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KANSAS STATE AGRICULTURAL COLLEGE
MANHATTAN, KANSAS

INFECTIOUS ABORTION INVESTIGATIONS

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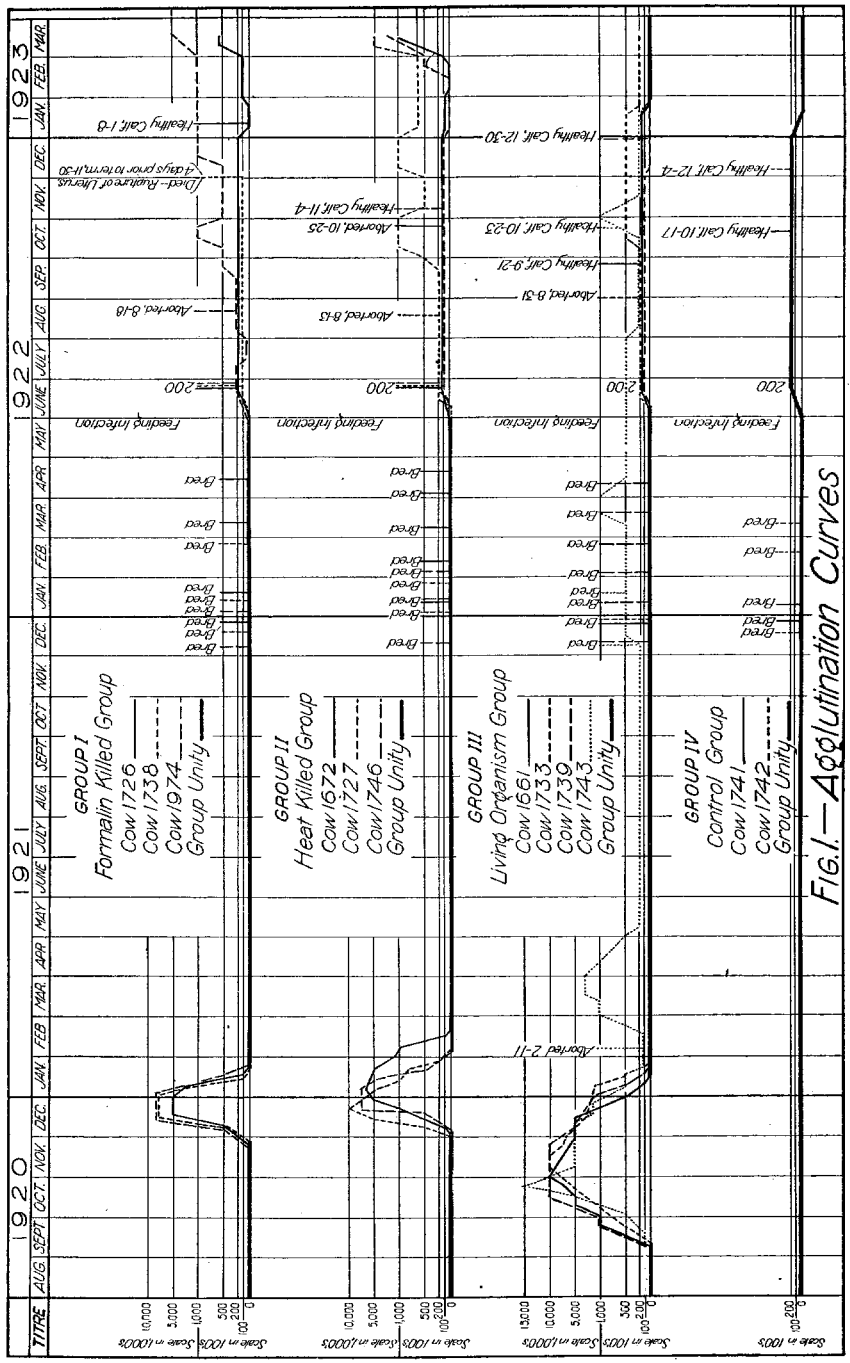
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INFECTIOUS ABORTION INVESTIGATIONS.[1]

H. F. LIENHARDT, C. H. KITSELMAN, AND C. E. SAWYER.

FOREWORD.

Infectious abortion of cattle has become a problem of world-wide importance. The cattle industry of Kansas, like that of every other state in the union, suffers from the effects of the disease. Because of the damage done by the disease, cattle breeders and practicing veterinarians are growing more insistent each year that a solution of the abortion problem be found.

The Kansas legislature of 1919 appropriated the sum of $5,000 a year to enable the Agricultural Experiment Station to study the disease. The appropriation was renewed by the legislatures of 1921 and 1923. This publication gives a brief description of the work done by the Agricultural Experiment Station on the abortion problem from July 1,1919, to June 30,1924.

STUDIES OF THE CAUSES OF ABORTION.

