

AN IMPROVEMENT IN THE METHOD OF ISOLATING AND
RECOVERING THE BACILLUS OF CATTLE ABOR-
TION THROUGH GUINEA PIGS.

By ERNEST W. SMILLIE, D.V.M.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical
Research, Princeton, N. J.)

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History of the Test.

For the isolation of *B. abortus* Bang up to 1912 there were available the culture methods of Bang and Nowak. Early in 1912 Theobald Smith and Fabyan¹ showed that *B. abortus* inoculated into guinea pigs produces a disease with well defined characters, and that the disease can be isolated from these guinea pigs after 3 or more months. This method proved a valuable addition inasmuch as culture methods frequently failed when badly soiled fetal membranes were objects of investigation.

This inoculation disease had already been recognized as an entity, distinct from tuberculosis, in 1894 by Smith,² and Schroeder and Cotton³ had the disease under observation since 1911 ascribing it, however, to a Gram-positive bacterium. This error was rectified in a publication issued in March, 1912.⁴ The disease in guinea pigs was more fully described by Fabyan⁵ in 1912. Since that date the guinea pig has been used by various workers for the isolation of *B. abortus* from fetal membranes and fetal tissues.

In view of the value of the method it seemed desirable to determine whether it might be possible to shorten the life period of the inoculated guinea pig without impairing the chances for obtaining cultures. In the following pages a number of experiments are described which were planned with this object in view.

¹ Smith, T., and Fabyan, M., *Centr. Bakteriolog., 1te Abt., Orig.*, 1912, lxi, 549.

² Schroeder, E. C., *U. S. Dept. Agric., Bureau Animal Industry, Bull.* 7, 1894, note by T. Smith, p. 80.

³ Schroeder, E. C., and Cotton, W. E., *Proc. Am. Vet. Med. Assn., 48th Convention*, 1911, 442.

⁴ *U. S. Dept. Agric., Bureau Animal Industry, Circular* 198, 1912.

⁵ Fabyan, M., *J. Med. Research*, 1912, xxvi, 441.

EXPERIMENTAL.

The material used to inoculate guinea pigs was derived from pure cultures as well as from tissues of fetuses and fetal membranes.

The guinea pigs were inoculated either into the subcutis or the abdominal cavity. When the animals were considered ready for bacteriological examination they were chloroformed, autopsied, the lesions noted, and the following procedure, which is the one regularly used in this laboratory for the cultivation of *Bacillus abortus*, was adopted throughout.

Ordinary veal peptone agar tubed and slanted is the medium employed. The surface of the organs from which cultures are to be made is, if necessary, seared with a heated spatula. Bits of tissue about the size of split peas are then torn out of the organ with sterile forceps, rubbed over the entire surface of the agar with a platinum loop, and finally pushed down into the condensation water. The cotton plugs are clipped off level with the tops of the tubes. The tubes at this point are thoroughly heated in the flame to kill any spores adhering to the contained cotton plugs as a result of handling, and after the tubes have cooled somewhat they are hermetically sealed with a layer of sealing wax. They are then incubated at 37°C. Colonies of *Bacillus abortus* are usually observed on the agar slant after 5 to 10 days incubation.

Cultures were prepared from lungs, liver, kidneys, and spleen in all cases; from the superficial inguinal lymph nodes when the animal had received a subcutaneous injection; from the retrogastric lymph nodes when the injection was intraabdominal; and from the testicle and the ovary or uterus. After an incubation of from 6 to 10 days, the cultures were examined and any growth was noted and studied. The colonies were counted on the agar surface. In case no growth appeared, the condensation water containing the bit of tissue was shaken so as to cover the agar surface and the tubes were replaced in the incubator. The object of this procedure was to give any bacteria growing out of the bit of tissue or in the condensation water an opportunity to multiply on the inclined surface. Probably one out of every ten to twenty tubes responded to this treatment.

