THE RELATION OF THE BACTERIAL FLORA OF THE UTERUS, TO THAT OF THE MECONIUM OF THE CALF

THESIS FOR DEGREE OF M. S.

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1919

THESIS

The relation of the bacterial flora of the uterus, to that of the meconium of the calf.

Thesis.

Submitted to the faculty of the Michigan Agricultural College in partial fulfillment of the requirements for the Degree of Master of Science.

By

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May 1919.

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THE RELATION OF THE BACTERIAL FLORA OF THE UTERUS, TO THAT OF THE MECONIUM OF THE CALF

Introduction.

This project was undertaken with the hope of throwing some light on two important phases of the abortion disease, first on the relation of bacterial infection of the uterus to that of the alimentary canal of the fetus, and second, on the persistance of the abortion bacilli in the uterus subsequent to abortion and parturition.

In the course of the work some data were collected with reference to the bacterial flora of cystic ovaries and of the uterus of sterile cows.

Review of Literature.

As early as 1885 Nocard (1) made a scientific investigation of infectious abortion at the request of the French Minister of Agriculture. He concluded among other things, that the disease was due to bacterial infection of the fetus and placenta, though he was unable to designate the specific organism. It was left for Bang (2), and his assistant, Stribolt, in 1895, to discover the organism and to demonstrate its definite relationship to the disease. A detailed account of this discovery and of the organism is given in their original publication (1897).

Nowak (3) made an important contribution to the study of infectious abortion by improving Bang's method of isolation and cultivation of Bang's bacillus under conditions of reduced

oxygen pressure brought about with the acid of B. subtilis in sealed jars. Nowak's method is described in a bulletin by Good (4) of Kentucky.

The more recent work on infectious abortion deals largely with the method of diagnosis, prevention, pathological changes, and the various organisms found in the uterus. Some investigators claim that there are other organisms besides Bang's bacillus that cause abortion.

Moussu (5) is of the opinion that certain strains of colon organisms will cause abortion. McFadyean and Stockman(6) claim that uterine infection with tubercule bacilli as well as with streptococci may result in abortion.

Good (7) reports that in one instance, he found the foetal membranes and amniotic fluid of an aborting cow teeming with the Staphylococcus pyogenes aureus in pure culture. The cow became weaker and weaker until it became evident that she would die. She was then slaughtered and autopsied. The uterus was filled with pus, which was found in numerous other internal organs, Staphylococcus pyogenes aureus being the invader. It is evident that this organism entered the uterus causing the expulsion of the fetus and afterwards becoming sufficiently generalized to kill the cow.

Williams (8), in his report on "Researches upon Abortion of Cattle" (1916), states that the bacillus of Bang is frequently found in the stomach and fetal blood or glands, but is never found in the alimentary tract. He claims this indicates that the primary invasion was through the chorion into the amniotic fluid.

Williams (9) reports that the amniotic fluid is being constantly swallowed by the calf. This being true, a bacteriological examination of the meconium should give an idea of the

organisms present in the amniotic fluid in case any are present.

In 1916, Hagan (10) began work on the meconium of new born calves in connection with the general problem of contagious abortion and allied diseases. The meconia of a total of fifteen new-born calves were examined. Fourteen of the calves were in two different herds, six being in the first and eight in the second, one was from an unborn calf obtained at an abattoir. The meconia from the six calves in the first herd all proved to be sterile. In the second herd, three were sterile, while the other five gave growths. Three of the cultures were of colon bacilli, one was a streptococcus, and one was mixed a streptococcus and a staphylococcus. The abattoir specimen gave B. coli.

Hagan gives the following result of the examination of the uterus of sixteen pregnant cows. The utero-chorionic space gave cultures in fourteen instances, or in about 87 percent, the fetal fluids gave cultures in five cases or in about 33 percent. The work of Hagan shows that there is a relation of the organisms found in the utero-chorionic space to those in the amniotic fluid and in the meconium.

A question in the minds of many investigators involves the length of time the abortion bacillus remains in the uterus after abortion. Schroeder and Cotton (11) injected cultures of the abortion bacillus into non-pregnant uteri of cows. The organisms were found to all disappear in the course of a few days. Examination of the uterine discharge of a cow which had aborted, shows that the abortion bacillus persists for a period of from twenty to thirty days, after which time the organism disappears. It is the opinion of Schroeder and Cotton that the abundance and period of persistence is intimately related to the magnitude of the lesions in the uterus attendent upon an abortion.

Schroeder and Cotton (12) have verified through their tests that aborted fetuses harbor Bang's bacilli in their stomach, liver, intestines, lymph glands, spleen and blood, and that in all cases in which the fetus was infected with abortion bacilli, the organism was found in the utero-chorionic space. Huddleson (13) reports the finding of Bact. abortus in the stomach, liver, spleen, kidney and blood of aborted fetuses.

