

## FURTHER STUDIES ON THE ETIOLOGICAL RÔLE OF VIBRIO FETUS.\*

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Among the twenty-six cases of abortion associated with *Vibrio fetus*,<sup>1, 2</sup> of which one also contained *Bacillus abortus*, there was none involving heifers. All were second or later pregnancies. All but three were purchased cows. More recently three cases of abortion in native heifers have been found associated with *Vibrio fetus*. These cases are of sufficient importance to be given in detail.

*No. 433.*—Native heifer; aborted October 24, 1919. Records state that she had been bred February 22, 1919. Male fetus, 28 inches long; color black and white. Coat of hair still lacking. The stomach fluids contained a few particles of meconium only. The contents of the large intestine were normal. The lungs were free from air. There were some small hemorrhages in the auriculo-ventricular valves. Besides a general fullness of blood in the organs there were no marked abnormalities in the fetus.

Cultures were made as follows: From the contents of the fourth stomach, which showed spirals in films, pure cultures of spirilla were obtained. Films from small and large intestines were negative, but cultures developed spirilla from contents of the colon and rectum. Cultures from the lungs and spleen also contained spirilla only. Those from the liver and kidneys remained sterile.

One guinea pig was inoculated with the contents of the fourth stomach, another with meconium, and a third with lung tissue. Killed after 7 weeks, none showed any signs of disease due to *B. abortus*, and the spleen cultures remained sterile.

A portion of the placenta representing the unoccupied horn, which had passed out, was cut from the still adherent and retained remainder. It was covered with shavings and without any odors of decomposition. During the washing to

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\* The tests relating to the milk were made by Miss Marion L. Orcutt.

<sup>1</sup> Smith, T., *J. Exp. Med.*, 1918, xxviii, 701.

<sup>2</sup> Smith, T., *J. Exp. Med.*, 1919, xxx, 313.

remove adherent bedding, yellowish, cheesy particles were carried off by the wash water from the cotyledons.

The chorion varies from a smooth, translucent, slightly injected membrane to one opaque, thickened, and leathery. The opacity is due to an infiltration which is in the form of slightly elevated plaques not removable by gentle scraping. In other places the infiltration is discrete, in the form of whitish opacities,  $\frac{1}{2}$  to 1 mm. in diameter. There are also scattering minute tufts of adventitious villi, completely cheesy. Edema of the subjacent tissue varies in thickness from place to place. The cotyledons are in part normal, in part diseased. Some of the latter are yellowish, pultaceous throughout. The still normal cotyledons contain, usually on the margin, necrotic, yellowish villi. More rarely such villi are scattered through the cotyledon.

Many films were examined without showing any bacilli of the type of *B. abortus*. There were, however, occasional larger rods and vibrios. A few were closely wound, the rest flatter, as is usual with *V. fetus*.

Sections of fixed and hardened material from various regions of the placenta show necrosis of villi, loss of surface epithelium with infiltration of the underlying tissue with leucocytes in certain areas. Where epithelium is present, no bacteria are found in them, as is the case when *B. abortus* is present. The endothelium of the capillaries has proliferated in places and it partly or nearly fills the lumina. Bacteria resembling vibrios are detected within these cells and in groups among necrotic villi. Inoculation of two guinea pigs with scrapings from the diseased placenta was negative as regards *B. abortus*.

Milk collected 3 days after the expulsion of the fetus, centrifuged according to the method previously described,<sup>3</sup> and injected into three guinea pigs also yielded negative results as regards *B. abortus*. Agglutinins for *B. abortus* were absent from the milk at this time.

The blood agglutinations of this heifer are significant in that they indicate subsequent infection with *B. abortus* during the second pregnancy. 2 days before abortion, October 22, 1919, the titer limit was 1:20, 20 days after abortion a trifle above 1:40. Nearly 6 months later samples taken 4 days apart were 1:640.

*No. 438.*—Date of breeding uncertain. Aborted November 9, 1919. The placenta was retained. Fetus, female, measures  $17\frac{1}{2}$  inches and weighs about 5 pounds. No subcutaneous edema or serous effusions. Autolytic changes of organs without any distinctly putrefactive odors present. The fourth stomach contains a thick mucoid pinkish fluid and films show spirilla. Films of contents of colon and rectum do not show any bacteria. One ventral lobe of the lungs shows under pleura fine, branching, grayish yellow lines made up of masses of spherical crystals.