To identify *Bacillus abortus* the colonies were examined both macroscopically and microscopically. The macroscopic characteristics of

such colonies are in themselves almost diagnostic. They vary considerably in size according to the numbers on the agar surface. When crowded they are relatively minute and as a rule discrete, except near the margin of the condensation water. When 1 to 2 cm. apart, they may become 3 mm. in diameter and strikingly convex. The microscopic examinations of these colonies were made in all instances to verify the macroscopic inspection, and the Gram test was applied to differentiate *Bacillus abortus* from Gram-positive organisms, such as *Bacillus pyogenes*.

The diagnosis of infection with *Bacillus abortus* was furthermore strengthened by a macroscopic examination of the organs of every guinea pig. As is now well known, in positive cases the spleen is much enlarged, highly congested, more or less nodular on the surface, and the Malpighian bodies may be enlarged and show through the distended capsule and on section as grayish foci of varying sizes. In addition to this characteristic lesion, one or both testicles may be attacked and the epididymis converted into an indurated, centrally necrotic mass. Minute nodules in the liver are found in most cases, usually not quite a millimeter in diameter, sometimes yellowish and probably representing necroses, sometimes grayish or pearly and then representing minute collections of cells as described by Smith and Fabyan and Fabyan.

Series I.

The first series of guinea pigs injected consisted of twelve, six males and six females, varying between 300 and 400 gm. in weight. They were injected September 26, 1917, with a stock culture recovered from a fetus after passage through a guinea pig. Three of each sex were inoculated subcutaneously and the same number intraperitoneally, each individual receiving 1 cc. of a suspension of the strain. The suspension was prepared by washing off the surface colonies of a 72 hour agar slant with sterile salt solution, diluting the resulting suspension to the approximate density of a 24 hour bouillon culture of *Bacillus typhosus* and then again diluting fifty times. Table I summarizes the results. The enlargement of the spleen is designated in bulk and not in dimensions.

TABLE I.
Results of Inoculation of Guinea Pigs with a Pure Culture of B. abortus Isolated through Guinea Pigs from a Fetus.

Guinea pig No.	Sex.	Method of inoculation.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight - gm.	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
1	Male.	Intraperitoneal.	15 days	+ 6	Six times normal size; adherent to ribs.	—	Spleen: luxuriant growth. Liver: good Testicle: " Spleen: " Regional lymph node: good growth.	+
2	Female.	Subcutaneous.	15	+ 14	Five times normal size; hyperemic and friable.	—	Kidney: good growth. Liver: few colonies. Spleen: 200 Regional lymph node: 150 Testicle: 18 Lung (contaminated): 18 Kidney: 2	+
3	Male.	Intraperitoneal.	21	+ 22	Eight times normal size; easily torn; surface nodular; nodules numerous.	Small necrotic foci in liver.	Spleen: 100 Regional lymph node: 200 Lung: 31 Kidney: 6 Ovary: 1	+
4	Female.	Subcutaneous.	21	+ 13	Eight times normal size, surface nodular.	Small abscess at point of injection.		+

5	Male.	Intraperitoneal.	29	+20	Slightly enlarged; very dark; surface nodular.	Minute necrotic foci in liver.	Spleen: 150 Retogastric lymph node: 50 Kidney: 7 Lung: 3 Liver: 2	+
6	Female.	Subcutaneous.	29	+52	Six times normal size; surface nodular.	Numerous minute necrotic foci in liver.	Spleen: 150 Regional lymph node: 125 Lung: 4 Ovary: 3 Liver: 2	+
7	"	Intraperitoneal.	35	-15	Eight times normal size; adherent to peritoneum and left kidney; tubercle in spleen at adherent point.	Few minute necrotic foci in liver. Capsule of left kidney thickened.	Spleen: 300 Retogastric lymph node: 300 Kidney: 300 Lung: 6 Liver: 3 Ovary: 2	+
8	Male.	Subcutaneous.	35	+87	Markedly enlarged; easily torn; rounded borders.	Liver enlarged; minute scattering tubercles.	Spleen: 14 Regional lymph node: 140 Lung: 9 Testicle: 6 Kidney: 5 Liver: 2	+
9	Female.	Intraperitoneal.	43	-29	Nine times normal size; surface smooth and easily torn.	—	Spleen: 100 Retogastric lymph node: 5 Kidney: 8 Lung: 5 Liver: 2 Uterus: 2	+

TABLE I—Concluded.

