27/25	510	8/18/25	Typical foci in spleen. Spleen 3 x normal abscess in both testacles	B.abortus cultured from spleen and testes	1;500
18			Control	637	"	563	"	Typical foci in liver. Spleen 5 x normal abscess in one testacle	B.abortus cultured from testes, liver and spleen	1:500
70	481	-	1/14/25	not exposed		571	"	All organs normal	No cultural findings	-
66	451	-	"	not exposed		642	"	Only one kidney old abscess in testicle. Seminal vesicles dark and watery	Yellow colonies from urine	-

Table IX shows the data obtained the ninth month or 271 days. This group was fed one agar slant a day for six days of culture C111. The vaccinated pigs showed an average loss of 73 grams between the time of exposure and autopsy. The controls showed an average loss of 54.5 grams. Two vaccinated pigs of this group showed typical Bact. abortus lesions. Both had enlarged spleens which were covered with white foci. The testes of Number 45 were completely atrophied. Bact. abortus was cultured from the liver of Number 45 butnot from Number 39. The other two vaccinated pigs were free from lesions and nothing was cultured from them. It is interesting to note that all of these pigs reacted 1:500 to the agglutination test while in most of the foregoing tests there were some which reacted only in low dilutions.

Both controls were heavily infected. The spleen of Number 17 was about five times normal size and showed typical foci. There were abscesses in both testes. Bact. abortus was cultured from testes and spleen. Number 18 showed typical foci in the liver, an enlarged spleen and an abscess in one testicle. Bact. abortus was cultured from the liver, testicle and spleen.

One of the vaccinated non-exposed pigs, Number 70 showed no lesions, no cultural findings and was negative to the agglutination test. The other pig, Number 66, had only one kidney. There was an old abscess in one testicle and the seminal vesicles were dark and watery. Staphylococcus aureus was cultured from the urine. The blood reacted negatively to the agglutination test.

TABLE X GROUP X

Pigs Exposed to Infection 303 Days After Immunization

No. Pig	Wt. before Vacc.	Agg. before Vacc.	Date of Vacc.	Wt. before Expos.	Date of 1st Expos.	Wt. 6 wks. after Expos	Date of autopsy	Anatomic Lesions	Bacteriologic results	Serologic findings B. abortus Positive
34	395	-	9/29/24	945	7/29/25	895	9/18/25	Spleen 2 x normal, atypical foci in liver lungs grown to body wall	No cultural findings	1:200
42	335	-	"	782	"	652	"	All organs normal	B. abortus cultured from kidney	1:500
43	365	-	"	830	"	762	"	Atypical foci in liver, abscess in testicles	No cultural findings	1:50
19			Control	360	"	422	"	Spleen 3 x normal, abscess in both testes	B. Abortus cultured from urine spleen, lungs, testes and kidneys	1:500
64	476	-	1/14/25	Not exposed		892	"	All organs normal	No cultural findings	-
69	414	-	"	Not exposed		922	"	All organs normal	No cultural findings	-

Table X shows the results for the tenth month or 306 days. There were only three pigs left to use in this group. They were fed one agar slant daily for six days of culture C111. The vaccinated pigs made an average loss of 46.5 grams between time of exposure and autopsy. The one control made a gain of 62 grams. This group of vaccinated pigs gave some very interesting results. The spleen of Number 34 was twice normal size, there were atypical foci in the liver and one lobe of the lungs was adherent to the body wall. No organisms were cultured from this pig and the agglutination reaction was 1:200. Number 43 presented a similar result with atypical foci in the liver and an abscess in one testicle. Nothing was cultured from this pig and the agglutination reaction was 1:50. In contrast to these two pigs Number 42 showed no lesions but Bact. abortus was cultured from the kidneys. The blood reaction was 1:500. The spleen of the control was three times normal size and there were abscesses in both testes. Bact. abortus was cultured from the spleen, lungs, testes, kidney, and urine. Both vaccinated non-exposed pigs were negative culturally, anatomically and serologically.