In working on this problem, it was important to find out as much as possible about the cause of the disease and what part of the infected animal harbors the germ. Also it seemed desirable to make a laboratory study of the germ which is supposed to cause the disease. This organism is known as *Bacterium abortum* (Bang). Most investigators are agreed that the germ is found in the uterus during pregnancy, but the fact is well known that the germ leaves the uterus within three weeks following an abortion and is then found in the udder. The aborted calf very commonly carries the germ, as does the after-birth or placenta. The bull rarely carries the germ, and when he is infected the germ occurs in an abscessed condition of the sexual organs. The digestive tract of the aborted calf, together with the spleen, kidneys, lungs, and occasionally the joints, show the presence of the germ upon bacteriological examination.

When a guinea pig is injected with material containing the organism, *Bacterium abortum* (Bang), a characteristic condition is often produced in the animal's spleen. This well-known fact gave rise to the belief that possibly a large animal, such as a cow, might also show a similar condition if injected with the germ.

1. Contribution No. 31 from the Department of Veterinary Medicine.

For the purpose of studying this feature of the problem, two heifers, under breeding age, were injected intraperitoneally with a large dose of germs which had been grown in pure culture on laboratory media. After 30 days had elapsed (the time required to produce the diseased condition of the spleen in the guinea pig) both heifers were killed and examined by careful microscopic and bacteriological methods. No change of a pathological nature could be seen by the naked eye or by the aid of the microscope. In one animal the germ was isolated and identified.

It has been commonly shown that certain disease germs upon entering an animal's body cause a marked or measurable increased production of substances in that animal's blood which aid in combating the disease. These are called anti-bodies and are of three kinds. When the agglutination test is used in the laboratory to measure their concentration, it is those anti-bodies called agglutinins that are measured. In both of the animals referred to there was a decided increase in the number of agglutinins.

A noninfected animal will not respond to this test when one part of its blood serum is placed in contact with 40 parts of treated germs in a test tube at body temperature for 24 hours. Both of these heifers were tested before being infected and were found to be negative or noninfected. Following the injection their blood serum would agglutinate, or give a positive reactive in a dilution of 1 part blood serum to 1,000 parts of the treated germ emulsion in one case, and 1 part of blood serum to 11,000 parts of the germ emulsion in the other case. It is, therefore, evident that although an animal may show response in its blood there may be no evidence of disease in any of its functional organs.

The germ which is believed to cause abortion is a very small rod-shaped organism, which is negative to the Gram stain but which readily takes most of the ordinary laboratory stains. The germ possesses no power of locomotion. When examined fresh from the animal body the organisms may be pleomorphic; that is, they may occur in single rods on some parts of the slide and in irregular groups or chains on other parts.

Some organisms have the power to ferment sugars, producing acid and gas. *Bacterium abortum* (Bang) does not produce acid or gas upon mannite, xylose, arabinose, lactose, saccharose, raffinose, innulin, galactose, glucose, levulose, inosite, maltose, salacin, sorbite, rhamnose, or adonite.

In this investigation the organism has not been grown on asparagin. No strain of *Bacterium abortum* (Bang) has produced indol in peptone water (Dunham) or acidified litmus milk. On the other hand, after a week in the incubator the color is deepened, showing the tendency of the organism to produce alkalinity in the medium.

There seems to be a tendency for certain strains of these organisms as they grow older to lose their power to produce lesions or diseased spleen in laboratory animals; that is, after a number of generations of culturing under artificial conditions. It has been observed that some organisms isolated from swine abortions have the power to cause diseased joint conditions in guinea pigs in addition to the aforementioned spleen lesions. In these investigations it has not been possible to cause pronounced joint lesions with a strain of *Bacterium abortum* (Bang) obtained from a cow.

A number of different kinds of media were used in an endeavor to find some relatively easy yet reliable method of isolating the germ from suspected material. Examinations were made of 119 cases of suspected material, all in a good state of preservation, cultured in this laboratory during the past five years. All of this material was cultured by the direct method; that is, petri dishes were inoculated and incubated, and by the indirect or guinea pig injection method. At the end of four weeks these injected animals were killed and tests made for the presence of *Bacterium abortum* (Bang). Each sample was cultured upon the following media: (1) Two per cent glycerine agar (liver infusion) plus 10 per cent sterile horse serum. The media had a final titre of 7.2 p. H., or slightly alkaline. (2) Same media with **2** per cent glucose added. (3) One-tenth of 1 per cent agar with a final titration of 7.4 p. H., or quite alkaline in reaction.

The following methods of incubation were used: (1) In a sealed jar, the oxygen of which had been exhausted by burning a candle. (2) In a sealed jar having all the air replaced by carbon dioxide. (3) In a sealed jar having all the air replaced by pure oxygen. (4) By attaching a slant seeded with *B. Subtilis or* Staphlococcus by means of a rubber tube to a slant of media after inoculating with the suspected material. (5) By growing *B. subtilis* plates in the same jar as the suspected plates. (See References: Nowak.)

