A STRAIN OF BACILLUS ABORTUS FROM SWINE.

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The economic significance of abortion among swine and the evidence already presented that the particular race or races of Bacillus abortus causing this disease may be involved in undulant fever of man have justified a rather detailed study of this disease and the strains of Bacillus abortus associated with it.

A review of the early work with the porcine disease was published in 1926. The evidence up to that date, though quite incomplete, pointed to an identity with the bovine races in terms of specific agglutinins but to differences in growth in sealed and unsealed cultures and in the lesions produced in guinea pigs. In these animals suppuration following the characteristic proliferation of monocytes into tubercle-like foci in this whole group was stressed as an important feature of the porcine races of Bacillus abortus. At that time no original strains were obtainable.

Late in May, 1927, our attention was called to an outbreak of swine abortion in the herd of a State institution. With the effective cooperation of the State authorities we were able to obtain a few fetuses. At the same time it was learned that the animals had not been fed cow's milk and that the garbage from the institution was not heated before it was fed. The yards were infested with rats. There had been no record of abortion in this herd prior to March of this year. When it occurred the aborted fetuses and membranes appeared decomposed to the attendants. A white viscid discharge appeared around the vulva of the affected sows. There had been no fresh pigs introduced

3 The writer is indebted to Dr. R. B. Little of this Department for data and material from this outbreak.
into the herd except a boar in 1926 which came from a herd free from the disease. Since the porcine disease has been subjected to but little detailed study, perhaps owing to its relatively infrequent occurrence, the notes on the several cases are briefly reproduced here.

**No. 401.**—Fetus of sow, reported to have been born dead and buried soon after. The matter was of sufficient importance at this time to warrant the recovery and study of this fetus, which had been buried overnight. Autopsy indicates that fetus had breathed. Several pieces of straw from bedding had penetrated into thorax and abdomen. Stomach empty, inflated with gas. Organs not noticeably affected. The placenta obtained in part shows irregular thin white patches of rather tough consistency, 1 to 2 cm. in diameter, which are readily peeled off the chorion. They resemble fat. Under the microscope the material composing them is amorphous and yields gas when 2 per cent HCl is passed under cover-slip. No exudates or ulcers noticed.

Owing to the condition of the fetal carcass only cultures from heart's blood and liver were made. In both blood cultures about 25 colonies appeared within 3 days. Colonies vary from ½ to 1½ mm. in diameter, and are convex, grayish, glistening. In the liver culture the same type of colony appeared. These were later identified as *B. abortus*.


*Fetus 402b*, identical as to size and condition with 402a. About one-half of placenta obtained. The surface of the chorion is overlaid with very thin isolated grayish patches, 2 to 3 mm. in diameter. A few lumps of exudate lying on surface. Films of scrapings show masses of cells, chiefly polymorphs, many containing three or more bacilli a trifle larger than the bovine type of *B. abortus*. Some are spindle-shaped. The subchorionic tissue is slightly edematous.

 Cultures from these fetuses gave the following result:

**402a.**—Cultures of spleen bit and stomach fluid sterile; of heart's blood contain a streptococcus; of lung and liver, rich growths resembling *B. coli*.

**402b.**—Cultures of stomach contents and liver remain sterile; of spleen contain a large coccus. A tube inoculated with heart's blood has a heavy growth above the condensation water; on the slope two small colonies later identified as *B. abortus*. Subcultures from these colonies develop in 24 hours without seal. The growth is moderately heavy in 48 hours.

Sections of hardened tissues from various organs of Fetuses 402a and b did not bring out any recognizable lesions. Similarly sections of the placentas were...
negative. In view of the definite lesions found in the fresh material, the sections probably did not pass through any diseased areas.

*Sow 403.*—Farrowed a litter of 9 small living pigs. On the assumption that the uterus might contain *B. abortus*, a swab was introduced into the vagina to collect a whitish viscid discharge which had appeared. This was stirred in bouillon and two guinea pigs inoculated subcutaneously with the clouded suspension. When killed 29 days later both animals had gained considerably in weight. The autopsy and spleen cultures were negative in both cases.