Guinea pig No.	Sex.	Method of inoculation.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight - gm.	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
10	Male.	Subcutaneous.	42	+90	Twice normal size; hyperemic and surface nodular.	One tubercle in liver 3 mm. in diameter.	Spleen: 80	+
							Regional lymph node: 60	
							Liver: 3	
							Kidney: 3	
							Testicle: 2	
11	Female.	Intraperitoneal.	52	-35	Enormously enlarged; adherent to peritoneum and left kidney; nodular surface.	Minute scattering tubercles and small necrotic foci in liver.	Lung: 0	+
							Spleen: 75	
							Retogastric lymph node: 50	
							Kidney: 12	
							Lung: 7	
12	Male.	Subcutaneous.	52	+106	Enormously enlarged; nodular surface; small glistening tubercles.	Minute irregular necrotic areas in liver.	Uterus: 4	+
							Liver: 0	
							Spleen: 75	
							Regional lymph nodes: 150	
							Kidney: 20	
							Liver: 3	
							Lung: 3	
							Testicle: 0	

Series II.

Six female guinea pigs, weighing between 325 and 375 gm. each, were used in the second series. They were inoculated September 26, 1917, three subcutaneously and three intraabdominally with a culture of *Bacillus abortus*, recovered from the placenta of a cow, after passage through a guinea pig. The method of preparation of the material to be injected and the dosage were the same as for the guinea pigs of Series I. A condensed description of Series II is given in Table II.

Series I and II were inoculated with the strains from a certain fetus and a certain placenta from two herds respectively, to determine whether or not there was an appreciable difference in the pathogenicity. It will be noted from the tables that the extent of the lesions produced, and the comparative colony counts for the two strains are approximately the same. As far as the guinea pig test is concerned the strains are evidently of the same level of virulence.

Series III.

The third series of guinea pigs consisted of six males and six females weighing between 350 and 450 gm. each. Three guinea pigs of each sex were inoculated subcutaneously, and the same number of each sex intraabdominally, on October 24, 1917, with a salt solution suspension of crushed cotyledons from the placenta of another cow.

The material to be injected was prepared in the following manner: Five cotyledons that were apparently affected were removed into sterile Petri dishes. After they had been thoroughly washed in running tap water from a deep well to remove particles of straw, etc., pieces varying in size from 1 to 2 gm. were clipped from each cotyledon, care being taken to cut down to the base of the villi. The bits of tissue were then ground up with sterile quartz sand in a sterile mortar. The ground mass was suspended in 0.85 per cent salt solution until a density was reached which compared with a 24 hour bouillon culture of *Bacillus typhosus*. Each animal was injected with 1 cc. of this suspension. Table III is a summary of the results.

An analysis of the three foregoing experiments indicates that the inoculation disease in guinea pigs due to *Bacillus abortus* runs a fairly definite course. The number of colonies appearing in cultures is

TABLE II.
Results of Inoculation of Guinea Pigs with a Pure Culture of B. abortus Isolated through Guinea Pigs from the Placenta of a Cow.

Guinea pig No.	Sex.	Method of inoculation.	Length of time after inoculation when Guinea pig was killed.	Gain in weight + Loss in weight -	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
13	Female.	Intraabdominal.	16 days	-26 gm.	Twice normal size; congested; easily torn.	—	Spleen: luxuriant growth. Retrogastric lymph node: luxuriant growth. Kidney: few colonies. Liver: " Lung: "	+
14	"	Subcutaneous.	16	+16	Normal in size and appearance.	Minute grayish necrotic foci in liver. Small abscess (8 mm. diameter) at site of injection.	Spleen: 200 Liver: 20 Kidney: 15 Lung: 3	+
15	"	Intraperitoneal.	28	+21	Five times normal size; surface nodular.	Scattering grayish (necrotic) foci in liver.	Spleen: 200 Retrogastric lymph node: 48 Liver: 10 Kidney: thin film. Lung: "	+

