

BACTERIUM ABORTUS INFECTION OF BULLS

[PRELIMINARY REPORT]

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Numerous investigators have called attention to the fact that *Bacterium abortus* agglutinins and complement-fixing bodies can frequently be demonstrated in the blood serum of bulls from abortion-infected herds. Such animals in consequence have frequently been referred to as being systemically infected. While the presence of these bodies constitutes strong evidence that abortion infection exists, or has been present, success has been reported in associating positive reactions with the causative infection in so few instances as to have resulted in a certain amount of speculation regarding the significance of these reactions in male animals.

Literature, it is true, records no great amount of investigative work in connection with bulls suspected of being infected with abortion disease where the object has been the isolation of the causative microorganism from the organs or tissues of the animals or the demonstration of lesions associated therewith.

Schroeder and Cotton ¹ in investigating this problem describe two cases that came under their observation. They state that one of the bulls at the time of autopsy showed the presence of an abscess involving the epididymis of one testicle from which *Bact. abortus* was isolated. The other animal was permitted to serve a cow that was considered to be free from abortion disease. Seminal fluid which was recovered from the vagina immediately following the service and injected into numerous guinea pigs produced *Bact. abortus* lesions in one of the experimental animals.

Rettger and White ² describe endeavors to associate the presence of the infection with positive serum reactions in three cases which they studied. In two of the animals neither abortus infection nor pathological changes could be demonstrated. In the third they call attention to the finding of two abscesses or cysts in the region of the groin, near the point of attachment of the scrotum; but from these abscesses they were unable to isolate the abortion organism, thus failing to obtain bacteriological evidence of the infection.

In view of the positive bacteriological findings of Schroeder and Cotton the present writers were prompted to undertake further investigations,

¹ SCHROEDER, E. C., and COTTON, W. E. SOME FACTS ABOUT ABORTION DISEASE. *In Jour. Agr. Research*, v. 9, no. 1, p. 9-16. 1917.

² RETTGER, I. F., and WHITE, G. C. INFECTIOUS ABORTION IN CATTLE. *Conn. Storrs Agr. Exp. Sta. Bul.* 93, p. 199-249. 1918. References, p. 246.

involving a considerable number of animals, in an endeavor to ascertain with what frequency abortus infection could be demonstrated in the generative organs of bulls giving positive or suspicious reactions to the agglutination test for this disease, and to determine whether or not pathological changes are commonly associated with such infection.

EXPERIMENTAL PROCEDURE

The procedure employed by the writers consisted in securing blood samples from the animals as they arrived at one of the abattoirs in close proximity to Washington, D. C., for slaughter. No information was available regarding the original source of the bulls or the exposure sustained. Each sample was given a number corresponding to the serial number of a tag that was attached to the animal's ear at the time of bleeding. The blood samples were then taken to the laboratory for the application of the agglutination test. At the time of slaughter, which was usually the following day, those animals giving positive or suspicious reactions were autopsied as carefully as abattoir conditions permitted and the organs of the genital system were secured for further study. Cultural work was depended upon as a means of detecting infection, the medium employed consisting of 3 per cent glycerin infusion agar to which approximately 5 per cent sterile blood serum was added. To reduce the oxygen tension the inoculated tubes were subjected to incubator temperature in closed jars in the presence of fresh cultures of *Bacillus subtilis*.

During the period from December 9, 1916, to July 7, 1918, the agglutination test for abortion disease was applied to 325 mature bulls. Of this number 288 gave negative results to the test. The manner in which the remaining 37 reacted is of considerable interest, inasmuch as the intensity of the reactions appeared to bear some relation to the cultural results.

The manner of applying the test consisted in the making of a 1 to 10 basic dilution of the blood serum. To the four tubes utilized for each case were added 0.4, 0.2, 0.1 and 0.05 cc. of this basic dilution. The amount of test fluid added to each tube was 1 cc.