*Sow 404.*—Farrowed a litter of 5 pigs of which 2 were born dead. These were buried together with parts of placenta. Next day, the fetuses were dug up and a uterine swab obtained from the sow.

*Fetus 404a.*—Male, 35 cm. long. Abdomen contains fluid blood. Lungs not inflated. No abnormalities noted. In making cultures bits of tissue and several drops of stomach fluid were transferred. Pure cultures of *B. abortus* were obtained from liver, heart's blood, lungs, and stomach fluid. Only a few colonies developed in spite of the large inoculum. All are convex, almost hemispherical droplets, grayish, glistening, partly translucent, measuring 1.5 to 2 mm. in diameter.

*Fetus 404b.*—Male, 32.5 cm. long. Left lung almost fully inflated; right airless. Stomach contains blood. Viscera normal in gross appearance. Both cultures containing heart's blood developed a mixed growth of several species. The liver culture remained sterile. In both tubes containing stomach contents growth appeared above the condensation water in 24 hours. On the 2nd day a heavy crop of minute colonies appeared on the slant, identified as pure cultures of *B. abortus*.

It appears from the foregoing protocols that bacilli resembling *Bacillus abortus* were isolated from the fetuses of 3 out of 4 sows. The fourth sow had a normal litter and was evidently not a carrier. Contrasting with bovine abortion, the distribution of bacilli in the fetuses was more general since cultures were readily obtainable from heart's blood and internal organs. In the bovine fetus the bacilli are in most cases limited to the digestive and pulmonary tracts and are sometimes wholly absent. The porcine fetuses examined showed no visceral lesions. The placentas obtained showed exudative processes resulting in thin excrescences. The nature of the peculiar fat-like expansions found on the placenta of No. 401 has not been elucidated.

Concerning the strains of *Bacillus abortus* obtained from the three sows or their litters, it may be stated briefly that they were identical so far as our comparative studies went. They all grew readily in
unsealed tubes and growth was visible within 2 to 3 days. The seal-
ing had a distinctly retarding effect before or after the colonies had appeared. The rate of growth is best illustrated in the following experiment.

From a colony (401) a trace of growth is transferred on a platinum wire to 5 cc.
bouillon and the latter thoroughly shaken. A loop of this suspension is trans-
ferred (a) to an agar slant which is sealed with sealing wax, and (b) to one having
the lower end of the cotton plug just dipped in paraffin. To (c) unsealed the plat-
ninum wire transfers directly a trace from the colony. After 28 hours the bouillon
suspension is distinctly clouded. (c) has a fairly good film. After 48 hours (a)
and (b) are now covered with a dense layer of minute colonies. Those in (b) are
2 or 3 times the size of those in (a).

Pathogenic Action on Guinea Pigs.—Strain 401 isolated directly
from a fetus was also recovered from guinea pigs as follows: The con-
tents of a coil of the small intestine of fetus were suspended in bouillon
and injected into two animals.

Guinea Pig 4180.—Weight 510 gm. Received subcutaneously 1 cc. suspension
of fetal intestinal contents. Killed after 30 days. All subcutaneous lymph
nodes swollen to about twice original dimensions. A necrotic focus in right
kneefold node. A moderate number of minute grayish points showing under
liver capsule. Spleen markedly congested. Dimensions twice normal. On
section follicles irregularly enlarged. In two agar slants containing a bit of spleen,
one sealed, growth appeared within 2 days in the unsealed tube. To hasten growth
the sealed tube was pierced with a hot iron rod. 2 days later growth had begun
in this tube above the bit of tissue.

Guinea Pig 4181.—Weight 470 gm. Chloroformed after 30 days. Lesions as
in preceding animal. Cultures containing spleen bits show after 2 days about 100
characteristic colonies.

Two guinea pigs were later on inoculated with suspensions of pure
cultures.