16	Female.	Subcutaneous.	28	+58	Four times normal size; nodular surface.	Minute necrotic areas in liver. Small subcutaneous abscess at site of injection.	Spleen: 175 Regional lymph node: 300 Lung: 8 Liver: 4 Kidney: 3 Ovary: thin film.	+
17	"	Intraperitoneal.	50	+41	Eight times normal size; congested; surface smooth and easily torn.	Minute (necrotic) areas in liver; walls of uterus injected.	Spleen: 100 Retrogastric lymph node: 40 Kidney: 4 Liver: 2 Lung: 1 Uterus: few.	+
18	"	Subcutaneous.	50	+116	Four times normal size; marked congestion; small tubercles; nodular surface.	A few scattering nodules in liver.	Spleen: 125 Regional lymph node: 10 Kidney: 6 Liver: 4	+

TABLE III.
Results of Inoculation of Guinea Pigs with Salt Solution Suspensions of Placenta from a Case of Abortion.

Guinea pig No.	Sex.	Method of inoculation.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight -	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
19	Female.	Subcutaneous.	15 days	+15 gm.	Twice normal size; congested; easily torn.	—	Spleen: 60 Regional lymph node: 200 Kidney: 20 Lung: 1 Liver: 1	+
20	Male.	Intraperitoneal.	16	- 9	Slightly enlarged; surface slightly nodular.	—	Spleen: 8 Retogastric lymph node: 5 Lung: 8 Liver: 2 Kidney: 1	+
21	"	Subcutaneous.	22	+17	Slightly enlarged; surface nodular.	Minute, scattering, refractile tubercles in liver.	Testicle: 0 Spleen: 150 Regional lymph node: count-less. Kidney: 90 Lung: 8 Testicle: 1 Liver: 1	+

22	Female.	Intraperitoneal.	22	+53	Slightly swollen; congestion; surface nodular.	A few minute scattering tubercles.	Spleen: countless. Retrogastric lymph node: 70 Kidney: 100 Liver: 5 Lung: 5 Uterus: 0	+
23	"	"	44	+153	Somewhat enlarged; congested; nodular surface.	Minute grayish necrotic foci in liver; few small tubercles.	Spleen: 35 Retrogastric lymph nodes: 10 Lung: 2 Uterus: 1 Kidney: 1 Liver: 0	+
24	Male.	Subcutaneous.	44	+125	Twice normal size; congested and very dark; nodular surface.	Fibrous induration of right testicle; disintegration of epididymis.	Spleen: 70 Regional lymph nodes: 10 Testicle: 1 Liver: 1 Lung: contaminated.	+
25	"	Intraperitoneal.	49	+151	Twice normal size; congested; prominent nodules on surface.	Minute necrotic foci in liver; large colon adherent in sublumbar region; abscess at adherent point.	Spleen: 30 Liver: 4 Lung: 1 Kidney: 1 Testicle: 1	+
26	Female.	Subcutaneous.	49	+98	Six times normal size; congested; smooth, easily torn surface.	Minute yellowish gray necrotic foci in liver.	Spleen: 45 Regional lymph node: 35 Liver: 1 Kidney: 1 Lung: contaminated.	+

TABLE III—*Concluded.*

Guinea pig No.	Sex.	Method of inoculation.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight -	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
27	Female.	Intraperitoneal.	28 <i>days</i>	+47	Twice normal size; surface nodular.	A few minute tubercles in liver.	Spleen: 250 Retrogastric lymph node: 80 Liver: 23 Uterus: 1 Kidney: many. Lung: contaminated.	+
28	Male.	Subcutaneous.	29	+11	Slightly enlarged; congested; surface nodular.	Minute necrotic foci in liver; a few scattering refractile tubercles; subcutaneous abscess at site of injection.	Spleen: 150 Regional lymph node: 125 Liver: 2 Kidney: 2 Lung: contaminated.	+
29	"	Intraperitoneal.	38	+99	Slightly enlarged; congested; nodular surface; fibrosis of the center.	Omentum adherent to peritoneum in two places.	Spleen: 90 Retrogastric lymph node: 75 Kidney: 4 Lung: 1 Liver: 0 Testicle: 0	+
30	Female.	Subcutaneous.	38	+125	Eight times normal size; congested; surface and borders nodular.	Scattering tubercles in liver; large colon adherent to dorsal wall.	Spleen: 35 Regional lymph node: 15 Kidney: 8 Lung: 7 Liver: 0 Uterus: 0	+

