A COMPARATIVE AND QUANTITATIVE STUDY OF THE VIRULENCE OF SEVERAL CULTURES OF *BRUCELLA ABORTUS* FOR THE GUINEA PIG AND COW

by

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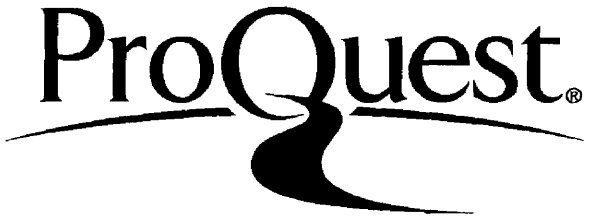

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In the study of an infectious disease it is always imperative that one know the minimum number of the causative microorganisms that is required to establish infection in the species of animal under study. Generally it is recognized that immunity to bacterial invasion is a relative state and may be overwhelmed by exposure to large numbers of microorganisms.

It is not surprising that more work has not been done to determine the minimum infective dose of *Brucella* for cattle as such studies are very costly. Apart from the economic consideration there are at least two other explanations for the lack of studies of this nature. One is that cattle frequently respond irregularly to either natural or experimental infection especially when the criteria of infection are the demonstration of *Brucella* in the fetus, fetal membranes or colostrum milk. This irregularity in response has consequently suggested that the size of the infective dose is of little, if any importance, especially when the animals are exposed *via* natural routes. The second explanation is that most workers have expected or hoped for the development of a more solid immunity in cattle than has thus far been demonstrated. Consequently a very severe challenging dose has been administered in most experimental attempts in immunization.

*This study is part of the cooperative project between the Bureau of Animal Industry, U. S. Department of Agriculture and the Michigan Agricultural Experiment Station.

Hagan (1 and 2) has stated that the infectivity of Br. abortus for guinea pigs was extremely great. It was computed that somewhat less than 100 organisms were required to infect most guinea pigs. Occasionally he encountered guinea pigs that had a greater resistance to infection than the average. The most resistant guinea pig encountered was infected by a number of organisms computed to be less than 10,000. Hagan also noted that the principal effect on the character of the disease, caused by varying the size of the infecting dose, was a change in the time relations. Very small doses produced an infection of a slower course than larger doses, but the eventual results were practically the same.

McEwen (6) in an extensive and carefully conducted experiment has estimated the suitable infective dose of Br. abortus for cattle to be 1460 x 10^4 organisms when deposited in the conjunctival sac. He exposed five groups of ten pregnant heifers each to different doses of Br. abortus by way of the conjunctiva. Group 1 received 1,460,000,000 bacteria; group 2, 14,600,000; group 3, 1,460,000; group 4, 146,000; and group 5, 1,460. In groups 1 and 2, nine heifers became infected; in groups 3, 4 and 5, seven, five and two heifers, respectively, became infected. According to these results the minimum infective dose of this strain for the average pregnant heifer was approximately 14,000,000 organisms.

It has long been known that pathogenic bacteria often undergo changes in virulence when cultivated on artifical media for long periods of time, and that in most cases their virulence can be enhanced by passage through suitable experimental animals. However, little concrete evidence can be found in the literature to substantiate the belief that the virulence of Brucella can be enhanced by passage through animals.

McEwen (4) made serial passages in guinea pigs of a strain of Br. abortus of low virulence. He measured the virulence for mice of the sixth, twentieth, thirtieth and fortieth passage and found that serial passages through guinea pigs resulted in an increased virulence. These same passages when inoculated into guinea pigs and pregnant cattle also showed an increase in virulence.

Hagan (1 and 2) observed that the virulence of Br. abortus for guinea pigs could be raised or lowered by appropriate passage through these animals.

Metzger and Stokes (5) have obtained some interesting results from long continued cultivation of Br. abortus in the live chick embryo. Two strains of Br. abortus were used; one virulent, the other of low virulence. The original virulent strain produced lesions and a high agglutination titer in guinea pigs and required carbon dioxide for growth on artificial media. At the thirty-ninth passage this virulent strain acquired the ability to grow artificially without carbon dioxide. A comparison of the forty-eighth and fiftieth passages showed that this strain had lost some of its virulence and ability to stimulate agglutinin production in the guinea pig. They state that the virulent strain remained smooth throughout the serial passages in chick embryos.

In view of the incompleteness of our knowledge pertaining to the minimum number of Br. abortus to produce infection, this study constitutes an effort to throw additional light on minimum infecting dosage, changes in virulence under artificial cultivation and by repeated guinea pig passage. In addition a less extensive effort has been made to determine the suitable infective dose of Br. abortus for pregnant heifers by way of the conjunctiva.

EXPERIMENTAL PROCEDURES AND RESULTS

Sources and Characteristics of Cultures

B. A. I. strain 19 - aerobic Br. abortus isolated from vaccine made by the U. S. Bureau of Animal Industry.

295 - aerobic, Br. abortus isolated from cow's milk in Michigan 5/31/30.

979 - aerobic, Br. abortus isolated from human blood in Iowa 9/3/30.