Guinea Pig 4221.—Received subcutaneously ½ cc. of a 1/375,000 dilution of an
agar slant. The dilution is prepared by washing the entire growth into the con-
densation water, which is equal to ½ cc. This is further diluted to the titer indi-
cated. Guinea pig killed after 26 days. Left kneefold and axillary nodes swollen
and contain firm, yellow, 1 mm. foci. Other nodes inconspicuous. Spleen
markedly congested. Dimensions twice normal. Minute grayish foci on section.
Under liver capsule minute grayish foci. Cultures from spleen positive. Tubes
contain several hundred typical colonies.
Guinea Pig 4209.—Receives the same culture but diluted only to 1/1250, into peritoneal cavity. Chloroformed in 25 days. Subcutaneous lymph nodes swollen. Spleen extremely full of blood. Dimensions 2½ to 3 times normal. Cultures as in preceding case.

Bacillus abortus was isolated from the uterine swab of Sow 404. The lesions produced are like those of Strain 401.

Guinea Pig 4232.—Receives subcutaneously ½ cc. of a turbid bouillon suspension of swab from Sow 404. Chloroformed in 21 days. At point of inoculation an abscess in the subcutis. Nearest kneefold lymph node, bean size, with still firm, necrotic foci. Spleen congested. Dimensions twice normal. Surface sprinkled over with minute grayish points. Spleen cultures contain several hundred colonies.

Guinea Pig 4231.—Receives 1 cc. of same suspension. Killed in 21 days. Lesions as in preceding animal with the addition of four small abscesses in omentum. Spleen and omentum cultures develop countless colonies. In cultures from both animals the growth in the sealed cultures was much retarded or else absent until seal penetrated and tubes reincubated.

Cultures of Bacillus abortus having identical characters in culture and in guinea pigs were thus isolated directly and through guinea pigs from 401 and 404. Material from 402 was not passed through guinea pigs.

Agglutination Reactions.—To determine the serological relationship between the swine strain and bovine types a culture recently isolated from a case of bovine abortion and serum from an old bovine carrier of Bacillus abortus, probably in the udder (No. 930), were used. The cow had carried agglutinins in her blood for a number of years. The swine sera were obtained by bleeding the sows from the tail. The absorption tests were performed as follows:

A Blake bottle containing nutrient agar was inoculated and incubated 48 hours. A suspension in normal salt solution was made which was 11 times the density of 2.4 on the Gates scale. 1 cc. of serum was mixed with 4 cc. of the suspension and centrifuged. The deposit was stirred up, incubated 2 hours, and centrifuged. The clear serum in a 1/5 dilution was tested in higher dilutions as indicated in Table I.

Reciprocal agglutination and absorption tests with a strain from swine are given in Table I.

The irregular inhibition of agglutination shown in this table is also evident in tests to be reported below. The serum of the cow is equally, but not completely, absorbed by cow and pig strain.
In Table II are recorded additional direct and cross-agglutination tests. Certain irregularities are also evident in these data. The serum from a normal pig was free from agglutinins towards swine strains. A test not tabulated showed complete absence of agglutinins towards the cow strain in dilutions as low as 1/10. The additional sows included in this and the following test are from the infected herd. No. 460 is a young pregnant sow, evidently not infected. No. 461 is also a young pregnant sow, showing in her serum a high agglutinin content. No. 462 is an old sow, also with a high agglutinin titer.
In Table III are given comparative tests of the swine sera with strains from swine and Cow 1373.

Here again the serum No. 460 appears free from specific agglutinins. The blood of infected Sow 402 is low in agglutinins. The rest are high. Both cow and swine strain show the irregular inhibitions with the maximum agglutination and subsidence of the bacteria around 1/160.