Of all the methods and kinds of media used the 2 per cent glucose, 2 per cent glycerine, 7.2 p. H. agar plus 10 per cent horse serum grown in a sealed jar with 10 per cent carbon dioxide gas, has yielded the most vigorous growing organisms.

The suspected material consisted of vaginal discharges, afterbirths, aborted fetuses, and milk from animals which had recently aborted.

Some of the cases did not show the presence of any organism capable of producing disease. Other cases readily yielded *Bacterium abortum* (Bang), the generally accepted cause of infectious abortion of cattle. The presence of *Bacterium abortum* (Bang) was shown in 34.5 per cent of the cases. The remainder, 65.5 per cent, did not show any evidence of this organism. (Table I.) The correlations between a positive agglutination reaction and the recovery of *Bacterium abortum* (Bang) was found to be an unreliable index. (Table II.) Still other cases yielded no colon-typhoid group of organisms. These are the organisms usually associated with scours in calves and sterility in cattle. (Table III.)

In view of the fact that all the specimens represented in the 119 cases came from animals having the symptoms or history of infectious abortion, the striking per cent mentioned above should not be passed over without considering the other organisms found, and attemping to associate them with this disease as causative or associated factors.

TABLE I.—Results of examination of suspected material for *Bacterium abortum* (Bang).

METHOD OF EXAMINATION.	Number of cases positive.	Per cent of cases positive.	Number of cases negative.	Per cent of cases negative.
By cultural method alone	12	10.0	.....	.....
By animal inoculation alone	4	3.4	.....	.....
By both cultural method and animal inoculation	25	21.1	78	65.5
Total of all methods	41	34.5	78	65.5

TABLE II.—Comparison of agglutination reactions with bacteriological findings.

	Number of cases positive for *Bacterium abortum* (Bang).	Per cent of cases positive.	Number of cases negative for *Bacterium abortum* (Bang).	Per cent of cases negative.
When cow's blood was positive	27	22.7	16	13.2
When cow's blood was negative	3	2.5	30	25.2
When no blood was tested	11	9.3	32	27.1
Total	41	34.5	78	65.5

TABLE III.—Summary of bacteriological findings from 119 examinations.

	Number of cases.	Per cent of cases.
Yielding *Bacterium abortum* (Bang) alone	21	17.6
Yielding *Bacterium abortum* (Bang) plus other pathogenes	20	16.8
Yielding pathogenes not *Bacterium abortum* (Bang)	59	49.6
Sterile (no organism isolated)	19	16.0
Total	119	100.0

BEHAVIOR OF BACTERIUM ABORTUM (BANG).

For the purpose of finding a difference, if any existed, between the biological reactions of different strains of *Bacterium abortum* (Bang), an experiment was planned using twenty stock strains of this organism isolated from cases of bovine abortion. It was thought that there might be some evidence of certain strains falling into two or more groups based particularly upon their agglutination reaction, which, if true, would be of considerable diagnostic importance.

PHYSIOLOGICAL REACTIONS.

No difference was discovered between strains of *Bacterium abortum* (Bang) in either morphology or sugar reactions, with but three exceptions. These were strains which had been carried in stock for about a year and had originally come from laboratories outside of Kansas.

A careful study was made of these using 16 different sugars. No gas was produced by any strain after 10 days incubation. One strain produced a slight (barely perceptible) amount of acid in saccharose and raffinose after five days incubation; one strain produced a like amount of acid in maltose, lactose, salacin, glucose, and levulose in the same length of time; and one strain produced a slight amount of acid in maltose, saccharose, and raffinose after five days incubation. Therefore, these strains were judged to be members of a colon subgroup and not true strains of *Bacterium abortum* (Bang).

SEROLOGICAL REACTIONS.

Seventeen strains of *Bacterium abortum* (Bang) and the three colon sub group strains noted above were used on cross agglutination tests. This work was done as follows: A separate rabbit was used for each strain to develop an immune serum. Injections were made at three-day intervals until the rabbit's serum would agglutinate the strain emulsion, antigen (An.), in dilution of 1-5,000, or higher.

Then each rabbit's serum was used to cross agglutinate each of the other strain antigens (bacterial emulsions). To illustrate, serum No. 1 was used on antigens Nos. 2, 3, etc., and serum No. 2 on antigens 1, 3, 4, etc., in addition to their own antigens.

No difference could be noticed among the behaviors of the 17 strains tested, so it was concluded that no difference existed, at least among this number of strains. (Table IV.) Strains Nos. 2, 5, and 18 were isolated from cases of abortion in cattle, but are not strains of *Bacterium abortum* (Bang) and seem to bear little if any relation to each other, with the exception that strain No. 18 agglutinates antigen No. 2 in dilution of 1-200. Serum No. 2 agglutinated *Bacterium abortum* (Bang), strain No. 9, in dilution of 1-2,000, although No. 2 is a member of a colon subgroup.

TABLE IV.—Results of cross agglutination tests.