1099T2 - aerobic, Br. abortus isolated from the fistula of a horse in Minnesota in 1930. This culture grows slightly on thionin dye plates.

1257 - aerobic, Br. abortus isolated from milk of an infected cow in Michigan 1/13/39.

1265 - Br. abortus isolated from lung of a bovine fetus in Illinois 3/9/40. This culture requires an increased CO_2 tension for growth.

1271 - Br. abortus isolated from human blood in Michigan 5/27/40. This culture requires an increased CO_2 tension for growth.

1280 - Br. abortus isolated from milk of an infected cow in Michigan 10/4/40. This culture requires an increased CO_2 tension for growth.

1282 - Br. abortus isolated from milk of an infected cow in Michigan 12/12/40. This culture requires an increased CO_2 tension for growth.

1284 - Br. abortus isolated from milk of an infected cow in Michigan 7/29/41. This culture requires an increased CO_2 tension for growth.

1285 - Br. abortus isolated from an aborted fetus in Michigan 2/18/41. This culture requires an increased CO_2 tension for growth.

1287 - Br. abortus isolated from an aborted fetus in Michigan 3/18/41. This culture requires an increased CO_2 tension for growth.

1677 - Br. suis isolated from human blood 5/3/40.

Method of Determining Numbers of Live Brucella

Considerable preliminary work was done to establish a method for determining the number of live organisms in a given suspension used in inoculating each group of guinea pigs. It was necessary to find a suitable suspending fluid, a method of standardizing the number of organisms in the suspension, and the proper dilutions of the standard suspensions.

The following four suspending fluids were studied:

Non-buffered physiological salt solution pH 6.4

Buffered physiological salt solution pH 6.8

1 per cent Tryptose, 0.5 per cent salt pH 6.9

0.1 per cent Tryptose, 0.5 per cent salt pH 6.9

The buffered salt solution was made by diluting a stock phosphate buffer of pH about 7.0 made according to the method of Clark and Lubs (7) to 1:6 with physiological salt solution.

The cultures used were suspended in these solutions and standardized by means of the Cenco photelometer using the #2 green filter to a galvonometer reading of 68.5. This density corresponds to tube 1 of the McFarland nephelometer. All suspensions, except the 1 per cent Tryptose broth were standardized to this density. The 1 per cent Tryptose broth had a definite yellow color and gave a galvanometer reading of 94 on the

photelometer as compared with 100 for the other suspending fluids. This necessitated a correction of 6 units on the galvanometer scale for this particular suspending fluid.

The standardized suspensions were diluted in their respective diluents to 1:1,000,000, 1:5,000,000 and 1:50,000,000. Different amounts of the final dilutions were placed in sterile Petri dishes in triplicate and Tryptose agar poured over them. Incubation was carried out for five days at 37°C. under aerobic conditions and in an atmosphere of increased CO_2 depending on the culture used. Counts were made of all plates.

After numerous trials it was found that suspensions made in 0.1 per cent Tryptose broth and diluted to 1:5,000,000 and 1:50,000,000 gave nearly repeatable plate counts. One cc. of 1:5,000,000 dilution of the original suspensions, one-half cc. of 1:5,000,000 and one-half cc. of 1:50,000,000 gave consistantly uniform plate counts of about 200, 100 and 10 colonies respectively.

Table 1 shows the results of suspending cultures 1265 and 1271 in both buffered and non-buffered physiological salt solutions and standardizing these suspensions to tube 1 McFarland nephelometer by the eye. Immediate dilution to 1:1,000,000 and plating in various amounts are shown as well as dilution to 1:1,000,000 after standing 24 hours at room temperature. It can be readily seen that a decrease in number of organisms occurs during the 24 hours in either buffered or non-buffered salt solutions. This would indicate that such solutions are unsuitable for suspending Brucella when live organisms are to be estimated or maintained.

The results of suspending cultures 1257 and 1271 in buffered salt solution and standardization of the suspension to 68.5 on the Cenco photelo-

TABLE I

Plate Counts of Cultures 1265 and 1271

Culture 1271

Plate No.	Buffered Salt Solution Immediate Plating			Non-Buffered Salt Solution Immediate Plating			Buffered Salt Solution 24 Hour Plating		Non-Buffered Salt Solution 24 Hour Plating	
	1/4c.c.	1/2c.c.	1c.c.	1/4c.c.	1/2c.c.	1c.c.	1/2c.c.	1c.c.	1/2c.c.	1c.c.
1	389	606	1089	447	752	1339	416	819	3	7
2	393	730	1289	479	685	1104	425	609	10	12
3	373	689	1298	419	736	1092				

Culture 1265

Plate No.	Buffered Salt Solution Immediate Plating			Non-Buffered Salt Solution Immediate Plating			Buffered Salt Solution 24 Hour Plating		Non-Buffered Salt Solution 24 Hour Plating	
	1/4c.c.	1/2c.c.	1c.c.	1/4c.c.	1/2c.c.	1c.c.	1/2c.c.	1c.c.	1/2c.c.	1c.c.
1	644	676	1219	298	688	1552	374	536	1	cont.*
2	cont.*	1168	1355	438	768	1358	381	546	0	10
3	501	844	1279	342	777	1365				

*Plate was contaminated.

meter are set forth in Table 2. Plate counts were made on various amounts of 1:1,000,000 dilution of original suspension. The counts obtained are relatively uniform and considerably lower than similar counts in Table I when the suspended cultures were standardized by eye to tube 1 of the McFarland nephelometer.