Moussu (5) was the first to make the statement that there were other organisms besides Bang's bacillus which caused abortion. Since this report of Moussu was made, other investigators have reported different organisms found in cattle subsequent to abortion. Egglink, reported by Ward (14), records a bacteriological investigation of 20 cases of Endometritis. In the 20 cases there occured:

Tubercle bacilli----------2
Bacillus pyogenes----------14
Streptococcus----------12
Colon bacillus----------6
Staphylococcus----------5
Bacillus proteus----------3
Bacillus subtilis----------1

In eleven cases reported by Wall (15) there occured:

Streptococcus----------7
Colon bacillus----------5
Bacillus pyogenes----------3
Anaerobic bacillus (in one of these
bacillus of malignant edema)--2
Necrosis bacillus----------1
Proteus bacillus----------1

Wall notes that of these only streptococcus and B. pyogenes have been found as pure infection, the others always being associated with other microorganisms. Wall suggested that the streptoccccus infection seems to be the most acute, as in a very short time it can cause a very deep and diffuse necrosis. The pyogenes infection proceeds more slowly and less intensely, but is of longer duration. Complication with other infections with putrefaction of the inflammatory exudate considerably increases the changes.

Different means of diagnosing the presence of abortion disease and of isolating Bang's bacillus have been used. Huddleson (16) inoculated guinea pigs intraperitoneally. After an incubation period of about twelve weeks, the antibody formation and the lesions of abortion were determined. Cooledge (17), Evans (18), Schroeder and Cotton (19) determined the presence of Bact. abortus to be common in milk by inoculation into guinea pigs. Whenever Bang's bacillus was present in the milk there was a slow development of lesions which are characteristic for this organism. The method of the above investigators in isolating Bang's bacillus by guinea pig inoculations is a very slow process. The latest method is by Smillie (2) and is considered by him to be much better.

Cystic ovaries have been studied to some extent in connection with abortion and sterility. Hiss, reported by Albrechtsen (21) believes that cysts may be the result of the disturbance of nutrition of the graafian follicle. Loeb (22) has recently shown that ovarian cysts may be produced in guinea pigs by underfeeding. In his experiments the feed was reduced to such an extent that the guinea pigs lost from 20 to 35 percent of their wright in from 7 to 14 days, and without exception cysts were found in the ovaries

No cysts developed in the control animals. This, however, was extreme underfeeding. Fitch (23) reports that in all cases where a cystic corpus luteum was cultured, vigorous growth was obtained. One ovary gave a pure culture of a long chained streptococcus. B. coli has been found in several ovaries. Micrococcus pyogenes has been found twice. A rod which seems to be a slight gas producer has been found three times. Albrechtsen (24) believes that ovarian cysts may result from direct infection from the uterus through the tubes, but that they are more frequently produced reflexly.

Method of Investigation.

Immediately after abortion or apparently normal parturition, (cases in which the calf lived) the buttocks and tail of the calf were thoroughly washed with mecurial soap and water. A sterile rubber glove was then put on the hand and by means of the first finger inserted into the rectum a small amount of meconium was obtained for bacteriological examination. If the fetus was dead at the time of abortion, or died in a few minutes afterwards, it was taken to the laboratory where the meconium could be obtained under strictly aseptic conditions. At the laboratory, the fetus was opened to expose the stomach and intestines, then a red hot spatula was held on an area of the stomach, as well as on a part of the colon to be opened. A sharp scalpel, that had been previously boiled in water, was used to open the stomach. A different scalpel was used for the opening of the colon. The material was taken from the stomach by a sterile 10 c.c. pipette which was forced through the small opening made by the scalpel.

The meconium was taken from the colon by a small spatula. Material from each was placed in a sterile Esmarch dish.

Material was collected from the uterus of the cow within a few hours after abortion or apparently normal parturition, also, on the following day, and then once each week for several weeks. The buttocks, tail and vulva of the cow were thoroughly washed with mecurial soap and water. The vagina was washed out with plenty of physiological salt solution. The uterus was retracted with uterine retractors, and if in any case the cervical canal was closed or too small for the metal catheter, it was first dilated with a pair of uterine dilators. The introduction of a pair of dilators into the cervical canal should be done with great caution, as the congested mucosa is easily perforated. Ordinarily fatal consequences do not result. After dilation of the cervical canal, a metal catheter was introduced into the uterine cavity. The catheter cannot be introduced to any considerable distance unless the end is turned to one side or the other, as the body of the uterus is comparatively short. If the catheter is inserted straight in, it will come in contact with the anterior wall separating the right and left horn.

The catheter was then connected with a force pump by means of a rubber tube, and the sterile physiological salt solution pumped into the uterus. A return flow catheter was not used in this work. It was necessary to compress the rubber tube and hold the solution in the uterine cavity until the uterus was well massaged per rectum. When the uterus was well massaged the rubber tube was removed from the catheter and a small amount of the solution allowed to escape. A sterile flask was used to

catch the remainder which was taken to the laboratory.