An.	Titre.	An.	Titre.	An.	Titre.	An.	Titre.
Serum No. 1.		**Serum No. 2.**		**Serum No. 3.**		**Serum No. 4.**	
1	1–10 000	2	1–4 000	3	1–10 000	4	1–10 000
2	Negative	3	Negative	4	1–10 000	5	Negative
3	1– 8 000	4	Negative	5	Negative	6	1–10 000
4	1–10 000	5	Negative	6	1–10 000	7	1–10 000
5	Negative	6	Negative	7	1–10 000	8	1–10 000
6	1–10 000	7	Negative	8	1–10 000	9	1–10 000
7	1– 8 000	8	Negative	9	1–10 000	10	1–10 000
8	1–10 000	9	1–2 000	10	1–10 000	11	1–10 000
9	1–10 000	10	Negative	11	1–10 000	12	1–10 000
10	1–10 000	11	Negative	12	1–10 000	13	1– 7 000
11	1–10 000	12	Negative	13	1– 7 000	14	1– 7 000
12	1–10 000	13	Negative	14	1– 7 000	15	1–10 000
13	1– 8 000	14	Negative	15	1–10 000	16	1–10 000
14	1– 8 000	15	Negative	16	1–10 000	17	1–10 000
15	1–10 000	16	Negative	17	1–10 000	18	Negative
16	1–10 000	17	Negative	18	Negative	19	1– 7 500
17	1– 8 000	18	Negative	19	1–10 000	20	1–10 000
18	Negative	19	Negative	20	1–10 000	1	1–10 000
19	1–10 000	20	Negative	1	1– 8 000	2	Negative
20	1–10 000	1	Negative	2	Negative	3	1–10 000
Serum No. 5.		**Serum No. 6.**		**Serum No. 7.**		**Serum No. 8.**	
5	1–5 000	6	1–15 000	7	1–15 000	8	1–20 000
6	Negative	7	1–10 000	8	1–15 000	9	1–20 000
7	Negative	8	1–10 000	9	1–15 000	10	1–15 000
8	Negative	9	1–15 000	10	1–10 000	11	1–15 000
9	Negative	10	1–10 000	11	1–15 000	12	1–15 000
10	Negative	11	1–10 000	12	1–10 000	13	1–10 000
11	Negative	12	1–10 000	13	1–10 000	14	1–10 000
12	Negative	13	1–10 000	14	1–10 000	15	1–20 000
13	Negative	14	1–10 000	15	1–15 000	16	1–20 000
14	Negative	15	1–10 000	16	1–15 000	17	1–20 000
15	Negative	16	1–20 000	17	1–15 000	18	Negative
16	Negative	17	1–10 000	18	Negative	19	1–20 000
17	Negative	18	Negative	19	1–15 000	20	1–15 000
18	Negative	19	1–15 000	20	1–15 000	1	1–15 000
19	Negative	20	1–10 000	1	1–10 000	2	Negative
20	Negative	1	1–15 000	2	Negative	3	1–15 000
1	Negative	2	Negative	3	1–10 000	4	1–20 000
2	Negative	3	1–20 000	4	1–10 000	5	Negative
3	Negative	4	1–15 000	5	Negative	6	1–15 000
4	Negative	5	Negative	6	1–15 000	7	1–15 000
Serum No. 9.		**Serum No. 10.**		**Serum No. 11.**		**Serum No. 12.**	
9	1–10 000	10	1–10 000	11	1–10 000	12	1–10 000
10	1–10 000	11	1–10 000	12	1–10 000	13	1–10 000
11	1–10 000	12	1–10 000	13	1–10 000	14	1–10 000
12	1–10 000	13	1– 8 000	14	1–10 000	15	1–10 000
13	1–10 000	14	1–10 000	15	1–10 000	16	1–10 000
14	1–10 000	15	1–10 000	16	1–10 000	17	1– 8 000
15	1–10 000	16	1–10 000	17	1–10 000	18	Negative
16	1– 7 000	17	1–10 000	18	Negative	19	1–10 000
17	1–10 000	18	Negative	19	1–10 000	20	1–20 000
18	Negative	19	1–10 000	20	1–10 000	1	1–10 000
19	1–10 000	20	1–10 000	1	1–10 000	2	Negative
20	1–10 000	1	1–10 000	2	Negative	3	1–10 000
1	1– 8 000	2	Negative	3	1– 8 000	4	1–10 000
2	Negative	3	1–10 000	4	1– 8 000	5	Negative
3	1–10 000	4	1–10 000	5	Negative	6	1–10 000
4	1– 7 000	5	Negative	6	1–10 000	7	1–10 000
5	Negative	6	1–10 000	7	1–10 000	8	1–10 000
6	1–10 000	7	1–10 000	8	1–10 000	9	1–10 000
7	1–10 000	8	1–10 000	9	1–10 000	10	1–10 000
8	1–10 000	9	1–10 000	10	1– 8 000	11	1–10 000

TABLE IV.—CONCLUDED.