The results of suspending culture 1257 in both buffered salt and 1 per cent Tryptose solution are tabulated in Table 3. These suspensions were standardized by eye to tube 1 McFarland and then diluted to 1:5,000,000 in their respective diluents. One-half and 1 c.c. amounts of each suspension were plated at intervals of 1, 6, 24 and 48 hours. The most striking feature of the results was the discrepancy in plate counts between the two suspensions made by eye in different diluting fluids, thus indicating that standardization by eye was not a desirable method. It was noted also that a sharp decrease in plate counts occurred at the 24 and 48 hour intervals in the buffered salt solution.

Table 4 shows the results of holding the 1:5,000,000 dilutions, made as described in Table 3, at room temperature for 24 hours and replating at the end of this time. Obviously the buffered salt solution exerted a marked bactericidal action on the organisms. The decrease in the number of organisms was much greater in the dilute suspension than in the original suspension. There was a slight increase in the number of organisms in the 1 per cent Tryptose solution.

Results similar to Table 3 appear in Table 5 except that cultures 295 and 979 were suspended in physiological salt solution rather than buffered physiological salt solution.

Table 6 is included to show the close agreement obtainable by standardizing suspensions of a culture in different suspending fluids,

TABLE 2

Plate Counts of Cultures 1257 and 1271

Culture 1257

Plate No.	Buffered Salt Solution Immediate Plating		
	1/4c.c	1/2c.c.	1c.c.
1	225	365	854
2	263	434	828
3	231	381	790

Culture 1271

Plate No.	Buffered Salt Solution Immediate Plating		
	1/4c.c.	1/2c.c.	1c.c.
1	263	539	739
2	257	450	835
3	234	425	800

TABLE 3

Effect of Buffered Salt Solution and One Per Cent Tryptose Solution on Culture 1257

Buffered Salt Solution

Plate No.	1/2 c.c.				1 c.c.			
	1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
1	70	121	73	44	203	213	159	95
2	70	98	89	54	168	cont*	159	114
3	98	105	73	57	222	190	178	114

One Per Cent Tryptose Solution

Plate No.	1/2 c.c.				1 c.c.			
	1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
1	206	193	244	206	390	422	333	396
2	184	200	238	219	371	371	397	365
3	238	209	203	215	371	441	489	419

*Plate was contaminated.

TABLE 4

Effect of Buffered Salt Solution and Tryptose on Culture 1257 in Low Dilution

Plate No.	1 c. c.			
	1a*	6a*	24a*	48a*
1	1	0	0	3
2	0	2	0	1
3	0	1	0	2

Plate No.	1 c. c.			
	1a*	6a*	24a*	48a*
1	Too Numerous	Too Numerous	Too Numerous	Too Numerous
2	"	"	"	"
3	"	"	"	"

*Diluted suspensions of Table 3 held 24 hours at room temperature before re-plating.

TABLE 5

Effect of Physiological Salt Solution and One Per Cent Tryptose Solution on Cultures 295 and 979

Culture 295 Non-buffered Salt Solution

Plate No.	1/2 c.c.				1 c.c.			
	1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
1	73	67	cont.	38	129	129	140	84
2	77	63	78	54	140	127	127	98
3	83	66	70	47	134	142	138	98

Culture 295 One Per Cent Tryptose Solution

Plate No.	1/2 c.c.				1 c.c.			
	1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
1	97	78	61	85	193	173	183	179
2	106	95	83	103	207	163	151	182
3	92	77	100	90	175	cont.	153	166

Culture 979 Non-buffered Salt Solution

Plate No.	1/2 c.c.				1 c.c.			
	1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
1	86	83	62	46	179	170	118	83
2	112	77	67	38	166	142	cont.	81
3	79	90	cont.	47	147	141	127	92

Culture 979 One Per Cent Tryptose Solution

Plate No.	1/2 c.c.				1 c.c.			
	1 hr.	6 hr.	24 hr.	48 hr.	1 hr.	6 hr.	24 hr.	48 hr.
1	97	84	cont.	93	204	195	182	212
2	104	87	94	115	206	196	191	208
3	107	94	107	113	192	194	180	177

TABLE 6

Comparison of Standardizing Different Suspensions on Photelometer

Plate No.	Immediate Plating Buffered Salt Solution	
	1/2 c.c.	1 c.c.
1	125	218
2	112	212
3	134	237

Plate No.	One per cent Tryptose Solution Immediate Plating	
	1/2 c.c.	1 c.c.
1	110	222
2	112	206
3	138	240

i.e. buffered salt solution and 1 per cent Tryptose solution. These suspensions were standardized to 68.5 and 62.5 respectively on the Cenco photelometer. One-half and 1 c.c. amounts of 1:5,000,000 dilution of the original suspensions were plated.