highest toward the 4th week. In animals killed later the number appears to diminish gradually. On the other hand, the lesions manifest to the naked eye appear to become more conspicuous and widespread as the number of bacteria tends to decline. One might therefore venture the general statement that for a diagnosis based on the isolation of *Bacillus abortus*, guinea pigs should be killed between the 3rd and the 4th week. For a diagnosis based on characteristic lesions they should be killed later, preferably after 7 or 8 weeks. These statements do not hold rigidly, since the progress of the disease depends largely on the dosage of the virus injected and cases have been found in this laboratory in which extensive lesions were found within 4 weeks. Table IV shows that in most instances the spleens become quite large in 4 weeks.

Series IV.

The material used in this series was from several different sources. The inoculated guinea pigs were handed over to me by Dr. Smith, who had already obtained cultures of *Bacillus abortus* directly from the material, and who inferred, therefore, that most if not all of the guinea pigs would yield positive cultures. The material came from six different cases and included fifteen guinea pigs (Table IV). They were autopsied in the 5th week. The fetal membranes of Cows 203 and 210 came from presumably full time calves. Fetuses 205a and 205b were twins from Cow 205 from which amniotic fluid had been collected at the time of delivery. Fetus 206 was of uncertain age, 28 inches long and probably between 7 and 8 months old.

The negative outcome of inoculations of meconium as compared with the positive results of inoculations of the contents of the fourth stomach should be noted. I am informed by Dr. Smith that direct cultures from meconium of Fetus 205a were positive, but the colonies were very scarce. The same was true of cultures from meconium of Fetus 205b. In these instances, therefore, direct cultures from the fetus were more reliable than guinea pig inoculations.

Series V.

This lot of seven male guinea pigs was injected with gradually increasing dilutions of a fresh culture of *Bacillus abortus*. The object of

TABLE IV
Results of Inoculation of Guinea Pigs with Fresh Material from Six Different Sources.

Guinea pig No.	Sex.	Source of material.	Method of inoculation.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight - gm.	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
31	Male.	Salt solution suspension (2 cc.) of meconium of Fetus 200.	Intraperitoneal.	31	+141	Slightly enlarged; nodular surface.	Minute necrotic foci in liver; hyperemia.	Spleen: 105 Liver: 0 Testicle: 0	+
32	"	1.5 cc. of thick salt solution suspension of 4th stomach contents of Fetus 200.	"	31	+106	Twice normal size surface quite nodular.	Fatty liver; necrosis of epididymis of both testicles; injection of vessels.	Spleen: 150 Testicle: countless. Liver: 0	+
33	"	0.3 cc. of salt solution suspension of cotyledon of Placenta 203.	Subcutaneous.	33	+111	Slightly enlarged; congested and dark.	Regional lymph nodes swollen; small abscess at injection site.	Spleen: 46 Regional lymph node: 75 Liver: 0 Spleen: 90 Liver: 0 Testicle: 0	+
34	"	0.3 cc. of salt solution suspension of cotyledon of Placenta 203.	"	33	+165	Twice normal size; congested; faintly nodular.	—	Spleen: 90 Liver: 0 Testicle: 0	+