In bad cases of pyometra, with abundance of pus, it was not necessary to use the physiological salt solution except for flushing the uterus as a therapeutic measure. Just massaging the uterus per rectum was enough to force out through the catheter a large quantity of pus, which was collected in a flask and used for the examination.

Media used and Method of Isolating Organisms.

The media used for isolating the organisms found in the material collected were:

Serum agar slants.

Serum agar plates.

Plain agar slants.

Plain agar plates.

Bouillon.

The serum was made by bleeding a cow or horse aseptically, allowing the blood to form a clot. The serum that rises to the top was drawn off and preserved with chloroform. The serum agar was made a day or two before using by melting tubes of plain agar and allowing them to cool to 40 or 45 degrees C. Serum was added in sufficient amount to equal one third of the amount of agar. If plates were to be used the mixture was poured into a petri dish and allowed to harden. The slants were made in the usual way. Ordinary plain agar which was made neutral or very slightly acid (1.5 percent normal) to phenolphthalein was used.

The same process was used in isolating the organisms from uterine material as from the meconium or cysts. The me-

conium was broken up and mixed well with a few cubic centimeters of physiological salt solution. A platinum loop was inserted into the material to be examined and then streaked across an agar plate four or five times. A second and a third plate were streaked in the same manner with the original material on the needle. The plates were incubated for 48 hours at 37 degrees C. The first plate might have to be discarded, but the second and third plates had many isolated colonies. Each colony was marked with a blue pencil and the plates were put in a Novy jar with plates of B. subtilis and incubated at 37 degrees C. for about four days. The cultural methods of Mowak (3) were used.

A macroscopic examination of the colonies was made with a good hand lens or the low power objective of the microscope. Transfers were made from all the different kind of colonies to agar slants. The different kind of colonies formed on the serum agar plates were transfered to serum agar slants and plain agar slants. The small transparent, dew drop colonies that developed on the plates while in the Novy jar were transfered to bouillon and incubated for two days. Then a transfer from this was made to another bouillon tube which was also incubated for one or two days at 37 degrees C. A transfer was made from the second tube of bouillon to a plain agar slant. Later in the work, it was found that dextrose agar slants gave better growth for Bact. abortus than the plain agar. It has been found, by running duplicate tests using plain agar in one case, and agar slants and bouillon, as described above in the other test, that the latter method gave a much better growth in a much shorter period of time and with less chance of losing the organism. The serum agar was used for isolating B. pyogenes.

The following media were used to identify the organisms isolated:

Plain agar.

Dextrose agar.

Serum agar.

Serum slants.

Blood agar.

Mannit agar.

Litmus lactose agar.

Gelatin.

Pork gelatin agar.

Litmus milk.

Plain milk.

Lactose bouillon.
Dextrose bouillon.
Saccharose bouillon.
} Placed in fermentation tubes and neutral red added.

Bouillon.

Esculin bile.

Potato slants.

Glycerine agar.

Soluble starch agar.

Dulcite agar.

Sorbin agar.

Arabinose agar.

Inulin agar.

Inulose agar.

The agar for the last six media, was made of agar-agar, water, salt and peptone, with one percent of the desired sugar added. Many organisms especially the different types of the colon group will ferment the ordinary plain agar.

Guinea Pig Inoculations.

Another method used to determine the presence of Bang's bacillus in the uterus and meconium was the injection of guinea pigs with the material collected. Two cubic centimeters of a solution of meconium was injected intraperitoneally into each of two guinea pigs. This was continued for about four washings covering a period of four weeks. The blood of the guinea pigs was tested for abortion every month after injection, for a period of three months. From twelve to fourteen weeks after the injection, the pigs were killed and examined for abortion lesions. The blood was tested by the complement fixation and agglutination tests. The technique employed in the complement fixation test is that of Hadly and Beach (25) of Wisconsin.

Table I.

Agglutination Test.

Tube	Antigen c.c.	Serum undiluted drops	Dilution
1	2	2	1-20
2	2	1	1-40
		Serum diluted 1-20 drop	
3	2	8	1-100
4	2	4	1-200
5	2	2	1-400
6	2	1	1-800

Shake all tubes well and incubate over night at 37 degrees C.

In abortion work it is not necessary to use the first tube which is a dilution of 1-20.

In the above table a dropper system was used. The capillary pipettes were graduated to deliver twenty drops to the cubic centimeter.

Experimental Work.

The experimental work was begun in June 1918. Each animal worked with is taken up individually. A history of the animal, if any could be obtained, is given.

Case 995 was purchased at the age of one year (1913).

Blood test August 20, 1913, negative.

Bred May 11, 1914.