The 1 per cent Tryptose solution proved to be superior to the other suspending fluid, but it had the disadvantage of foaming upon shaking and also necessitated a correction on the photelometer reading to obtain the desired turbidity reading. It was decided to use 0.1 per cent Tryptose plus 0.5 per cent salt solution to eliminate both the color and undesirable foaming. In Table 7 are set forth the results of plate counts made from the three suspending and diluting fluids, i.e., 0.1 per cent Tryptose, buffered salt, and non-buffered salt solution. Culture 1257 was suspended in the 3 solutions and standardized to 68.5 on the Cenco photelometer. The 3 suspensions were diluted to 1:5,000,000 in their respective diluents at intervals of 1, 6, 24 and 48 hours. One c.c. amounts of the 1:5,000,000 dilutions were plated in triplicate. It was noted that the 0.1 per cent Tryptose solution suspension gave relatively constant plate counts over the entire 48 hour period while both the buffered and non-buffered salt solution suspensions gave decreasing plate counts. Each of the 1:5,000,000 dilutions made at intervals of 1, 6, 24 and 48 hours was held for 24 hours and replated in 1 c.c. amounts. Here again it was noted that the buffered and non-buffered salt solutions exerted a marked bactericidal action on the organisms. There was a slight multiplication of the organisms in the 0.1 per cent Tryptose solution.

The results of suspending cultures 1287 and 1284 in 0.1 per cent Tryptose and standardizing the suspensions to 68.5 on the photelo-

TABLE 7

Comparison of Effects of 0.1 Per Cent Tryptose Solution, Buffered Salt Solution and Non-Buffered Salt Solution on Culture 1257

1257 in 0.1 Per Cent Tryptose pH 6.9

Plate No.	1 hr.	6 hr.	24 hr.	48 hr.	1a*	6a*	24a*	48a*
1	153	130	189	184	203	308	269	217
2	121	126	198	174	191	cont.	248	241
3	182	218	176	168	214	254	243	236

1257 in Buffered Salt Solution pH 6.8

Plate No.	1 hr.	6 hr.	24 hr.	48 hr.	1a*	6a*	24a*	48a*
1	146	152	163	81	4	10	16	35
2	108	153	181	95	10	cont.	17	34
3	132	171	99	cont.	8	0	7	23

1257 in Non-Buffered Salt Solution pH 6.4

Plate No.	1 hr.	6 hr.	24 hr.	48 hr.	1a*	6a*	24a*	48a*
1	134	147	100	52	17	4	0	0
2	135	112	93	69	41	cont.	0	2
3	117	170	122	80	55	2	0	2

*1:5 million dilution held 24 hours at room temperature and replated in 1 c.c. amounts

meter are set forth in Table 8. The suspensions were diluted to 1:5,000,000 and 1:50,000,000 and plated in triplicate as follows: 1 c.c. of 1:5,000,000 dilution of original suspension, 1/2 c.c. of 1:5,000,000 and 1/2 c.c. of 1:50,000,000 dilution. This procedure gave plate counts of about 200, 100 and 10 colonies respectively and was used in the guinea pig inoculation experiments as standard procedure.

In Vivo Virulence Studies

Most of the experiments carried out to determine virulence and increased virulence due to animal passage have been made by using large numbers of viable Brucella organisms. It was considered desirable to use very small numbers of organisms in this study to definitely determine the virulence of a culture as well as any increase in virulence due to animal passage. By using small numbers of organisms and grading the size of the injections it seemed probable that a minimum infecting dose could be established and enhancement of virulence by animal passage determined.

The minimum infecting dose was indicated by the smallest number of organisms that produced infection as demonstrated by the agglutination response and cultural findings. Any increase in virulence of a culture due to animal passage was measured by its ability to produce infection in doses smaller than the original culture or by production of more extensive gross lesions and a greater agglutination response in the same dosage as the original.

Eight cultures of Brucella were studied with respect to their virulence for guinea pigs and resultant increase in virulence due to

TABLE 8

Results of Standardizing Culture 1287 and 1284 on Cenco Photelometer Using 0.1 Per Cent Tryptose

Culture 1287

Plate No.	1 c.c. of 1:5M	1/2 c.c. of 1:5M	1/2 c.c. of 1:50M
1	202	114	8
2	209	106	10
3	224	103	15
4	212	104	11

Culture 1284

Plate No.	1 c.c. of 1:5M	1/2 c.c. of 1:5M	1/2 c.c. of 1:50M
1	194	96	10
2	181	101	9
3	197	83	10
4	cont.*	105	8

*Plate was contaminated with mold.

subsequent guinea pig passage.

Seven passages were made through guinea pigs with culture 1257, six with culture 1280, five with culture 1287, three with culture 1284, and one with cultures 1285, 1099T_2, and 1677. Culture 19 was used in eight different doses before infection could be established.

In all cases each dose of organisms was injected subcutaneously into three guinea pigs of approximately the same size. The various groups of guinea pigs were placed in separate cages isolated from all other groups. At the end of 30 days each group was sacrificed and autopsied. At autopsy heart blood was collected for the agglutination test, lesions were noted, and cultures were made of the spleen and liver on Tryptose agar plates containing crystal violet in a 1:1,000,000 dilution. All plates were incubated for five days at 37°C.