35	Male.	1 cc. of sterile amniotic fluid of Cow 205.	Subcutaneous.	29	+181	Twice normal size; congested; surface nodular.	Minute scattering tubercles in liver.	Spleen: Regional lymph node: 50 Testicle: 0 Liver: 0	+
36	"	2 cc. of salt solution suspension of amniotic fluid of Cow 205.	"	31	+109	Four times normal size; severe congestion; smooth, easily torn surface.	Minute scattering tubercles in liver.	Spleen: Regional lymph node: 70 Liver: 80	+
37	"	1 cc. of salt solution suspension of meconium of Fetus 205a.	Intraperitoneal.	29	+129	Normal.	—	Spleen: 0 Liver: 0 Testicle: 0	-
38	"	4th stomach contents of Fetus 205a 0.5 cc. + 0.5 cc. of salt solution.	"	30	+25	Twice normal size; surface nodular; nodules numerous.	Minute scattering tubercles in liver.	Spleen: 18 Testicle: 6 Liver: 0	+
39	"	4th stomach contents of Fetus 205b 0.5 cc. + 0.5 cc. of salt solution.	"	33	+80	Four times normal size; marked congestion; nodular surface.	Disintegration of epididymis of both testicles; vessels injected.	Spleen: 16 Testicle: count- less. Liver: 0	+
40	"	1 cc. of salt solution suspension of meconium of Fetus 205b.	"	33	+139	Twice normal size; nodular surface.	—	Spleen: 0 Testicle: 0 Liver: 0	-
41	"	4th stomach contents of Fetus 206 in salt solution.	Subcutaneous.	30	+70	Three times normal size; surface nodular.	Minute necrotic foci in liver; testicles congested.	Spleen: 350 Testicle: 0 Liver: 0	+

TABLE IV—*Concluded.*

Guinea pig No.	Sex.	Source of material.	Method of inoculation.	Length of time after inoculation when guinea pig was killed	Gain in weight + Loss in weight - gm.	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
42	Male.	1.5 cc. of salt solution suspension of lung of <i>Festus</i> 206 ground with sand.	Subcutaneous.	30 days	+114	Three times normal size; markedly congested; easily torn.	Minute scattering tubercles in liver.	Spleen: 150 Liver: 2 Testicle: 0	+
43	"	0.6 cc. of salt solution suspension of cotyledon of Placenta 210.	"	34	+72	Five times normal size; congested; surface nodular; nodules prominent.	Minute tubercles in liver. Epididymis of both testicles disintegrated; abscess in left testicle; congestion.	Spleen: thin film. Liver: 150 Testicle: 2	+
44	"	0.3 cc. of salt solution suspension of cotyledon of Placenta 210.	"	34	+208	Eight times normal size; congested; distended capsule; easily torn.	Numerous minute tubercles in liver.	Spleen: 80 Liver: 1 Testicle: 0	+
45	"	0.5 cc. of salt solution suspension of cotyledon of Placenta 210.	"	34	+162	Five times normal size; congested; nodular surface; nodules numerous.	Few minute tubercles in liver.	Spleen: 70 Liver: 1 Testicle: countless. Regional lymph node: 17	+

the experiment was to determine whether or not there is a marked difference in the gross lesions produced, and in the number of organisms present, indicated by colonies found in cultures prepared from the spleens, in the guinea pigs injected with low dilutions and those injected with high dilutions. In other words, I wished to find any difference in the severity of the disease in guinea pigs inoculated with material heavily loaded with *Bacillus abortus*, and in those inoculated with mildly infected material, all animals being autopsied within the 4th week after injection.

The culture was obtained from Dr. Smith, who had recovered it from Cow 214, after passage through a guinea pig. A 72 hour agar slant was washed off with 2 cc. of sterile salt solution. After removal to a sterile tube the material was diluted to a comparative density of a 24 hour bouillon culture of *Bacillus typhosus*. 1 : 50 of this original dilution was injected into the first guinea pig, and the dilution doubled for each succeeding animal to the seventh, which received a dilution of 1 : 3,200. All the guinea pigs were injected into the peritoneal cavity. Table V is a summary of the results. Owing to an error, cultures prepared from organs of Guinea Pigs 50 and 52 were both labeled Guinea Pig 50; therefore a definite statement is impossible, but there was an average of about 100 colonies from the spleens of both guinea pigs.