An.	Titre.	An.	Titre.	An.	Titre.	An.	Titre.
Serum No. 13.		**Serum No. 14.**		**Serum No. 15.**		**Serum No. 16.**	
13	1–7 000	14	1–10 000	15	1–10 000	16	1–10 000
14	1–8 000	15	1–10 000	16	1–10 000	17	1–10 000
15	1–8 000	16	1–10 000	17	1–10 000	18	Negative
16	1–8 000	17	1–10 000	18	Negative	19	1–10 000
17	1–8 000	18	Negative	19	1–10 000	20	1–10 000
18	Negative	19	1–10 000	20	1–10 000	1	1–10 000
19	1–7 000	20	1– 9 000	1	1–10 000	2	Negative
20	1–8 000	1	1–10 000	2	Negative	3	1–10 000
1	1–8 000	2	Negative	3	1–10 000	4	1–10 000
2	Negative	3	1–10 000	4	1–10 000	5	Negative
3	1–7 000	4	1–10 000	5	Negative	6	1–10 000
4	1–7 000	5	Negative	6	1–10 000	7	1–10 000
5	Negative	6	1– 9 000	7	1–10 000	8	1–10 000
6	1–7 000	7	1– 9 000	8	1–10 000	9	1–10 000
7	1–7 000	8	1– 9 000	9	1–10 000	10	1–10 000
8	1–7 000	9	1– 8 000	10	1–10 000	11	1–10 000
9	1–7 000	10	1– 8 000	11	1–10 000	12	1–10 000
10	1–7 000	11	1– 8 000	12	1– 8 000	13	1–10 000
11	1–7 000	12	1– 8 000	13	1–10 000	14	1–10 000
12	1–7 000	13	1– 8 000	14	1–10 000	15	1– 9 000
Serum No. 17.		**Serum No. 18.**		**Serum No. 19.**		**Serum No. 20.**	
17	1–10 000	18	1–5 000	19	1–10 000	20	1–10 000
18	Negative	19	Negative	20	1–10 000	1	1–10 000
19	1–10 000	20	Negative	1	1–10 000	2	Negative
20	1–10 000	1	Negative	2	Negative	3	1–10 000
1	1–10 000	2	1–200	3	1– 9 000	4	1–10 000
2	Negative	3	Negative	4	1–10 000	5	Negative
3	1–10 000	4	Negative	5	Negative	6	1–10 000
4	1–10 000	5	Negative	6	1–10 000	7	1– 8 000
5	Negative	6	Negative	7	1–10 000	8	1–10 000
6	1–10 000	7	Negative	8	1–10 000	9	1–10 000
7	1–10 000	8	Negative	9	1–10 000	10	1–10 000
8	1–10 000	9	Negative	10	1–10 000	11	1–10 000
9	1– 8 000	10	Negative	11	1–10 000	12	1–10 000
10	1–10 000	11	Negative	12	1–10 000	13	1–10 000
11	1–10 000	12	Negative	13	1–10 000	14	1–10 000
12	1–10 000	13	Negative	14	1–10 000	15	1– 7 000
13	1–10 000	14	Negative	15	1–10 000	16	1–10 000
14	1–10 000	15	Negative	16	1–10 000	17	1–10 000
15	1–10 000	16	Negative	17	1–10 000	18	Negative
16	1–10 000	17	Negative	18	Negative	19	1–10 000

NOTE.—An., antigen.

AEROPHILIC RELATIONS.

Pure Oxygen.

Some work was done in isolating *Bacterium abortum* (Bang) from aborted fetuses by using pure oxygen. The oxygen was generated chemically and the Novy jar containing culture plates was connected to the generator and all of the air was replaced by oxygen. About ten attempts were made using this method. Control jars seeded, by the Nowak method, with *B. subtilis* were also used at the same time. The results were so much better with the Subtilis jar method that the oxygen method was discontinued.

Substitution of an Inert Gas for Oxygen.

This phase of the work was done in an endeavor to find some more favorable atmosphere to use in isolating *Bacterium abortum* (Bang) than the Nowak (Subtilis jar) method and consisted in replacing the normal air content of the Novy jar with carbon dioxide gas from a drum. In this method, as well as in all experiments upon cultural technique, a standard method (Nowak) was used for routine as well as for checks. The number of isolations of *Bacterium abortum* (Bang) by the use of 100 per cent carbon dioxide gas was very low and unsatisfactory.

Partial Anaerobiosis.

Culture plates were placed in a Novy jar together with a lighted candle and the jar was sealed, the intention being to convert the oxygen in the jar into carbon dioxide. This method gave a higher per cent of cultures than either of the others, and was used until Huddleson published his modified method of replacing 10 per cent of the air in a closed jar with carbon dioxide gas. Very satisfactory results are obtained by this method.

A modification of the *B. subtiliis* jar method was made and found fairly satisfactory. This consisted of the following: A large tube containing a 2 per cent agar slant was streaked with the suspected material, the upper one-third of the tube had an upright vial containing bouillon seeded with *B. subtilis*. The tube was sealed and incubated for three to five days. The action of this method was identical with that of the Nowak method and yielded about the same per cent of strains of *Bacterium abortum* (Bang).