To identify the various passages of the cultures the original passage was given the culture number, the subsequent passages were numbered with the culture number followed by a, b, c, d, etc. The time elapsing between isolation of a culture and its subsequent reinjection into guinea pigs was seven days.

Tables 9 to 12 show the result of seven passages of culture 1257 through guinea pigs. Passages of 1257a, 1257d, and 1257e are not shown since these were used on another group of guinea pigs in relatively higher doses than was the practice in this work. However, the passages served their purpose in enhancing the virulence of the culture. It was noted that the original culture 1257 produced irregular infection in doses of 27 and 11 organisms, but that the 1257c passage produced infection to a high degree in doses of 31 and 11 organisms. The lesions observed in passages 1257b, 1257c, and 1257f were much more extensive

TABLE 9

Injection of Br. Abortus No.1257

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	188	Liver, lymph nodes, spleen x 1.5	1:400	Positive	Negative
2	188	Lymph nodes, spleen x 3	1:400	Positive	Positive
3	188	Lymph nodes, spleen x 1.5	1:100	Positive	Negative
4	57	Liver, lymph nodes, spleen x 4	1:200	Positive	Positive
5	57	Liver, lymph nodes, spleen x 1.5	1:400	Positive	Negative
6	57	Liver, lymph nodes, spleen x 1.5	1:400	Positive	Positive
7	27	None	Negative	Negative	Negative
8	27	None	I 1:100	Negative	Negative
9	27	Spleen slightly enlarged	1:100	Positive	Negative
10	11	None	I 1:50	Positive	Negative
11	11	None	Negative	Negative	Negative
12	11	None	I 1:200	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 10

Injection of Br. Abortus No.1257b

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	2³⁄₄	Liver, lymph nodes, spleen x 1.5	1:400	Positive	Negative
2	2³⁄₄	Liver, lymph nodes, spleen x 6	1:400	Positive	Positive
3	2³⁄₄	Lymph nodes, spleen x 1.5	1:200	Positive	Negative
4	56	Liver, lymph nodes, spleen x 7	1:400	Positive	Positive
5	56	Liver, spleen x 2	1:100	Positive	Negative
6	56	None	Negative	Negative	Negative

TABLE 11

Injection of Br. Abortus No. 1257c

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	31	Lymph nodes, spleen x 5	1:400	Positive	Positive
2	31	Liver, lymph nodes, spleen x5	1:400	Positive	Positive
3	31	Liver, lymph nodes, spleen x 4	1:400	Positive	Positive
4	11	None	Negative	Negative	Negative
5	11	Liver, spleen x 1.5	1:400	Positive	Positive
6	11	Liver, spleen x 4	1:400	Positive	Positive

TABLE 12

Injection of Br. Abortus No.1257F

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	199	Spleen x 4	1:200	Positive	Positive
2	199	Spleen x 2	1:100	Positive	Positive
3	199	Liver, lymph nodes, spleen x 8	1:400	Positive	Positive
4	115	Spleen x 4	1:100	Positive	Positive
5	115	Spleen x 2	1:200	Positive	Negative
6	115	Liver, spleen x 5	1:200	Positive	Positive
7	13	Liver, spleen x 2	1:50	Positive	Positive
8	13	None	Negative	Negative	Negative
9	13	Spleen x 1.5	I 1:100	Positive	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

than those observed with the original passage.

The results of five passages of culture 1287 through guinea pigs are shown in Tables 13 to 17. It was noted that this culture produced irregular infection as determined by cultural findings, agglutination response, and gross lesions. However, culture 1287b produced positive cultural findings in all doses, while cultures of 1287c and 1287d in doses comparable to those higher doses of 1287, 1287a and 1287b produced a consistently high agglutination response, and extensive lesions, as well as positive cultural findings. This indicated an enhancement of virulence for this culture after three passages.

The results of six passages of culture 1280 through guinea pigs in various doses are set forth in Tables 18 to 23. It can be seen that this culture gave a most feeble response in the first three passages, but the fourth passage produced a more marked agglutination response and positive cultural findings in all doses. The passage of 1280d and c were made in an attempt to produce more extensive lesions. It was noted that these last two passages did elicit more pronounced gross lesions as well as positive agglutination and cultural response.

Tables 24 to 25 show the results of progressively increased doses of culture 19 in an attempt to produce infection in the guinea pig. It required a massive infective dose of 470,000,000 organisms to produce infection with this culture.

In Tables 26 to 28 are tabulated the results of three passages of culture 1284 through guinea pigs. It appears that the third passage showed an enhanced virulence as indicated by more extensive lesions and consistent positive cultural findings.