**TABLE III.**

*Agglutination of Strains from Cow 1373 and Sow 401 by Various Sera from Infected Herd.*

<table>
<thead>
<tr>
<th>Source of infection</th>
<th>Serum dilutions</th>
<th>Strain used</th>
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<tbody>
<tr>
<td></td>
<td>1/10</td>
<td>1/20</td>
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<tr>
<td>B</td>
<td>++++</td>
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<tr>
<td>C</td>
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<td>461</td>
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<tr>
<td>462</td>
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</tbody>
</table>

*The Effect of Feeding Cultures from This Outbreak on Swine.—Only one experiment has been completed to date.*

Sow 344, born June, 1925. Last litter May 4, 1927. Late in September the sow was put into an outdoor enclosure provided with a small house for shelter on soil not overrun by swine for at least 15 years. A Blake bottle containing an agar layer was seeded with strain from Swine 401, now 5 months under cultivation, and incubated for 2 days. On Oct. 8, 1927, the sow was fed with the salt solution suspension of the entire culture mixed with dissolved powdered milk.
The sow gave birth to two normal young on October 19. Placental material was not recovered nor any material indicating the birth of dead pigs. The agglutinin reactions of the blood of the sow on the day of feeding and the day following farrowing are given in Table IV.

Following the farrowing, a swab was inserted into the uterus and the attached material suspended in normal saline and injected into the peritoneal cavity of two guinea pigs. Both were chloroformed after 46 days. Autopsy and cultures were negative. At the same time two guinea pigs received an intraperitoneal injection of the sow's milk. Both, killed after 46 days, had large spleens and other lesions diagnostic for Bacillus abortus infection. Pure cultures of Bacillus abortus were obtained from spleen tissue. The guinea pig lesions resembled closely those produced originally by the culture fed. The significant result of this experiment is contained in the fact that the organism fed became located in the mammary gland and not in the uterus of the pregnant sow. The agglutinin test before infection suggests possible infection of the sow with Bacillus abortus, but tests on other sows of the herd yielded nearly the same figures. The herd has been free from abortion and recent tests of the milk from four sows on guinea pigs were entirely negative.

In the spring of 1928, the attention of this Department was called to an outbreak of swine abortion in another State institution. The sows gave birth to large litters of which most died on the same day. Material from uterine swabs and sow's milk were injected into a large number of guinea pigs. All of these when killed 4 to 6 weeks later

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TABLE IV.

Agglutination Reactions of Sow before and after Infection.

<table>
<thead>
<tr>
<th>Blood drawn</th>
<th>Serum dilutions</th>
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<tbody>
<tr>
<td></td>
<td>1/10</td>
</tr>
<tr>
<td>October 8...</td>
<td>+++++</td>
</tr>
<tr>
<td>&quot; 20....</td>
<td>+++++</td>
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</tbody>
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4 The writer is indebted to Dr. Henry W. Dustan for material from this outbreak.
were normal and cultures of the entire, small spleen negative. Similarly cultures made from the organs of four new-born pigs were negative.

In this outbreak *Bacillus abortus* evidently played no part. The work is mentioned here so that those entrusted with the investigation of such outbreaks on the spot do not fail to consider causes other than infection with *Bacillus abortus*.

**SUMMARY.**

The outbreak of infectious abortion in swine, probably the first reported from the eastern United States, was associated with a strain of *Bacillus abortus* growing rapidly on ordinary nutrient agar slopes without seal and presenting certain slight pathological deviations from the bovine form of disease in guinea pigs such as the occurrence of necrotic, suppurating foci in spleen and lymph nodes. Agglutination tests, comprising both cross-agglutination and absorption procedures failed to distinguish the strain from the bovine type. The gross appearance of the fetuses from this outbreak was normal. The shreds of placentas obtainable indicated slight erosion of the chorionic epithelium and some exudation. The specific bacilli were quite widely disseminated in the tissues of the fetuses. The pathogenic action of this swine strain on guinea pigs was evidently much feebler than that of most earlier swine strains as reported and it approached more closely that of bovine strains. The culture fed to a pregnant sow failed to produce abortion, possibly because of the advanced stage of pregnancy. The organism was not recovered from the uterus but was found in the sow's milk.