The vesiculæ seminales, vasa deferentia, testes, and epididymides were secured from the 37 bulls whose blood serum showed the presence of *Bact. abortus* agglutinins. From 15 to 20 tubes of medium were utilized for culturing the various organs from each bull. These investigations resulted in the demonstration of the presence of *Bact. abortus* infection in four animals—No. 88, 98, 136, and 409—and in the detection of marked lesions in bulls 98 and 409.

A brief description of the work performed and the findings in these cases follow.

The agglutination reactions of the animals appear in the following table.

TABLE I.—Results of agglutination tests

Animal No.	Suspected serum.				Animal No.	Suspected serum.			
	0.04 cc.	0.02 cc.	0.01 cc.	0.005 cc.		0.04 cc.	0.02 cc.	0.01 cc.	0.005 cc.
21.....	Sl	Sl	—	—	168.....	+	Sl	Sl	—
84.....	+	Sl	—	—	177.....	Sl	—	—	—
86.....	Sl	Sl	—	—	178.....	+	Sl	—	—
88.....	+	+	+	Sl	179.....	Sl	Sl	—	—
89.....	Sl	Sl	—	—	189.....	+	Sl	—	—
98.....	+	+	+	Sl	198.....	+	+	Sl	—
103.....	+	+	+	+	265.....	+	Sl	—	—
105.....	+	Sl	—	—	271.....	+	+	Sl	—
109.....	+	Sl	—	—	280.....	+	Sl	—	—
133.....	+	+	+	+	301.....	Sl	Sl	—	—
134.....	Sl	Sl	Sl	—	319.....	+	Sl	—	—
136.....	+	+	+	Sl	326.....	+	+	Sl	—
137.....	+	+	Sl	Sl	338.....	Sl	Sl	—	—
143.....	Sl	—	—	—	348.....	Sl	—	—	—
146.....	+	Sl	—	—	409.....	+	+	+	+
147.....	Sl	—	—	—	451.....	+	+	+	—
150.....	+	+	Sl	—	453.....	+	Sl	Sl	—
154.....	Sl	Sl	—	—	454.....	+	+	Sl	—
165.....	Sl	Sl	—	—					

+ = Complete agglutination.
 — = No agglutination.
 Sl = Partial clumping of bacteria.

EXAMINATION AND FINDINGS OF BULL 88

February 12, 1918. Agglutination test: 0.04 cc. +, 0.02 cc. +, 0.01 cc. +, 0.005 cc. Sl.

February 14, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Fluid of left seminal vesicle turbid in appearance and slightly more excessive in amount than that contained by other organ. No indication of abnormal conditions noted elsewhere.

BACTERIOLOGICAL FINDINGS.—Of the 16 tubes of medium inoculated from the various organs enumerated, after four days' incubation three tubes developed from 40 to 60 colonies of an organism suggestive of *Bact. abortus* and subsequently identified as such. These inoculations were from the left seminal vesicle.

EXAMINATION AND FINDINGS OF BULL 98

March 8, 1918. Agglutination test: 0.04 cc. +, 0.02 cc. +, 0.01 cc. +, 0.005 cc. Sl.

March 9, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Marked pathological changes involved the left seminal vesicle. The organ was increased from 8 to 10 times

its normal size. On cross section of the vesicle numerous hemorrhagic areas were observed, as well as a number of necrotic centers, the latter being confined chiefly to the more central portions of the organ. So softened were some of these foci that the necrotic material assumed a semifluid character. The capsule of the organ showed considerable thickening. (See Pl. 22.)

HISTOLOGICAL EXAMINATION.—Sections from the left seminal vesicle showed varying stages of the diseased process, ranging from exfoliation of the epithelial lining of a few of the acini to complete obliteration of the normal glandular structure. There was marked proliferation of the interstitial tissue with round cell infiltration, which was more pronounced immediately surrounding the acini and just beneath the epithelial lining of the acini. In those areas exhibiting the more pronounced pathological changes many of the acini were filled with detached epithelial cells and cell débris. In other areas where the mere outline of the acini could be traced, a homogeneous substance was present, together with more or less granular detritus. There were hemorrhages into and between the acini. Occasionally large areas of degeneration and necrosis were observed. As a result of the inflammatory changes little normal glandular structure was recognized in many of the sections examined (Pl. 24, A and B). Plate 23 A, representing a normal seminal vesicle, is inserted for comparison with Plate 23 B, and Plate 24, A and B.