TABLE 13

Injection of Br. Abortus No.1287

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	200	None	I 1:50	Positive	Negative
2	200	Spleen x 1.5	1:200	Positive	Positive
3	200	Died			
4	107	None	I 1:200	Positive	Negative
5	107	None	1:100	Positive	Positive
6	107	None	Negative	Negative	Negative
7	10	None	Negative	Negative	Negative
8	10	None	Negative	Negative	Negative
9	10	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 14

Injection of Br. Abortus No. 1287a

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	212	None	I 1:200	Positive	Positive
2	212	Spleen x 1.5	1:200	Positive	Negative
3	212	Died			
4	107	Spleen x 2	1:100	Positive	Positive
5	107	Spleen x 1.5	I 1:100	Positive	Negative
6	107	Died			
7	11	Spleen x 1.5	1:100	Positive	Positive
8	11	None	Negative	Negative	Negative
9	11	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 15

Injection of Br. Abortus No.1287b

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	235	Spleen x 1.5	1:400	Positive	Negative
2	235	None	Negative	Negative	Negative
3	235	None	1:100	Positive	Negative
4	108	None	I 1:25	Positive	Negative
5	108	None	I 1:100	Positive	Negative
6	108	None	Negative	Negative	Positive
7	10	Spleen x 1.5	Negative	Positive	Positive
8	10	Spleen x 1.5	Negative	Negative	Positive
9	10	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 16

Injection of Br. Abortus No.1287c

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	227	Liver, spleen x 3	1:200	Positive	Negative
2	227	Liver, spleen x 4	1:200	Positive	Negative
3	227	Died			

TABLE 17

Injection of Br. Abortus No.1287d

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	157	Liver, lymph nodes, spleen x 6	I 1:100	Positive	Positive
2	157	Lymph nodes, spleen x 3	1:400	Positive	Negative
3	157	Spleen x 1.5	1:400	Positive	Positive

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 18

Injection of Br. Abortus No.1280

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem *	Cultural Findings	
				Spleen	Liver
1	177	None	I 1:50	Positive	Negative
2	177	None	Negative	Negative	Negative
3	177	Died			
4(a)	89	None	I 1:50	Positive	Negative
5(a)	89	None	I 1:50	Positive	Negative
6	89	Liver, spleen x 3	Negative	Negative	Negative
7(b)	9	Spleen x 2	Negative	Negative	Negative
8(b)	9	Liver, spleen x 3	Negative	Negative	Negative
9(b)	9	Liver, spleen x 3	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

(a) Brucella from these guinea pigs grew aerobically and apparently represented cross infection as 1280 is an anaerobic abortus.

(b) Enlarged spleens apparently due to a staphylococcus not Brucella.

TABLE 19

Injection of Br. Abortus No.1280a

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	207	None	Negative	Positive	Negative
2	207	None	Negative	Positive	Negative
3	207	None	Negative	Negative	Negative
4	113	None	Negative	Negative	Negative
5	113	None	Negative	Negative	Negative
6	113	None	Negative	Negative	Negative
7	11	None	Negative	Negative	Negative
8	11	None	Negative	Negative	Negative
9	11	None	Negative	Negative	Negative

TABLE 20

Injection of Br.Abortus No.1280b

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem *	Cultural Findings	
				Spleen	Liver
1	163	Spleen x 1.5	Negative	Positive	Negative
2	163	None	Negative	Positive	Negative
3	163	None	Negative	Negative	Negative
4	78	None	Negative	Positive	Positive
5	78	None	I 1:50	Negative	Negative
6	78	Spleen x 1.5	I 1:25	Positive	Positive
7	8	None	Negative	Negative	Negative
8	8	None	Negative	Negative	Negative
9	8	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 21

Injection of Br. Abortus No.1280c

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem *	Cultural Findings	
				Spleen	Liver
1	180	Spleen x 1.5	I 1:100	Positive	Negative
2	180	None	Negative	Negative	Negative
3	180	None	I 1:25	Positive	Negative
4	113	None	1:50	Positive	Positive
5	113	None	1:50	Positive	Positive
6	113	None	I 1:50	Positive	Negative
7	18	Spleen x 1.5	1:50	Positive	Negative
8	18	None	I 1:50	Negative	Negative
9	18	None	Negative	Positive	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 22

Injection of Br. Abortus No.1280d

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	136	Liver, lymph nodes, spleen x 5	1:400	Positive	Negative
2	136	None	Negative	Negative	Negative
3	136	Died			

TABLE 23

Injection of Br. Abortus No.1280e

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	105	Lymph nodes, spleen x 1.5	1:400	Positive	Negative
2	105	Liver, lymph nodes, spleen x 3	1:400	Positive	Negative
3	105	Liver, lymph nodes, spleen x 3	1:400	Positive	Negative

TABLE 24

Injection of U.S.B.A.I. Strain 19

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	9	None	Negative	Negative	Negative
2	9	None	Negative	Negative	Negative
3	9	None	Negative	Negative	Negative
4	81	None	Negative	Negative	Negative
5	81	Spleen x 1.5	Negative	Negative	Negative
6	81	None	Negative	Negative	Negative
7	154	None	Negative	Negative	Negative
8	154	None	Negative	Negative	Negative
9	154	None	Negative	Negative	Negative
10	500	None	I 1:200	Negative	Negative
11	500	None	I 1:25	Negative	Negative
12	500	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 25