Cultures from contents of the fourth stomach contain only spirilla. The same is true of cultures of the colon contents. One culture from the rectum contains

<sup>3</sup> Smillie, E. W., Little, R. B., and Florence, L., *J. Exp. Med.*, 1919, xxx, 341.

cocci, the other only spirilla. Cultures made with bits of spleen, liver, and lungs develop only spirilla. One tube from the kidney contains large bacilli, the other only spirilla.

Four guinea pigs, inoculated respectively with a suspension of material from a uterine swab, with fourth stomach contents, meconium, and lung tissue, were negative as to *B. abortus* when killed and cultured after 6 to 7 weeks.

A sample of milk drawn 9 days after expulsion of the fetus was inoculated into three guinea pigs. *B. abortus* was not isolated from any of them. The titer limit of the agglutinations for *B. abortus* of this heifer 4 days after abortion was 1:40, the milk titer was negative when tested 9 days after. 5 months after abortion the titer limit of the blood was 1:80, of the milk below 1:20.

*No. 449.*—Aborted during the night of December 1 to 2, 1919. Date of breeding unknown. Female fetus, 16 inches long. Lower jaw and part of upper jaw eaten away by some animal during the night. Some coils of the small intestine protruding from the opening at the umbilicus. General suffusion of tissues with hemoglobin. Autolytic changes indicated by appearance of tissues. Skin readily peeled away from subjacent muscular tissue.

Contents of the fourth stomach turbid. They contain epithelial growths from amnion, swallowed by the fetus. Films from the fourth stomach contents contain numerous spirilla. One culture from the contents contains only spirilla. In the other they are associated with bacilli. Cultures from the liver contain only spirilla. Those from the lungs contain a mixture of rods and cocci.

Only one guinea pig was inoculated with the contents of the fourth stomach. The result was negative as to *B. abortus*.

Milk collected 1 day after expulsion of the fetus was tested on three guinea pigs for *B. abortus* with negative result.

The agglutination titer of this heifer also indicated absence of *B. abortus*. On February 12, 1919, and April 1, it was 1:80. On November 25 it was 1:20. On the date of abortion, December 2, it was 1:40.  $4\frac{1}{2}$  months later it was 1:80. The agglutination titer limit of the milk 1 day after abortion was 1:40,  $4\frac{1}{2}$  months later it was below 1:20.

#### *Agglutination Tests.*

It was stated in an earlier publication<sup>4</sup> that the agglutinability of recently isolated strains of *Vibrio fetus* is low or absent and that it rises under artificial cultivation to a certain maximum. This is not always true, however, as Table I shows. The vibrios of the three aborting cases are titrated against the serum of a rabbit treated repeatedly with cultures of Strain 67. In all cases a high agglutina-

<sup>4</sup> Smith, T., and Taylor, M. S., *J. Exp. Med.*, 1919, xxx, 299.

tion titer is demonstrated, although the strains are still in the early generations. The table also shows a general serological relationship among the strains tested.

The outcome of several tests of the blood serum of the three reported cases is summarized in Table II. Owing to a slight spontaneous agglutination in the controls, the high titers with *Vibrio* 356 will have to be reduced one or two degrees. The results, while not uniform, give encouragement that agglutination tests may be of use should this type of infectious abortion become more widespread. In

TABLE I.  
*Agglutination Tests with Serum from a Rabbit Immunized with Cultures of Vibrio 67.*

Vibrio No.	No. of transfer.	Serum dilutions.								Control.
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	Vibrios in salt solution.
433	11th	C.*	C.	C.	C.	C.	C.	+++	+++	—
438	9th	"	"	"	"	"	"	C.	+++ <sup>1</sup>	—
449	5th	"	"	"	"	"	"	"	+++	—
67	125th	"	"	"	"	"	"	"	+++	—

\* C. indicates complete agglutination, +++ nearly complete agglutination, ++ marked agglutination, + slight agglutination, = doubtful, and — no agglutination.

<sup>1</sup> when following a symbol denotes a degree of agglutination between it and the next higher symbol.

only one case (No. 449) was serum antedating the abortion on hand, and this was negative. We have, therefore, no way of knowing at present whether a high titer precedes abortion, as is almost regularly true in the presence of *Bacillus abortus*. By choosing some easily agglutinable strain which has been tested against normal sera it may be possible to detect this type of infection as readily as the other. All of the strains included in the table were tested against the serum of a normal heifer and no clumping was observed in dilutions ranging from 1:20 to 1:1,280.