BACTERIOLOGICAL FINDINGS.—Eighteen tubes of medium were utilized for the culturing of the different organs. The six tubes from the left seminal vesicle after three days' incubation developed from 75 to 150 colonies of an organism that appeared typical of *Bact. abortus*. All tubes inoculated from other sources remained sterile, although incubated for several additional days. Subsequent work with the organism isolated established its identity as *Bact. abortus* and indicated that pure cultures of the organism were isolated in all instances.

EXAMINATION AND FINDINGS OF BULL 136

May 15, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

May 16, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—The right seminal vesicle showed slight enlargement. The fluid contained by this organ presented a turbid appearance. No lesions were elsewhere detected.

BACTERIOLOGICAL FINDINGS.—Five of the 18 tubes of medium inoculated from the different organs developed from 40 to 60 colonies of an organism that was subsequently identified as *Bact. abortus*. These inoculations were from the right seminal vesicle.

EXAMINATION AND FINDINGS OF BULL 409

August 15, 1918. Agglutination test: 0.04 cc. +, 0.02 cc. +, 0.01 cc. +, 0.005 cc. +.

August 16, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Left seminal vesicle showed evidence of disease. This organ was approximately twice the size of the right and was incised with considerable difficulty on account of fibrous tissue proliferation. The fluid contained was decidedly turbid. Other organs presented a normal appearance.

MICROSCOPIC EXAMINATION.—Sections from the left seminal vesicle showed marked proliferation of the interstitial tissue with areas of round-cell infiltration. Degeneration and exfoliation of the epithelial cells lining the acini were observed. A few of the acini contained cells and cell detritus; others had been completely obliterated as a result of the inflammatory process. (See Pl. 23 B.)

BACTERIOLOGICAL FINDINGS.—Two of the 20 tubes of medium that were inoculated from the various organs developed colonies typical of *Bact. abortus*. The colonies were few in number and appeared on but 2 of 5 tubes that were sown with material from near the same point. These tubes were from the left seminal vesicle. All tubes inoculated from other sources remained sterile. The infection was subsequently established as *Bact. abortus*.

It has been previously suggested that the intensity of the serum reactions appeared to bear some relation to the cultural results. Of the 37 bulls exhibiting agglutinating properties for a *Bact. abortus* suspension, the blood serum of but 7 caused perfect agglutination of a suspension with 0.01 cc. of the serum. It may be observed that 4 of these 7 animals yielded positive cultural results and that in no instance was the presence of the infection demonstrated in animals when their blood serum failed to cause perfect agglutination with such an amount of serum.

EXAMINATION AND FINDINGS OF GUERNSEY BULL

Since the isolation of *Bact. abortus* infection from the cases previously described, the writers have had an opportunity to demonstrate the presence of the infection and observe lesions in a fifth bull where the isolation of abortion bacteria was carried out under different conditions and where it was possible to obtain a somewhat more complete history in regard to the development of the pathological changes that were associated with the infection.

This pure-bred Guernsey, 8 years of age, was acquired by the present owner in June, 1918, and appeared at the time to be in perfect physical condition.

The writers were informed that during the following January an asymmetrical enlargement of the scrotum was noted. Mechanical injury was

suspected which had prompted the application of fomentations and counterirritants. When the condition failed to respond to this treatment and an area of softening that appeared to involve the left testicle was later detected, a canula had been introduced through which had been evacuated a considerable quantity of a semifluid material. It was furthermore stated that the animal had at times discharged through the urethra a substance bearing some resemblance to that removed by the surgical procedure.

On April 22, 1919, about three months after the swelling was first observed, a sample of blood was secured for the application of the agglutination test for abortion disease. The specimen caused clumping of a *Bact. abortus* suspension with 0.0001 cc. of the serum.