GENERAL DISCUSSION

This experiment was undertaken to determine how long immunity to Bact. abortus infection would last in guinea-pigs which had been vaccinated with a non-virulent strain. The monthly records give the results obtained. When the conditions under which this experiment has been run are noted it will be seen that the protection produced by vaccination with this organism was subjected to a more severe test than would take place under natural conditions. As will be pointed out later, in several cases the pigs were subjected to virulent organisms for a period of six weeks. If this experiment were run on cattle instead of guinea-pigs it would be comparable to placing immunized cattle in a box stall for six weeks with cattle which were eliminating virulent organisms constantly. This would be a condition seldom, if ever, approached in the field. When the small number of organisms required to cause infection in a guinea-pig are considered, the severity of this test is made more emphatic. According to Hagan (12) less than 100 organisms are sufficient to cause infection, dependent somewhat upon the resistance of the animal.

It is somewhat difficult to make a definite statement as to the length of time this immunity will last, but it seems safe to state that protection is evident at the end

of six months. After this time there seems to be a gradual lowering of resistance to infection. Some protection, however, is evident at the end of ten months, for as is shown in table X one pig out of three vaccinated pigs showed infection. When the individual resistance of the pigs is considered it would be natural to expect that some pigs would show a higher resistance than others. Topley, Wilson and Lewis (9) in their work on mice showed that there was a wide variation in animals. Out of 185 mice fed cultures of B. aertrycke 92 died, 61 showed presence of organisms in the feces and 32 showed no symptoms. When these 32 were injected intraperitoneally along with 30 controls they showed a mortality of only 12.5 per cent while the controls showed a death rate of 76.6 per cent. The remaining 25 were injected along with 25 controls and in 11 days all controls had died and only 3 of the immunized pigs had died. In 68 days 44 per cent of the 25 had died. So it is not unreasonable to suspect that some animals would fall short of a definite length of time and others would exceed this length of time.

There is another factor which makes it difficult to say whether an animal has become infected and therefore has lost its immunity or power of resistance. The only means of telling whether an animal is infected or not are the lesions produced and the cultivation of the organism. Even these cannot always be given as positive proof. Other organisms produce lesions which resemble those of Bact. abortus

very closly and there is some chance of misinterpreting these results. It seems possible that lesions may be produced when the animal is first exposed but later the animal's resistance is able to overcome the infection and the organisms are thrown off or destroyed. Smillie (10) has shown that the number of living bacteria in the spleen of the guinea-pig which he had infested with Bact. abortus was larger at three to four weeks than later although the microscopic lesions tended to become more pronounced as the bacteria declined.

On the other hand there are guinea-pigs which show no lesions but Bact. abortus can be cultured from their internal organs. It can be seen from their data that the controls were constantly eliminating large numbers of organisms in the urine. Most of the time the drinking water and feed contained considerable amounts of feces and litter, not-with-standing the fact that the containers were emptied, cleaned and refilled daily. If the food and water contained living organisms they would be taken into the system of the animals and pass to the various organs. If these organs were semared on media when containing these organisms, growth might be obtained.

Besides giving some light on the topic for which this experiment was planned other interesting results can be pointed out from the data obtained. The question of the relation between the presence or absence of agglutinins and immunity is one which has caused much discussion. Most

immunologists are agreed that agglutinins are not an indication of the presence or absence of immunity while some still speak of them as such. Topley, Wilson, and Lewis (9) have shown by experimenting with mice that the presence of agglutinins is no measure of resistance. On the other hand, in cases where agglutinins were present an increasing resistance was associated with an increasing proportion of positively reacting mice as judged by agglutination tests.

Fleishner, Meyer and Shaw (11) in their work on cutaneous hypersensitiveness claim that a high degree of immunity was established in all of their animals as was evidenced by the constantly positive agglutination reaction. These data do not bear out this statement. As was shown by the non-exposed vaccinated pigs the agglutination reaction of most of these pigs was below 1:50 or negative before they were exposed to virulent culture. Yet there was immunity present as was shown by the absence of lesions and cultural findings. After they were exposed there was a rise in the agglutination titre. The variation on the agglutination titre cannot be accounted for. This varied from negative in one case to 1:500 in most of the pigs. No higher dilutions than this were run. With the exception of one guinea-pig all the controls reacted 1:200 or more to the agglutination test.

It is not shown by these data how soon the agglutination began to disappear in the vaccinated pigs, as no pigs which had been exposed were killed under four months.