A low per cent agar was also used in isolating from the organs of an aborted fetus. This consisted of a slightly alkaline bouillon containing one-tenth of 1 per cent agar in suspension. This method yielded a good per cent of cultures, but difficulties were encountered in getting the cultures to grow. In transplanting to solid media it

sometimes required two or three intermediate soft agar media (four-tenths of 1 per cent) transplantations before a strain would grow on 2 per cent agar.

SUMMARY.

1. In the attempt to produce experimental lesions in heifers, it was found that their blood reacted in high dilution to the agglutination test following a single injection of living organisms.

2. No lesions were found upon slaughtering these animals.

3. *Bacterium abortum* (Bang) did not produce acid or gas on any sugar used.

4. No indol was produced in Dunham's peptone water by any strain of *Bacterium abortum* (Bang).

5. Glycerinated serum agar containing glucose is the best medium for isolating *Bacterium abortum* (Bang) when the atmosphere of the container is 10 per cent carbon dioxide gas.

6. About 16 per cent of all the cases of abortion examined were bacteriologically sterile.

7. No difference could be ascertained between different strains of *Bacterium abortum* (Bang) of bovine origin.

ATTEMPTED PRODUCTION OF IMMUNITY.

An experiment was begun in September, 1920, to determine the relative values of certain bacterial products as preventives of the abortion disease. The facilities available limited the size of experimental herd to 12 animals. These animals were purchased on the open market at an average age of six months. Nothing is known of their previous history. The breeding is mixed in most of the cows, although several are grade Holsteins or grade Shorthorns. The bull used is a grade Hereford.

The herd was divided into four groups, and the following broad principles were outlined and carried out in considerable detail: All the animals were tested by the agglutination and the complement fixation tests at the time of purchase, and subsequently at weekly intervals by the agglutination test. Graphs were drawn from the results of the agglutination tests. Three of the groups were vaccinated, bred, and then fed *Bacterium abortum* (Bang) contaminated feed and water. The control group was handled the same as the others, except that no vaccines were administered. The groups were treated as follows:

Group 1. *Treated with Heat-killed Organisms:* Three animals were injected subcutaneously at weekly intervals with three doses

of physiological saline suspension of *Bacterium abortum* (Bang) heated to 60°C. for one-half hour or until the germs were killed.

Group II. *Treated with Formalin-killed Organisms:* Three animals were injected subcutaneously at weekly intervals with three doses of a physiological saline suspension of *Bacterium abortum* (Bang) which had been exposed to a 1 per cent solution of formalin until the organisms were killed.

Group III. *Treated with Living Organisms:* Four animals were injected subcutaneously at weekly intervals with five doses of living *Bacterium abortum* (Bang) suspended in physiological saline suspension.

Group IV. *Control group, untreated:* Two animals were left unvaccinated as controls.

It was thought that, there might be some difference between the immunity conferred by treatment with a bacterin killed with heat and that conferred by treatment with one killed with a chemical, such as formalin. As soon as the animals were vaccinated they were removed to a separate lot. When a definite diagnosis of pregnancy could be made, all the animals were brought together for the feeding infection. This consisted of the use of massive bouillon cultures of several strains of *Bacterium abortum* (Bang) grown in the laboratory. The feed and water were infected three times weekly with the bouillon cultures. Pregnancy was determined by the cessation of oestrum for two periods and by manual palpation of the uterus per rectum.

The animals were all vaccinated before attaining sexual maturity. The blood of every animal, when vaccinated, changed from negative to a high positive titre. A careful record of breeding troubles and abortions was kept and correlations were made, when possible, between them and the blood titre as shown by the agglutination test.

At the time of writing all the animals have completed their first lactation periods and some have completed their second. It is intended to run this experiment over two successive lactation periods.

RESULTS OF FIRST CALF CROP.

In Group I, all the animals showed a decided response of 1-7,000, or higher, to the agglutination test immediately after their third injection. The titre remained positive for a period of about seven weeks before again becoming negative. There were two abortions in this group, or 66.6 per cent. (Group I, fig. 1.)

In Group II, one cow aborted, or 33.3 per cent. One cow died,

four days prior to her full period, from rupture of the uterus. As the fetus was full time and cultured negative for *Bacterium abortum* (Bang), it is not considered as an abortion. All the animals of this group responded to the vaccination by showing as high a titre as 1-5,000 or higher, to the agglutination test. They remained positive about as long as did those in Group I. (Group II, fig. 1.)

In Group III, one cow aborted, or 25 per cent. The calf in this instance was born alive but was premature and very weak. All the animals in this group responded to vaccination by reacting in dilution as high as 1-10,000, or higher, to the agglutination test. Their titres remained at the peak for a longer time than did those of Groups I and II. (Group III, fig. 1.)