When the animal was examined on the following day with the object of obtaining material for bacteriological work, the enlargement involving the left testicle was found to be four or five times the size of the normal organ. When a needle was passed into its lateral wall, little resistance was encountered after the instrument had been inserted for about $1\frac{1}{2}$ inches. Through the needle were aspirated from 400 to 500 cc. of a gray-colored substance of the consistence of heavy cream. At the same time from 20 to 30 cc. of a turbid fluid were obtained from the urethral opening. This material was secured by exerting pressure on the urethra and by massaging the seminal vesicles through the walls of the rectum. During this procedure it was detected that the seminal vesicles differed markedly in size, enlargement of the right organ being pronounced.

CULTURAL RESULTS.—Eight tubes of serum agar were inoculated with the semifluid substance aspirated from the interior of the enlargement involving the diseased testicle. Numerous dilutions were made of the fluid recovered from the urethra with physiological salt solution, and serum-agar tubes were sown with these dilutions. When the tubes were examined after six days' incubation one colony of abortuslike appearance was observed on one of the tubes from the substance obtained by aspiration. The infection was later established as *Bact. abortus*. Further inoculations of medium with like material resulted in the isolation of additional abortus colonies, although fewer in number than were anticipated from the extent of the lesions. No *Bact. abortus* was isolated by cultural methods from the material secured from the urethra, but excessive contamination made these results inconclusive.

On May 9, or about two weeks after the condition was diagnosed as abortus infection, an opportunity was afforded for the making of a more thorough examination of the diseased process involving the external genitals and for further bacteriological work, for the affected testicle with its coverings were at this time removed and forwarded to the Pathological Division.

The weight of the mass of tissue was 5½ pounds. On section it was found to consist of an outer wall or capsule of from 1½ to 2 inches in thickness. This abnormal structure had evidently resulted from proliferative changes involving mainly the connective tissue coverings of the testicle. Firmly embedded in this external layer could be distinguished areas of tissue that upon microscopic examination were identified as epididymis that had undergone severe inflammatory changes. The cavity formed by this dense fibrous wall contained a considerable quantity of a grayish-colored, semifluid material identical with the substance previously obtained by aspiration. Floating free in the cavity was also a mass of tissue that was recognized as the remains of the testicle, it having the same general form although somewhat reduced in size. Blood vessels no longer communicated with the organ, and the serous membranes which normally envelop it had seemingly been entirely obliterated. The close resemblance existing between the semifluid substance and softened portions of the testicle strongly indicated that the organ was undergoing liquefaction necrosis.

MICROSCOPIC EXAMINATION.—The thick wall surrounding the testicle consisted largely of dense fibrous tissue with a certain amount of round-cell infiltration. Different portions of the epididymis which were embedded in this mass showed extensive interstitial proliferation, which had resulted in a wide separation of the tubules. Chronic inflammatory changes were noted in sections from the testicle proper. Many tubules were surrounded by zones of round-cell infiltration. There was exfoliation and more or less disintegration of the epithelium lining the tubules, causing the latter to be largely occupied by cell débris. Advanced degenerative changes, verging on necrosis, were observed in all the sections examined, the peripheral portion of the organ exhibiting little more than a mere outline of the testicle structure.

BACTERIOLOGICAL FINDINGS.—Tubes of serum agar that were inoculated with the exudate at this time developed numerous colonies of an organism that was identified as *Bact. abortus*.

It has been suggested by writers on abortion disease that *Bact. abortus* infection when acquired by bulls remains active for a comparatively brief period, the resistance offered being sufficient for its destruction. The encountering of a considerable number of animals giving slight agglutination reactions and the isolation of *abortus* infection from only a small percentage of the bulls cultured would tend to strengthen the theory that the infection may commonly terminate in this manner. On the other hand the extensive pathological changes and the chronic character of the lesions exhibited by three of the five bulls where *abortus* infection was demonstrated suggest that it may be unwise to assume that long-standing cases of infection never exist.