The difference in the number of colonies present in the organs of inoculated guinea pigs, indicated by colonies counted on agar slants, is not marked between those receiving injections of heavy and light suspensions of the bacillus of abortion. It is of considerable significance, however, that positive cultures can be obtained from the organs between the 3rd and the 4th weeks after inoculation with material that harbors very few organisms.

Series VI.

In order to compare the relation of the lesions and growth of cultures prepared from inoculated guinea pigs autopsied 4 weeks after injection and those kept for 4 months, the three animals given in Table VI were chloroformed and examined. Guinea Pig 53 showed no characteristic lesions, and the bacterial count was low. Guinea Pigs 54

TABLE V.
Results of Inoculation of Guinea Pigs with a Pure Culture of B. abortus, Isolated through Guinea Pigs from Cow 214, the Number of Organisms Injected Being Gradually Diminished by Increasing the Dilution.

Guinea pig No.	Sex.	Dose injected.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight - gm.	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
46	Male.	1 cc. of 1:50 the original dilution.	23 days	+	Possibly very slight increase in bulk; hyperemic; nodular surface.	Minute necrotic foci in liver; spermat-ic vessels injected.	Spleen: 170 Retrogastric lymph node: 100	+
47	"	1 cc. of 1:100 the original dilution.	23	+154	Twice normal bulk; mild congestion; surface nodular.	Few minute necrotic foci in liver.	Spleen: countless. Retrogastric lymph node: 48	+
48	"	1 cc. of 1:200 the original dilution.	23	+105	Hyperemic; no enlargement.	Few minute necrotic foci in liver.	Spleen: 50 Retrogastric lymph node: 15	+
49	"	1 cc. of 1:400 the original dilution.	23	+152	Twice normal bulk; congested.	Liver slightly enlarged; few minute necrotic foci. Sper-matic vessels in-jected.	Spleen: 215 Retrogastric lymph node: 25	+
50	"	1 cc. of 1:800 the original dilution.	23	+167	Slight enlargement; hyperemic.	Two or three minute yellowish foci in liver; kidneys very dark.	Colonies present in all cultures.	+
51	"	1 cc. of 1:1,600 the original dilution.	23	+142	Normal.	Small intestine con-gested.	Spleen: 140 Retrogastric lymph node: 125	+
52	"	1 cc. of 1:3,200 the original dilution.	23	+92	Hyperemic.	A few minute yellow-ish areas in liver.	Colonies present in all cultures.	+

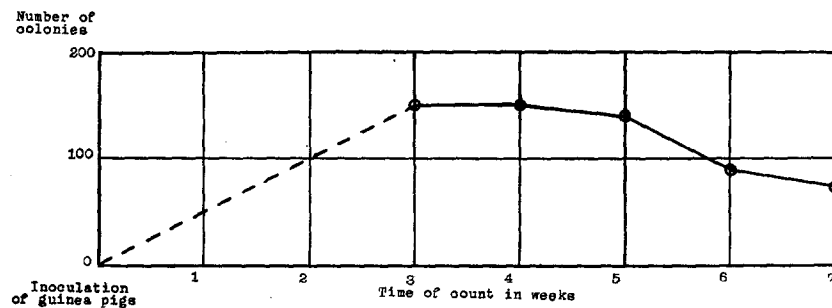
TABLE VI.
Results of Inoculation of Three Guinea Pigs with Fresh Material from Different Sources. Autopsies Performed about 4 Months after Injection.

Guinea pig No.	Sex.	Method of inoculation.	Length of time after inoculation when guinea pig was killed.	Gain in weight + Loss in weight - gm.	Condition of spleen.	Other lesions.	No. of colonies on agar slant.	Result.
53	Male.	Intraperitoneal.	123 days	+293	Twice normal bulk; hyperemic; surface faintly nodular.	Liver dark; few small circumscribed tubercles. Vessels supplying testicles injected.	Spleen: 16 Liver: 1 Testicle: 0 Kidney: 0	+
54	"	"	118	+215	Bulk increased at least ten times; very dark; surface nodular and easily torn.	Few minute tubercles in liver. Induration of epididymis of both testicles; small necrotic center to the mass.	Spleen: 45 Liver: 2 Testicle: 0 Kidney: 0	+
55	"	"	110	+291	Bulk increased at least ten times; severe congestion; surface nodular; capsule easily torn.	Few minute circumscribed tubercles in liver. Atrophy of testicles; induration of epididymis; vessels injected.	Spleen: 90 Liver: 2 Testicle: 0 Kidney: 0	+