At the end of four months agglutination reactions were 1:50. At the end of seven months the agglutinins had entirely disappeared. Hagan states that a fall in the agglutinin titre begins about the tenth week of infection.

Other interesting points presented in these data are the distribution of lesions and infections as determined by cultural findings. Meyer (13) found that the enlargment of the spleen was most striking and constant in guinea-pigs injected with body fluids or tissues infected with bovine Bact. abortus. Smillie (10) also states that the spleen was the organ in which the bacteria were regularily present in large numbers. The data in this paper conform very closly to these statements as is shown in table XI and XII.

TABLE XI

Numbers of Immunized Guinea-pigs Showing Lesions and Cultural Findings

No. of Days	No. of Pigs	B. abortus Lesions in liver	spleen	lungs	kidney	testes	urinary bladder	gall bladder
35	4							
65	4							
92	4					2		
112	4	1						
150	4							
180	4	2	1					
211	4	3	2					
243	4	1	1					
271	4		2			1		
303	3	2						
Total	39	9	5			3		

		B. abortus cultured from						
35	4							
65	4							
92	4							
112	4		2					
150	4							
180	4							
211	4		1					
243	4	1	2		1	2		
271	4	1						
303	3				1			
Total	39	2	5		2	2		

TABLE XII

Number of Control Guinea-pigs Showing Lesions and Cultural Findings

No. of Days	No. of Pigs	B. abortus Lesions in liver	spleen	lungs	kidney	testes	urinary bladder	gall bladder
35	2	2	1					
65	2	1	1			1		
92	2	2	2					
112	2		1			1		
150	2	2	1			2		
180	2	1	2			1		
211	2	2	2					
243	2	2	1			1		
271	2	1	2			2		
303	1		1			1		
Total	19	13	14			9		

		B. abortus cultured from						
35	2							
65	2	1	2			1		
92	2	1	2					
112	2		2		1	1	1	
150	2		1			1	1	
180	2				1	1		
211	2		2	1		1	1	
243	2		1	1		1		
271	2	1	2			2		
303	1		1	1	1	1	1	
Total	19	3	13	3	3	9	4	

In the vaccinated pigs the liver showed the most changes, but Bact abortus was cultured from the spleen in a great number of cases. In the controls the spleen was more constant in showing lesions and the presence of Bact. abortus. The liver was second in number of lesions but the testes yielded a greater number of cultural findings. The liver, spleen and testes were the only organs showing lesions, yet Bact. abortus was cultured from practically all the organs except the gall bladder. Meyer has reported the culturing of B. melitensis from the gall baldder in large quantities but no reports show a record of Bact. abortus being cultured from this organ.

Some experimenters report difficulty in infecting guinea-pigs orally with Bact. abortus. Hagan (12) claims that the susceptibility of guinea-pigs to infection through ingestion of Bact. abortus is relatively slight. Schroeder and Cotton (14) were not able to infect guinea-pigs at all, with bovine strains of abortus, when given orally to them. They obtained 58 per cent infection with a swine strain of Bact. abortus. This experiment does not confirm their resutls. Of eighteen control pigs which were fed bovine strains of Bact. abortus all showed lesions and the organism was recofered from them. Only in one case was Bact. abortus not recovered and this one showed typical lesions. This gives a rate of 94.7 per cent infection. Very little emphasis can be placed on the comparison of weights in this emperiment. The controls used were much younger than

the vaccinated pigs, therefore, they made larger gains after being exposed to virulent cultures. The average initial weight of the vaccinated pigs was 367 grams. The average weight of the control pigs was 415.4 grams. The average weight of the vaccinated pigs before exposure was 720.17 grams. The average weight of the vaccinated pigs after exposure was 697 grams. The average loss in weight of the vaccinated pigs was 23.17 grams. The average weight of the controls after exposure was 455.6 grams.

SUMMARY

Guinea-pigs vaccinated with a non-virulent strain of Bact. abortus are protected against infection for a period of six months.

Agglutinins are not an indication of immunity in guinea-pigs.

Guinea-pigs can be easily infected by feeding virulent Bact. abortus organisms on their food.

Bact. abortus infections are most common in the spleen of guinea-pigs.

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