

BACILLUS ABORTUS (BANG) AS AN ETIOLOGICAL FACTOR IN INFECTIOUS ABORTION IN SWINE

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So far as we are able to learn from the literature, the cause of infectious abortion in swine has never been determined. Lynch in his "Diseases of Swine," says, "Infectious abortion results from the infection of the genital passages by some specific germ, the true nature of which is as yet undetermined. The disease is not nearly so common as in mares and cows, and, while it may run through an entire herd, it is not likely to be spread from one farm to another except in unusual instances." He further states, "The infectious type of the disease is especially mild in its symptoms, and unless the animals are carefully watched the pigs may be slipped without any notice of the fact until several weeks later, when it is found that the sow is no longer pregnant." He also says, "The nearer to full term the sow is at the time of abortion, the less dangerous the occurrence and the more mild the symptoms. Signs of threatening abortion are loss of appetite, restlessness, making of the bed, shivering, trembling of the muscles, dulness, and in some cases very severe labor-pains."

While considerable work has been done with regard to infectious abortion in mares and cows in this laboratory, only three outbreaks of infectious abortion among sows, have come to our investigation. We have found that it is no uncommon occurrence for one or two sows in a herd to abort. Numerous cases of this kind have been studied by the writers, with no etiological results, which would lead to the opinion that such abortions were due to some accident.

In the large outbreak investigated by the writers early this year, some twenty sows aborted in rather quick succession. On visiting the place, it was found that one sow had aborted the night before, and two of the fetuses together with the attached afterbirth were brought to the laboratory for examination. The fetuses were fairly well developed, although not far enough along to have any hair. On one of the afterbirths there were noted numerous brownish, villus-like projections ranging in size from 1 to 3 mm. in diameter. They were so numerous in some places as to become confluent. On opening these, a dark serous fluid was noted. In our investigations upon the etiology of infectious abortion of animals, streak dilutions on agar or agar serum are always made and incubated under aerobic, Nowak, and anaerobic conditions. In this instance, streak dilutions were made on a series of agar plates, the agar being slightly alkaline to phenolphthalein, with material from the nodules on the afterbirth, from bits of the afterbirth from both pigs, and from the contents of the umbilicus, heart, liver, stomach and kidney of each fetus. From appearances, the kidneys of the pigs were very much enlarged and gorged with blood. As these pigs had lain on straw for a few hours, several of the plates showed on incubation in the air the growth of a considerable number of contaminating organisms, such as *B. coli*, *B. subtilis*, etc. This, of course is what would be expected on plating the afterbirth as the material had lain on straw for several hours before being secured by us. Cultures from the internal organs of the fetuses, however, were nearly sterile. The clear places on the plates were marked with India ink, after which they were subjected to the cultural method of Nowak.¹ The material was incubated at 37°C. for four days and removed from the jars. On casual examination of the petri dishes, we were led to believe that they contained nothing but the growth of bacteria usually encountered in plating material which had lain on the ground for some hours. Upon examining one of the plates carefully with a Coddington lens, however, there were noted in some of the clear spaces of the dishes a few very

¹ E. S. Good, Investigations of the Etiology of Infectious Abortion of Cows and Mares, Bull. No. 165, Ky. Agri. Exper. Sta., 1912, p. 249.

small dew drop like colonies, which on being examined with the microscope resembled in every respect colonies of the *B. abortus* of Bang. Examined by the aid of the hand lens they were nearly water clear to direct light and of bluish tint to reflected light. They were round and raised, with exceptionally well defined borders. Most of these minute dew drop colonies were homogeneous, with the exception that a few of them had a few granules in the center, typical of many colonies produced by the *B. abortus*. Viewing the larger colonies under the microscope, it was seen that the centers had assumed a granular consistency, while the outer portion was homogeneous and transparent. The colonies on some of the plates were so large that they might have been taken for some other species, measuring $1\frac{3}{4}$ mm. in diameter. These conformed, however, to similar colonies of *B. abortus* derived from the tissues of the cow. To reflected light they had assumed an amber color, the centers having a whitish appearance. On examining stained preparations of these colonies, the morphology of the organism was identical, so far as we could determine, with *B. abortus*. On examining the plates carefully with a Coddington lens and microscope, colonies resembling those mentioned and measuring from a pinpoint to 1.5 mm. in diameter were seen on streaks made from the blood of the umbilicus, on the streaks made from the small nodule-like growths on one of the afterbirths, and from the afterbirth and internal organs of the fetuses. Eighty-four colonies in all were counted on streaks made from one of the small nodules. Some of these colonies were so small that they could not have been seen with the naked eye. There were one or two colonies on the streaks made from the livers. The plates streaked with the amniotic fluid were almost completely covered with contaminating bacteria, but, in the clear spaces five dew drop colonies were noted. The streaks of the contents of the stomachs of both pigs showed numerous small dew drop colonies. Streaks from the kidneys were negative. Stained preparations had been made from the different organs mentioned but were not examined until the cultures had developed. Upon examining these preparations, germs identical with the *Bacillus abortus* (Bang) were seen in large numbers in those made from the stomach contents of the pigs.