CONCLUSIONS

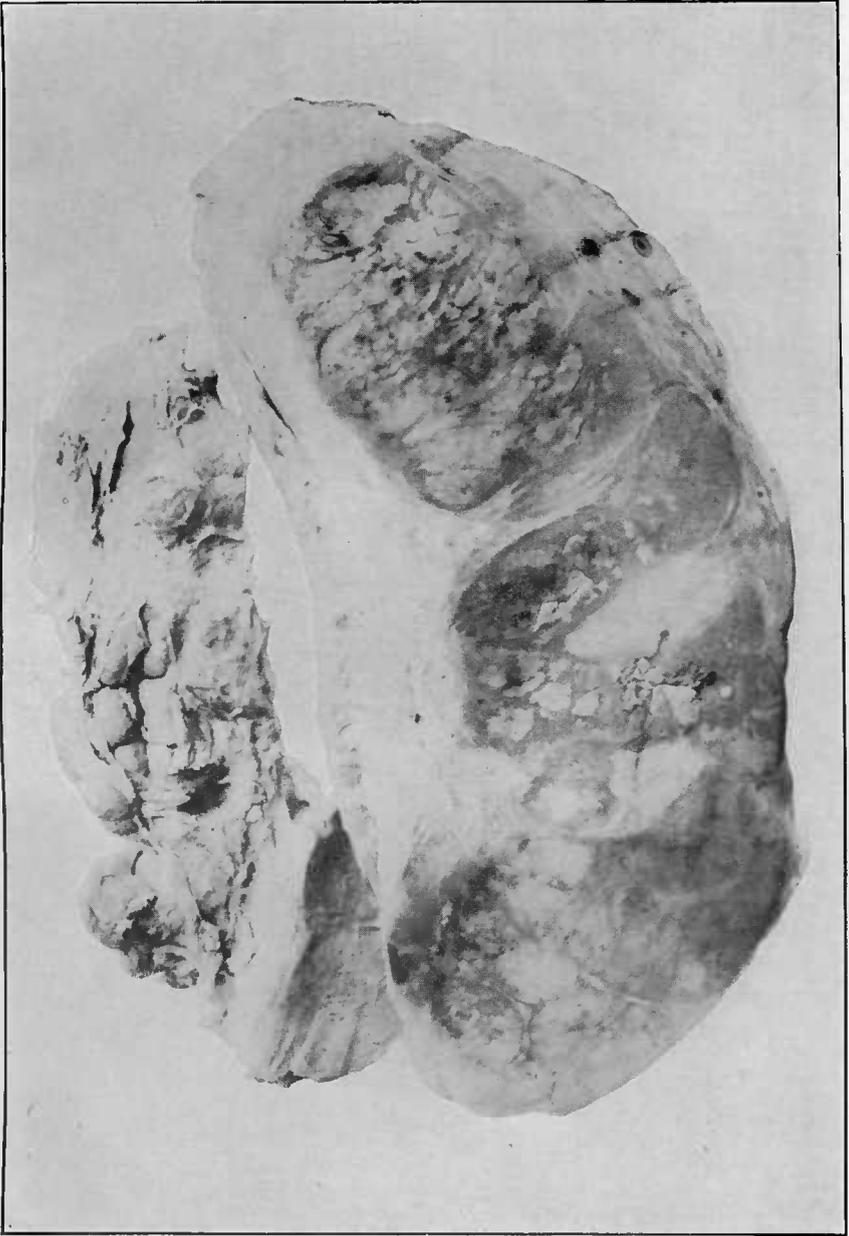
Bact. abortus infection may involve organs of the generative apparatus of bulls, producing chronic inflammatory changes.

Of the generative organs, the seminal vesicles appear to furnish the most favorable site for the lodgment and propagation of abortion infection.

The presence of *Bact. abortus* infection in bulls appears to be more strongly indicated by relatively marked than by slight reactions to the agglutination test for this disease.

PLATE 22

Photograph of normal and diseased seminal vesicles of bull 98, showing the marked increase in size and the gross pathological changes of one of the organs.