and 55 presented the characteristic enormously enlarged, nodular spleen, atrophy of the testicles, and induration of the epididymis. In all three the number of colonies, counted in agar slant cultures of the spleen, was less than those counted on cultures prepared from guinea pigs autopsied between the 3rd and 4th weeks after inoculation.

Analysis of Results.

The foregoing experiments tend to confirm earlier work in demonstrating an inoculation disease in guinea pigs due to *Bacillus abortus* of Bang which is regularly associated with an enlarged, congested spleen. Other less constant lesions affect the testicles, kidneys, and bones.



The broken line indicates no count made; the solid portion indicates the beginning of the count.

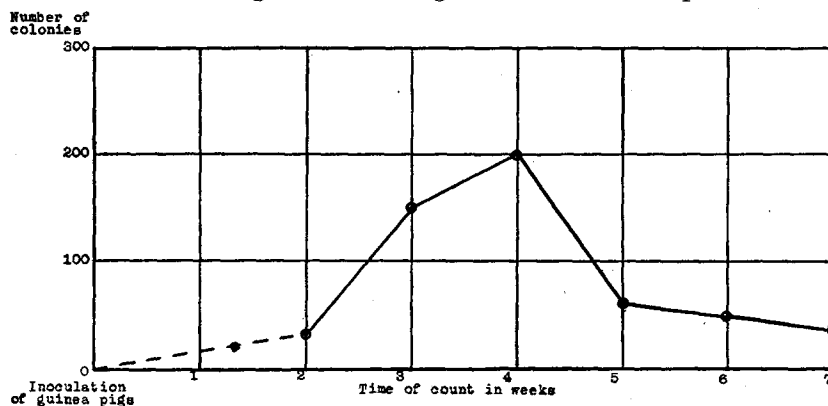
TEXT-FIG. 1. Guinea pigs of Series I inoculated with *B. abortus* 86. Colonies counted in cultures from spleens.

Minute foci frequently occur in the liver. There is, as a rule, no local lesion after subcutaneous inoculation. The animal regularly gains in weight. There is no appreciable difference in the result, whether the infection is introduced subcutaneously or into the abdomen. Nor does the size of the infecting dose within certain limits affect the result. Amounts varying between a standard dose and one-sixty-fourth of the same produced nearly the same results.

The immediate object of the investigation was to determine how far the incubation period in the guinea pig could be shortened. The results given above and Text-figs. 1 and 2 show that cultures of *Bacillus abortus* are regularly recovered from inoculated guinea pigs within

3 to 4 weeks. The figures obtained show that the number of living bacteria in the spleen of the guinea pig is larger at that time than later, although the macroscopic lesions tend to become more prominent as the bacteria decline. It remains to be seen whether the period cannot be shortened still more.

At least two or three culture tubes should be inoculated with bits of spleen tissue. In the series described such cultures were successful in 53 out of 55 cases. The two negative inoculations came from guinea pigs not affected with the disease; *i.e.*, they did not receive *Bacillus abortus* in the material inoculated. For diagnostic purposes there is no need of inoculating tubes from organs other than the spleen.



TEXT-FIG. 2. Guinea pigs of Series III inoculated with Placenta 146. Colonies counted in cultures from spleens.

CONCLUSIONS.

1. *Bacillus abortus* Bang can be regularly recovered from guinea pigs inoculated with material containing the bacillus within 3 to 4 weeks.
2. The method is especially useful in recovering the organism from fetal membranes which, as a rule, are obtained after having come in contact with fecal matter, bedding, etc.
3. The spleen is the organ in which the bacteria are regularly present and in largest numbers. Cultures must be made from it to ensure success.