TABLE II.  
Agglutination Tests with Blood Serum of the Three Cases.

Case No.	Date of abortion.	Date of withdrawal of blood.	Vibrio used as antigen.		Date of test.	Serum dilutions.								Controls.				
			Case No.	Transfer.		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	Vibrios in salt solution.	Normal serum.			
433	Oct. 24, 1919	Nov. 13, 1919	433	6th	Dec. 1, 1919	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—	
		" 13, 1919	347	27th	" 1, 1919	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—	
		Apr. 15, 1920	433	16th	Apr. 19, 1920	C.	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
		" 15, 1920	356	32nd	" 19, 1920	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
438	Nov. 9, 1919	Nov. 13, 1919	438	4th	Dec. 1, 1919	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—	
		" 13, 1919	347	27th	" 1, 1919	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—	
		Apr. 15, 1920	449	16th	Apr. 19, 1920	C.	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
		" 15, 1920	356	32nd	" 19, 1920	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
449	Dec. 1, 1919	Nov. 25, 1919	449	7th	Jan. 31, 1920	—	—	—	—	—	—	—	—	—	—	+	—	
		" 25, 1919	347	33rd	" 31, 1920	++	+	—	—	—	—	—	—	—	—	+	—	
		Dec. 2, 1919	449	5th	" 19, 1920	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
		" 2, 1919	347	33rd	" 31, 1920	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
		Apr. 15, 1920	449	13th	Apr. 19, 1920	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—
		" 15, 1920	356	32nd	" 19, 1920	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+++ <sup>1</sup>	+	—

## GENERAL SUMMARY.

The data bearing on these three cases are quite sufficient to rule out *Bacillus abortus* as the agent. Not only the cultures and guinea pig tests of fetal tissues and contents of the digestive tract, but also the agglutination and guinea pig tests of the milk, were negative. The same is true of the agglutination tests of the blood serum. Only in one case was the placenta obtained in part. The stained films and the sections from various regions showed no abortion bacilli. Guinea pig tests of placental tissue were negative for *Bacillus abortus*. On the other hand, minute organisms resembling vibrios were detected in the cytoplasm of endothelial cells within capillaries in the edematous subchorionic tissue. Subsequently the agglutination titer of the blood serum of one of these cases rose to a level indicating infection with *Bacillus abortus* during the second pregnancy.

The peculiar distribution of abortions due to *Vibrio fetus* among older cows and heifers in this herd, resulting at first in cases among older cows and latterly passing to young stock, may be explained by certain occurrences in the herd itself. It may be assumed that the infection was originally brought in by purchased cows. The young stock is kept segregated from these in a special barn, and when 6 months old it is pastured on outlying farms until returned in an advanced stage of pregnancy. The heifers during the first pregnancy were thus kept away from vibrio carriers until after the first calf was born.

In June and July, 1919, 55 older cows, purchased and native, were placed on the young stock pasture. The three cases of abortion in heifers due to *Vibrio fetus* occurred October 24, November 9, and December 2, 1919. The age and condition of the fetuses accord very well with the assumption that *Vibrio fetus* was introduced among the young stock in June or July of the same year.

The information gathered thus far concerning vibrionic abortion in this herd enables us to formulate a tentative hypothesis subject to modification with increasing knowledge of this type of infectious abortion. The infectious agent was probably introduced by purchased cows in 1917 or earlier. It gained a certain headway up to 1919, then the number of cases declined so that between May, 1919,

and May, 1920, only the above three cases in heifers, and one case of mixed infection with *Bacillus abortus* in an older cow, were detected. During the same period cases due to *Bacillus abortus* continued undiminished. The greater resistance of *Bacillus abortus* manifested in cultures as compared with *Vibrio fetus* is thus reflected in its behavior in nature. The temporary dying out of the infection indicates that natural immunization of a herd to *Vibrio fetus* proceeds quite rapidly. Another outbreak may be expected when the immunity of the herd has declined in the absence of the infecting agent and the latter is reintroduced from without, or it may reappear at any time when a vibrio of higher virulence is brought in.