Injection of U.S.B.A.I. Strain 19

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	5000	None	I 1:50	Negative	Negative
2	5000	None	I 1:25	Negative	Negative
3	5000	None	Negative	Negative	Negative
4	90,000	None	Negative	Negative	Negative
5	90,000	None	Negative	Negative	Negative
6	90,000	None	Negative	Negative	Negative
7	900,000	None	Negative	Negative	Negative
8	900,000	None	Negative	Negative	Negative
9	900,000	None	Negative	Negative	Negative
10	470,000,000	Liver, spleen x 2	1:400	Positive	Positive
11	470,000,000	Liver, lymph nodes, spleen x 6	1:400	Positive	Positive
12	470,000,000	Liver, lymph nodes, spleen x 6	1:400	Positive	Positive

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 26

Injection of Br. Abortus No.1284

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	210	None	1:100	Positive	Negative
2	210	Spleen x 2	1:400	Positive	Negative
3	210	Liver, spleen x 2	1:400	Positive	Negative
4	108	Lymph nodes, spleen x 1.5	1:100	Positive	Negative
5	108	None	Negative	Negative	Negative
6	108	Spleen x 1.5	Negative	Negative	Negative
7	10	Lymph nodes, spleen x 1.5	I 1:100	Positive	Negative
8	10	Lymph nodes	I 1:50	Positive	Negative
9	10	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 27

Injection of Br. Abortus No.1284a

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem *	Cultural Findings	
				Spleen	Liver
1	191	None	1:100	Positive	Positive
2	191	Liver, spleen x 2	1:200	Positive	Positive
3	191	Lymph nodes, liver, spleen x 1.5	1:200	Positive	Negative
4	96	Lymph nodes, liver, spleen x 2	1:200	Positive	Negative
5	96	Lymph nodes, spleen x 2	I 1:100	Positive	Positive
6	96	Spleen x 2	I 1:50	Positive	Negative
7	9	None	Negative	Negative	Negative
8	9	None	Negative	Negative	Negative
9	9	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 28

Injection of Br. Abortus No.1284b

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem *	Cultural Findings	
				Spleen	Liver
1	207	Liver, spleen x 5	1:100	Positive	Positive
2	207	Spleen x 2	1:100	Positive	Negative
3	207	Spleen x 2	I 1:100	Positive	Positive
4	95	Spleen x 1.5	1:200	Positive	Positive
5	95	Spleen x 1.5	1:200	Positive	Positive
6	95	Liver, spleen x 1.5	I 1:100	Positive	Positive
7	9	Liver, spleen x 3	1:400	Positive	Negative
8	9	Spleen x 1.5	I 1:100	Positive	Negative
9	9	None	Negative	Negative	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

The results of one passage of culture $1099T_2$ through guinea pigs in various doses are tabulated in Table 29. This culture obviously was very virulent to begin with so further passages were not made.

Table 30 shows the results of one passage of culture 1285 through guinea pigs in various doses. This culture was very virulent as indicated by the agglutination response and positive cultural findings. However, extensive lesions were not produced.

The results of one passage of culture 1677 through guinea pigs in various doses are shown in Table 31. This culture was the only Br. suis used and it was noted that it possessed a very high degree of virulence for guinea pigs. It produced the greatest agglutination response, most extensive gross lesions and positive cultural findings of all.

Exposure of Heifers to Br. Abortus

Two groups of heifers were used in an effort to determine a satisfactory conjunctival infective dose of a virulent culture of Br. abortus for use in exposing pregnant heifers for other experimental purposes. By preliminary plating studies it was found that 1 drop (from a pipette standardized to number 14 in a B. and S. wire gauge) of a 1/10 dilution of a suspension standardized to 50 on the Cenco photelometer contained 4,500,000 viable Brucella organisms. Group 1 was composed of 7 pregnant and 1 non-pregnant heifer. Group 2 was composed of 6 pregnant heifers. Group 1 received 3 drops of the described suspension of a recently isolated culture of Br. abortus #1282. Group 2 received 1 drop of a like suspension of a recently isolated culture of Br. abortus #1284. Both of these cultures were isolated from the same cow in the same herd, but at a different time. It was considered advisable to use a recently isolated culture from the same cow in order to avoid any decrease in

TABLE 29

Injection of Br. Abortus No.1099T_2

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	145	Spleen x 2	1:400	Positive	Negative
2	145	Lymph nodes, spleen x 1.5	1:400	Positive	Negative
3	145	Spleen x 1.5	1:400	Positive	Negative
4	81	Spleen x 1.5	1:400	Positive	Negative
5	81	Spleen x 3	1:400	Positive	Negative
6	81	None	1:200	Positive	Negative
7	9	Lymph nodes, spleen x 2	1:400	Positive	Negative
8	9	None	1:400	Positive	Negative
9	9	Spleen x 1.5	1:100	Positive	Negative

*All titers indicated are complete unless preceeded by an I which indicates incomplete agglutination.