In Group IV, the animals were not vaccinated, and their titres remained negative until feeding infection was commenced. Both the animals in this group had normal calves, or 100 per cent calf crop. (Group IV, fig. 1.)

SUMMARY.

The work on this phase of the project is not yet completed. The small number of animals used does not warrant the drawing of final conclusions. Attention should be directed, however, to certain results of this experiment in the hope that further work can be done either to substantiate them or to prove them erroneous.

1. The herd bull reacted negatively throughout the experiment.

2. The attempt at feeding the infection was to adhere as closely as possible to natural infection or the manner in which most cows are believed to pick up their infection on the farm, through contaminated feed and drinking water. All the animals, with but one exception, responded exactly the same to the feeding infection; that is, their titre rose from negative to 1-200, which is a positive titre. (Fig. 1.)

3. In no case did a cow herald approaching abortion by a sudden rise in her blood reaction. However, three of the animals which did abort showed a rise in their blood reaction about five weeks later.

4. One animal was accidentally impregnated by a neighboring bull. As this fact was unknown at the time, this cow was vaccinated with the other animals in her group. The fact that she did not react positively until after her vaccination and the occurrence of her abortion several months later, lead one to consider this incident as a good check upon the abortion-producing power of the strains of *Bacterium abortum* (Bang) used in these experiments.

5. If the length of time the various group reactions remained positive in this experiment is a reliable index of the immunity produced, then the living organism vaccine should be given precedence. It is doubtful, however, whether the evidence in this case is sufficient to warrant definite comparisons,

6. Since in Groups I, II, and III attempts at immunization were made by using either bacterins or vaccines, and abortions occurred, while in the control group no such attempts were made, and no abortions occurred, the efficacy of the three supposed preventives must be doubted.

7. Attention should be drawn to the bacteriological findings shown in Table III.

8. The results of isolation from the experimental herd under examination are found in Table V.

TABLE V.—Results of bacteriological examinations.

GROUP.	Cow No.	Material.	Direct method.	Indirect method.	Pig No.	Blood test.	
						Pig.	Cow.
Living organism.	1661	Milk	Sterile	*Staphlococcus albus*	1284	1–500	1–200
		Placenta,	*Staphlococcus aureus*	*Staphlococcus aureus*	1278	1–1,000	
			Staphlococcus citreus	*Staphlococcus pyogenes bovis*	1279	.....	
	1733	Milk	Sterile	*Staphlococcus albus*	1273	1–1,000	1–200
		Calf	*Colon* Group	*Colon* Group	1287	Negative	
				Gram + Coccus	1276	.....	
		Placenta,	*Colon* Group	*Bacterium abortum* (Bang)	1270	Negative	
					1271	1–200	
	1739	Milk	*Streptococcus lacticus*	Sterile	1471	.....	1–200
		Placenta,	*Aspergillus niger*	*Aspergillus niger*	1416	.....	
	1743	Milk	Sterile	.....	.....	.....	.....
		Mucus, vaginal,	Sterile	*Bacterium coli communior*	1403	.....	1–500
Control.	1741	Milk	*Streptococcus lacticus*	*Staphlococcus citreus*	1293	Negative	1–200
		Placenta,	*Staphlococcus albus*	Sterile	1292	Negative	
	1742	Milk	*Bacterium acidi lactici*	Sterile	1415	Negative	1–200
		Calf	Sterile	.....	.....	.....	
Heat killed.	1672	None	.....	.....	.....	.....	1–200
	1727	Milk	.....	*Bacterium abortum* (Bang)	1272	1–200	1–200
					1285	1–1,000	
					1256	1–200	
		Fetus	*Bacterium abortum* (Bang)	*Bacterium coli communior*	1258	.....	
		Placenta,	*Staphlococcus albus*	*Staphlococcus albus*	1265	1–200	
				Bacterium abortum (Bang)	1257	1–200	
	1746	Milk	Sterile	*Staphlococcus albus*	1402	.....	1–200
		Placenta,	*Bacterium coli communior*	*Gram—Coccus*	1400	Negative	
	1726	Milk	*Strephtococcus lacticus*	*Colon* Group	1422	.....	Negative
		Placenta,	*Colon* Group	.....	.....	.....	

Formalin killed.	1738	Milk.....	*Streptococcus lacticus*.....	Sterile.....	1409	Negative	1–200
		Fetus....	*Staphlococcus citreus*.....	*Staphlococcus pyogenes bovis*.....	1408	.....	
		Placenta,	*Staphlococcus lacticus*.....	Sterile.....	1407	Negative	
	1974	Milk.....	*Bacterium acidi lactici*.....	*Bacterium coli aerogenes*.....	1277	.....	1–200
				Bacterium abortum (Bang).....	1286	.....	
			Bacterium abortum (Bang).....		1404	1–500	
				Colon Group.....	1259	.....	
				Staphlococcus albus.....	1266	.....	
		Placenta,	*Bacterium abortum* (Bang).....	*Bacterium abortum* (Bang).....	1274	.....	