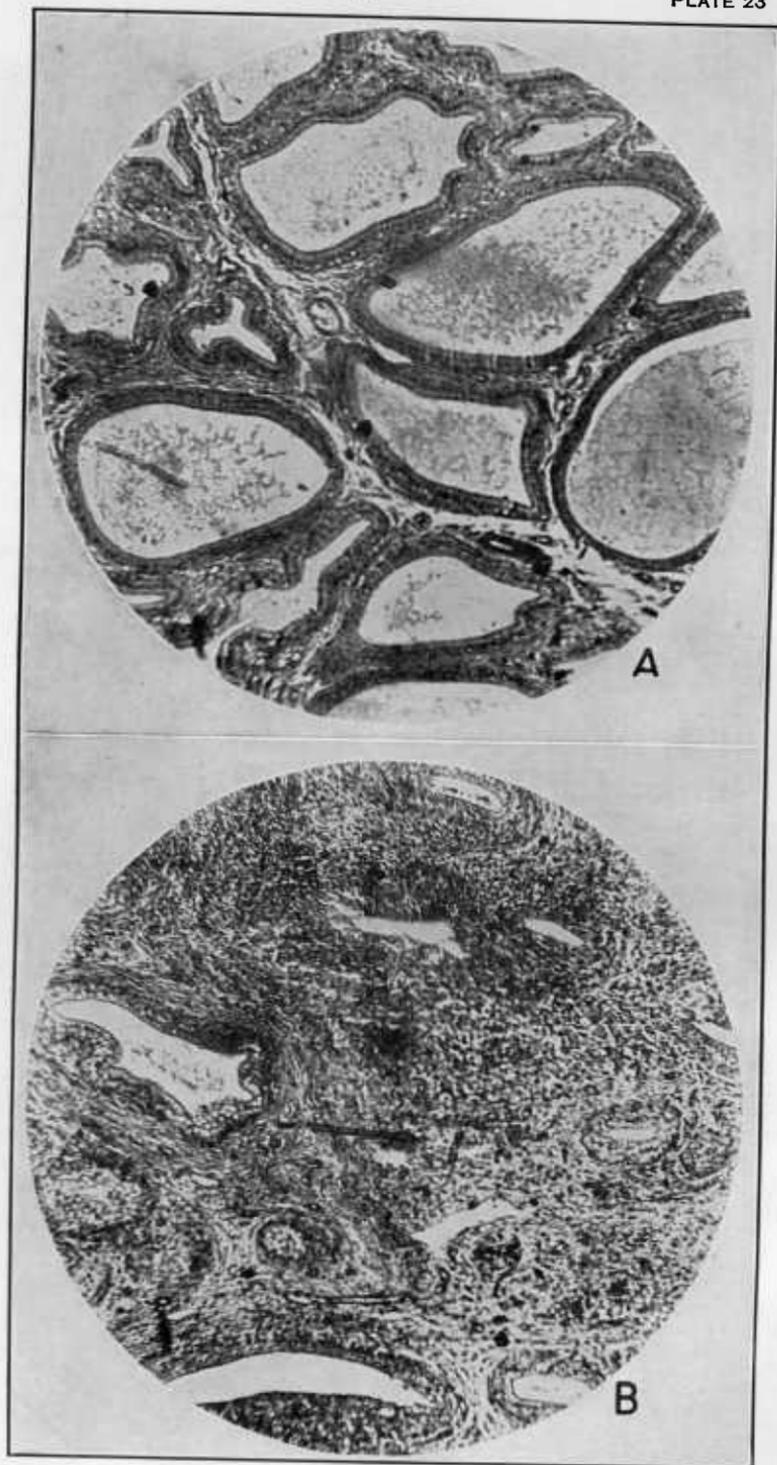


PLATE 23

A.—Photomicrograph of a section from a normal seminal vesicle of bull. $\times 92$.

B.—Photomicrograph of section from seminal vesicle of bull 409, showing inflammatory changes. $\times 92$.

PLATE 24

A.—Photomicrograph of section from seminal vesicle of bull 98, showing tissue proliferation and exfoliation of epithelium lining acini. $\times 92$.

B.—Photomicrograph of section from seminal vesicle of bull 98, showing advanced pathological changes with cell degeneration and necrosis. $\times 92$.