Typical colonies were streaked on agar slants, some of which were incubated by the Nowak method and others in the air. At the end of twenty-four hours, no visible growth was noted on the streak cultures incubated in the air. At the end of forty-eight hours, however, some little growth could be detected even with the naked eye, and at the end of seventy-two hours, quite a luxuriant growth of the organism was obtained, in appearance identical with streak cultures made with the *B. abortus*. On examining the tubes which had been incubated under the Nowak method, we found that they had made but slightly better growth than those incubated in the air. Streaks on agar at room temperature showed no growth in the time mentioned. We came to the conclusion that if this organism was the *B. abortus* and grew in the air after the first generation it was different from any we had ever isolated. The organism responded to the following tests in the following manner: It was found to be non-motile; gram negative; did not produce gas in either lactose or glucose; did not coagulate milk; grew readily in plain bouillon, showing a fair degree of cloudiness at the end of seventy-two hours; and did not liquefy gelatin. Serum-agar tubes heavily inoculated with this organism and quickly solidified in ice water and incubated in the air, developed a growth characteristic of the *B. abortus*, as noted by this laboratory, in that a narrow ring of growth appeared as a slight haze 3 mm. beneath the surface of the medium at the end of sixty hours, and eventually extended to the top of the medium. All the above tests conform to the biological and cultural characteristics of the Bang bacillus.

We were not, however, satisfied that the organism isolated was the *Bacillus abortus* on account of its growing in the air so readily, so we subjected the culture to the agglutination and complement fixation tests, using the serum from a rabbit made immune to the Bang bacillus, which agglutinated in high dilutions. It was found that this serum agglutinated our organism in a dilution of 1:1200, which was exactly the same dilution in which the serum agglutinated an antigen made of a well known strain of the *B. abortus*, which had been obtained from an aborting cow. Using as an antigen the organism isolated from the

sows and the immune serum mentioned, the complement was completely fixed with 0.02 cc. of serum. We were thus convinced that this organism was identical with the germ that produces abortion in the cow, the only difference being that it grew readily in the air after the first generation, while the cultures we have derived from the cow usually do not grow in the air until after being cultured for several generations by the Nowak method. An exception to this rule, was discovered by Dr. Frank M. Surface,² who accidentally inoculated a cow with a culture of the Bang bacillus which had repeatedly been transplanted for some two years from agar to agar, or from agar to plain peptone broth, and then back to agar. He had obtained this particular strain while in Denmark. The cow injected, aborted and Surface isolated the organism, the first generation of which developed in the air. He was able to determine definitely, by using this organism as an antigen in the complement fixation test with an immune serum, that it was the Bang bacillus. Surface states that the growth obtained in a Novy jar (Nowak method) was in no respect better than that obtained in the free air.

We cannot state whether the organism isolated from the aborting sow would have grown directly from the tissues, as we cultured none of the material in that way for any length of time. Upon re-culturing the original material, which had been kept in the ice box, we found that the contaminating bacteria had become so numerous as to make streak dilutions impossible.

INOCULATION EXPERIMENTS WITH THE ORGANISM OBTAINED FROM THE ABORTING SOW

To test this organism further, a streak culture on an agar slant was washed with 5 cc. of physiological salt solution and 2 cc. of this material was diluted in 3 cc. of normal salt solution and injected intravenously into a pregnant sow, no. 1, on February 25, 1916. On March 13, seventeen days after the inoculation, this sow aborted five fetuses. The only symptom

² Surface, F. M., A Note on the Maintenance of Virulence by Bacillus Abortus Bang, *Journal of Infectious Diseases*, 1913, **12**, p. 359.