A STUDY OF AGGLUTINATION TESTS IN EXPERIMENTAL ANIMALS.

WEEKLY TESTS IN ADULT COWS.

The result of the blood tests are shown on the graphs. Every animal was negative when put on the experiment and then was tested at weekly intervals. A variation is shown from week to week, with no apparent reason in some cases. The only explanation offered is that certain animals obtained a larger quantity of organisms in their feed or drinking water prior to the slight variation in their reaction. Some of the cows would voluntarily take the bouillon from the mouth of the flask when opportunity offered. Attention should be called to the uniformity of response when the feeding infection commenced. (Fig. 1.)

YOUNG ANIMALS SUBJECTED TO TEST AT BIRTH.

The attempt was made to obtain a blood sample from each calf as soon as possible after it was born; that is, before it suckled the dam. Since there was no caretaker on hand during the night this was not possible in all cases. It was found that some calves were born with positive reactions; that is, their blood reaction was positive before they ingested milk. In the cases when an animal was born negative in reaction and the dam was positive, the calf's blood took on the same reaction as the dam soon after ingesting milk from the dam. (Table VI.)

TABLE VI.—Blood reactions of calves.

GROUP.	Cow No.	Preliminary test.	Peak of vaccination.	Feeding infection.		Time of calving.	Six weeks later.	Calf at birth.	Condition of calf.	Remarks.
				Before.	After.					
Living organisms,	1661	Negative	1–10,000	Negative	1–200	C 1–200	1–200	Negative	White scours	Serum given
	1733	Negative	1–10,000	Negative	1–200	Ab 1–200	1–500	1–200	O. K.	
	1739	Negative	1–10,000	Negative	1–200	C 1–200	1–100	1–100	O. K.	
	1743	Negative	1–15,000	1–500	1–200	C 1–200	1–1,000	1–500	O. K.	
Heat killed.	1672	Negative	1– 7,000	Negative	1–200	Ab 1–200	1–200	.....	No calf obtained	
	1727	Negative	1–10,000	Negative	1–200	Ab 1–200	1–1,000	Negative	Born dead	
	1746	Negative	1– 8,000	Negative	1–200	C 1–200	1–200	Negative	Slight attack of scours	No treatment
Formalin killed.	1726	Negative	1– 5,000	Negative	1–200	C–Negative	1–100	Negative	O. K.	
	1738	Negative	1– 8,000	Negative	1–200	D 1–200	.....	Negative	Dead on Caesarian section	
	1974	Negative	1– 8,000	Negative	1–200	Ab 1–200	1–500	1–100	Couldn't suckle. Died	
Control.	1741	Negative	.....	Negative	1–200	C 1–200	1–200	Negative	O. K.	
	1742	Negative	.....	Negative	1–200	C 1–200	1–200	Negative	White scours	Serum given

NOTE.—C, calved normally; Ab, aborted; D, died.

This again shows the ease with which an animal may assume a positive blood titre from ingestion.

COMPARISON OF AGGLUTINATION REACTION OF MILK AND SERUM.

An effort was made to correlate the reaction of the cow's milk and serum immediately after the birth of the calf or the occurrence of an abortion. The milk sample was a composite sample of all four quarters. The results are shown in Table VII.

TABLE VII.—Comparison of milk and serum reaction to the agglutination test.

GROUP.	Cow No.	Blood.	Milk.
Living organism	1661	1–200	Negative
	1733	1–200	1–200
	1739	1–100	1–100
	1743	1–500	1–500
Heat killed	1672	1–200	None
	1727	1–200	1–100
	1746	1–200	Negative
Formalin killed	1726	1–200	Negative
	1738	1–200	Negative
	1974	1–200	1–100
Control	1741	1–200	Negative
	1742	1–200	Negative

It will be seen that a cow may have higher reaction blood serum than milk, and also a cow may show a decided positive reaction in her blood serum, and still remain negative in her milk titre.

SUMMARY.

1. A newly born calf may have a different blood reaction from its dam, but a day or so after nursing the dam its blood reaction tends to approach that of the dam.

2. Cross agglutination failed to show any existing difference between strains of *Bacterium abortum* (Bang).

NOTES.

1. Histologic sections were made from the following organs of the heifers used in the attempted production of lesions: Lungs, liver, spleen, kidneys, uterus, vagina, mammary glands, and supra-mammary lymph glands.

2. The calf of cow 1742 (control group) died four days after birth from exposure during a blizzard.

3. It was possible to isolate *Bacterium abortum* (Bang) from cows 1743 and 1974 at weekly intervals over a period of about six weeks. It seemed that drenches of formalin successfully eliminated the organisms from the milk. At least attempts to isolate them after the second dose of formalin were not successful.

4. Cow 1672 aborted when about six months pregnant and no trace of either the fetus or the placenta could be found, consequently no bacterial cultures were made. No milk had formed in her udder at that time.

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