TABLE 30

Injection of Br. Abortus No.1285

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem*	Cultural Findings	
				Spleen	Liver
1	197	Spleen x 1.5	I 1:100	Positive	Negative
2	197	None	1:100	Positive	Positive
3	197	None	1:100	Positive	Negative
4	114	Spleen x 2	I 1:100	Positive	Positive
5	114	None	I 1:100	Positive	Negative
6	114	None	1:200	Positive	Negative
7	18	None	I 1:50	Positive	Negative
8	18	None	Negative	Negative	Negative
9	18	None	I 1:100	Positive	Negative

TABLE 31

Injection of Br. Suis No.1677

Guinea Pig No.	No. of Organisms Injected	Post Mortem Lesions	Serum Agglutination at Post Mortem	Cultural Findings	
				Spleen	Liver
1	190	Liver, lymph nodes, spleen x 8	1:400	Positive	Positive
2	190	Liver, lymph nodes, spleen x 5	1:400	Positive	Positive
3	190	Died			
4	104	Liver, lymph nodes, spleen x 3	1:400	Positive	Positive
5	104	Liver, lymph nodes, spleen x 12	1:400	Positive	Positive
6	104	Liver, lymph nodes, spleen x 6	1:400	Positive	Positive
7	13	Liver, lymph nodes, spleen x 5	1:400	Positive	Positive
8	13	Liver, lymph nodes, spleen x 3	1:400	Positive	Positive
9	13	Liver, lymph nodes, spleen x 3	1:400	Positive	Positive

virulence since the two groups of heifers were not exposed at the same time.

Rapid agglutination tests were made on both groups of heifers for several weeks prior to exposure. All heifers used failed to give a positive reaction in 1:25 dilution with the exception of 883. Heifer 883 apparently was exposed to infection to a mild degree previous to the conjunctival exposure as her agglutination reaction was positive in 1:100 dilution 83 days prior to exposure, but became negative in all dilutions 28 days before exposure.

After exposure, agglutination tests were made until the heifers aborted, calved normally, or were sold. Agglutination tests were made on colostrum milk and cultures were made of colostrum milk, fetal organs and fetal membranes. Guinea pigs were not injected with milk or tissue extracts in all cases since it was deemed unnecessary if positive cultures were obtained.

The heifers were maintained in the same barn separate from other cattle and were allowed access to a separate pasture. The heifers were placed in box stalls at the time of parturition or abortion and held until sold or discharges ceased.

The results of inoculating the heifers in Group 1 with 13,500,000 viable Br. abortus organisms by way of the conjunctival sac are shown in Table 32. In this group 6 of the heifers developed a serum agglutination titer of 1:100 within 41 days after exposure while 2 required 82 days to develop an agglutination titer of 1:100. Three of the heifers in Group 1 were sold prior to the termination of pregnancy to make room for further experimentation. However, all three of these animals were infected as determined by the agglutination response. Br. abortus was cultured

TABLE 32

Exposure of Pregnant Heifers to Br. Abortus

	Heifer Number	Stage of Pregnancy At Exposure	Agglutination Titer Days After Exposure									Termination of Pregnancy
			13	27	41	53	68	82	99	114	186	
Group 1	889	6 mo.	-	50	100	100						Aborted 236 days
	53075	7 mo.	25	50	100	400	400					Live calf 268 days
	882	5-1/2 mo.	100	400	400	400						Aborted 215 days
	856	4 mo.	50	400	400	400						Aborted 170 days
	3406120		50	400	400	400	400	400				Not pregnant
	537273	2 mo.	100	100	100	100	100	200				Sold prior to termination
	879	3-1/2 mo.	-	-	25	-	-	100				Sold prior to termination
	885	6 mo.	-	-	-	25	25	100				Sold prior to termination
Group 2	297	6 mo.		25		400			400			Aborted 237 days
	479	5-1/2 mo.		50		25			400			Aborted 246 days
	883	6 mo.		-		-				-	-	Normal calving
	851	5-1/2 mo.		25		-			100	100		Aborted 244 days
	854	4-1/2 mo.		25		25			200	200		Aborted 207 days
	570132	4 mo.		100		200			200	200		Aborted 195 days

from the colostrum and fetuses of all of the heifers which calved or aborted.

The heifers in Group 2 were inoculated with 4,627,500 viable Br. abortus organisms by way of the conjunctival sac. The results are shown in Table 32. The previously mentioned heifer 883 must have developed a high degree of immunity by natural exposure as indicated by a lack of agglutination response and a normal parturition. All other heifers in Group 2 developed a serum agglutination titer of 1:100 by the 99th day after exposure. With the exception of 883, all heifers in Group 2 aborted and showed Br. abortus in the colostrum and fetuses.

SUMMARY

A suitable suspending or diluting fluid for making plate counts of Brucella was prepared from 0.1 per cent Tryptose and 0.5 per cent sodium chloride in distilled water. The pH after sterilization was 6.9.

Guinea pigs were readily infected with Br. abortus and Br. suis in very small numbers. Ten organisms were sufficient to produce infection in most of the guinea pigs.

Continued passage of Br. abortus through guinea pigs resulted in an increase in virulence of all cultures.

Strain 19 B.A.I. exhibited the lowest virulence of any culture of Br. abortus studied.

Susceptible pregnant heifers were readily infected with 4,627,500 viable Br. abortus organisms deposited in the conjunctival sac.

The time of the agglutination response of the two groups of pregnant heifers exposed to two different doses of organisms varied inversely with the number of organisms used.

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