shown by this sow before aborting was that she did a great deal of rooting a day or two before slipping her pigs. As soon as she aborted she ceased rooting. The aborted fetuses, while quite well developed, were not haired over. Stained slides were made from the contents of the different organs. Streak dilutions were made of the heart, liver and stomach contents of each pig, as well as of the afterbirth, on 2 per cent agar poured in petri dishes and solidified. Some of the dishes were incubated in the air, while others were cultured according to Nowak. These fetuses were numbered 1 to 5. The organism with which this sow was injected was obtained from the heart, liver and stomach of pig no. 1; from the heart of pig no. 2; from the heart, liver and stomach of pig no. 3; from the heart and liver of pig no. 4; and from the heart and stomach of pig no. 5. Streak dilutions of these organs grown in the air showed no growth at the end of twenty-four hours. After forty-eight hours, however, the growth was distinctly visible to the naked eye, and at the end of seventy-two hours it was abundant. The streak dilutions grown under diminished oxygen (Nowak method) showed no more growth than that obtained in the air. Upon microscopical examination of the contents of the stomachs of pigs no. 1 to 5, it was seen that these organs harbored the germs in exceedingly large numbers.

On February 29, 1916, a pregnant sow (no. 2) was fed in ship-stuff the growth of the organism, obtained from the aborting sow, on two large agar slants washed off with 40 cc. of sterile normal salt solution. This sow was kept in an inclosure separate from sow no. 1. On March 10, 1916, she received the contents of five small agar tubes in a similar feed. On March 17, the attendant informed us that this sow was going to abort because she was acting like the other sow, previous to aborting, in vigorously rooting the ground floor of her pen. On March 19, nineteen days after being fed the initial dose of the organism, the sow aborted. We obtained three of the pigs. She had eaten the afterbirth, and in all probability had also eaten some of the pigs, as she had bitten out a large piece from the side of one of the pigs secured. After a long series of dilutions, we were able to isolate the original organism from the stomach contents

of one of these pigs. The bacillus was present in this instance in very small numbers.

On March 27, 1916, the tails of these sows were carefully washed, shaved and disinfected, the ends cut off, and 25 cc. of blood taken. The blood serum of each of these sows caused complete agglutination of a known culture of *B. abortus* Bang in a dilution of 1:100, with 75 per cent agglutination in a dilution of 1:250, and the complement was completely fixed with 0.02 cc. of the serum. Serum from a normal hog tested at the same time did not agglutinate the agglutinating fluid in any dilution, nor did it fix the complement.

The slipping of the pigs produced no after effects upon the sows that we could notice. They will be kept under observation for some time.

Taking into consideration all the results mentioned in this paper, we may conclude that the *Bacillus abortus* (Bang) is an etiological factor in infectious abortion of sows. Whether or not it is the only etiological factor, will have to be determined by further investigations. This is the second time, so far as we know, that the *Bacillus abortus* has been associated naturally with aborting animals of a species other than the cow. The first was discovered by Dr. Surface³ when he found the disease epizootic among guinea pigs which were being reared in an inclosure in which inoculation experiments were being carried on with the Bang bacillus. Some of the litter from the cages containing the inoculated pigs had gotten into the pens of breeding pigs and caused the spread of the disease.

SUMMARY

1. Epizootic infectious abortion occurs occasionally among sows, though not so frequently as among cows and mares.
2. Previous to the time of this investigation, no etiological factor connected with the disease in the sow had been discovered.
3. In this investigation the *B. abortus* of Bang, the organism

³ F. M. Surface, Bovine Infectious Abortion Epizootic Among Guinea Pigs, *Journal of Infectious Diseases*, 1912, 11, no. 3, p. 464.

causing the disease of infectious abortion in the cow, was isolated from the afterbirth of an aborting sow and from the contents of the umbilicus, heart, liver and stomach of two aborted fetuses.

4. The strain of *Bacillus abortus* isolated from the sow responded to all the biological and physiological tests of the strains isolated from the uterine exudate of aborting cows by this laboratory, with the exception that the original culture grew in the air after the first generation.

5. Pregnant sow no. 1, inoculated intravenously with 2 cc. of an agar slant culture of the bacillus secured from the aborting sow, washed off with 5 cc. of normal salt solution, aborted five fetuses seventeen days after the injection, and the organism was isolated from the afterbirth and internal organs of the fetuses. The bacillus in this instance grew directly from the tissues under strictly aerobic conditions. Pregnant sow no. 2, on being fed the organism derived from the aborting sow, aborted nineteen days afterwards. The sow ate the afterbirth and presumably some of the pigs. The organism was secured from the stomach contents of one of the fetuses obtained.

6. The blood serum of each of these sows, after aborting, completely agglutinated a strain of *Bacillus abortus* (Bang) derived from an aborting cow, in a dilution of 1:100. The complement was fixed in each case with 0.02 cc. of the serum. The serum of a normal hog did not agglutinate in any dilution, nor did it fix the complement.