November 15, 1913, she received a 10 c.c. culture of dead Bact. abortus, also, in December 1913, January and February 1914. Complement fixation and agglutination tests made after these injections showed a positive reaction until May 29, 1914. November 20, 1916 and December 10, 1916, she was given subcutaneously 5 c.c. and 10 c.c. dead culture of Bact. abortus. February 12, 1917, she was given 20 c.c. of live culture of Bact. abortus subcutaneously. She developed a positive reaction February 12, 1917 and has remained positive until the present time.

Calved January 15, 1916.

Bred October 17, 1917.

Calved June 24, 1918 the 249th day of the period of gestation. Following this, a uterine infection developed. The discharge from the uterus which was mixed with mucus, was of a thick yellowish non-purulent nature. The contents from the first

washing (the day of parturition) were centrifuged and 0.5 cubic centimeters of the sediment injected into each of two guinea pigs. A microscopic examination of the first washing revealed many short rods, a few long rods, and cocci, also, a few lymphocytes. July 3, 1918, after collecting material for examination, the uterus was treated with a 25 percent Lugol's iodine solution. A microscopic examination of the uterine washing at this time revealed many short rods and cocci, also, some lymphocytes and epithelial cells. July 8, 1918, the discharge was more the nature of mucus and was not as abundant as when previously treated. After collecting material for examination, the uterus was again treated with 25 percent Lugol's iodine solution.

Results obtained from the guinea pigs; One pig died of an infection of B. coli communior. The second pig was bled and then autopsied. The pig had a slightly enlarged spleen, but Bang's bacillus could not be isolated. The complement fixation and agglutination tests of the guinea pig blood was negative with Bact. abortus antigen.

The organisms isolated from uterine washings were:

B. subtilis.

B. coli communior.

Staphylococcus pyogenes aureus.

Staphylococcus pyogenes bovis.

Streptococcus pyogenes.

B. 121.2421032 (see table VI)

The cow was bred July 26, 1918.

Doctor Hallman examined this cow for pregnancy January 21, 1919. External os was open to such an extent that the thumb could be introduced as far as the second fold. She was killed for

beef January 30, 1919, and the following notes were taken. Pregnant in left horn. Posterior presentation. The head of the calf was bent backward and to the left, with the point of the head lying on the left shoulder. Cervical canal was slightly open at the posterior end. The canal was quite well filled with mucus of pregnancy which was protruding back into the vagina. In the body of the uterus and posterior ends of both horns, the chorion was attached to the uterine mucosa by a diffuse development of cotyledonous tissue. This cotyledonous tissue consisted of tufts varying in size from one centimeter to nodules three centimeters in diameter by five centimeters in length. The area involved was approximately fourty centimeters long. Along the dorsal wall of the pregnant horn there was apparently a diminition in the number of cotyledons. In other portions of the uterine cavity the development of cotyledons was apparently normal. The cotyledons varied from three to eight centimeters in length. In the utero-chorionic cavity there was a small quantity of the so called abortion exudate. Material was taken from the utero-chorionic space, amnionic fluid, and meconium of fetus for bacteriological examination. No organisms could be grown from any of the above.

Case 995 B., the calf of cow 995, born on the 249th day of gestation, was very weak, but lived. Meconium was obtained for examination. Two c.c. of a suspension of meconium was injected intraperitoneally into each of two guinea pigs.

The organisms isolated were:

B. coli communior.

Staphylococcus pyogenes aureus.

On September 26, 1918 the pigs were bled and then autopsied. No abortion lesions were found. The complement-

fixation and agglutination tests of the guinea pigs blood were negative.

Case 801 was purchased September 1, 1917, Her blood reaction at that time was negative. She was bred November 3, 1917. At week intervals, beginning on May 29, 1918, she received intravenously four injections of 5, 10, 20, and 20 cubic centimeters respectively of live culture of Bact. abortus. On June 7, 1918, one week after injection of Bact. abortus, she developed a strong positive reaction to the complement fixation and agglutination test respectively. She aborted July 17, 1918, the 257th day of the period of gestation. The calf was very weak and died in about one hour. The following day the fetal membranes had to be removed. At this time the uterus was treated with Septicosolution and boric acid. On July 20 and 26 and August 1st, the uterus was washed out with physiological salt solution and the samples taken to the laboratory for examination. A microscopic examination of the various washings revealed many small rods and cocci, also, a few lymphocytes and epithelial cells.

In the bacteriological examination, the following organisms were isolated:

B. coli communior.

Streptococcus pyogenes.

Staphylococcus pyogenes bovis.

Bact. abortus.

Bact. 122.4222034 (See table VI).

Case 801A, the calf of cow 801, was aborted on the 257th day of gestation, and lived only about one hour. Some of the meconium and contents of the stomach were obtained for bacteriological examination and for injection into guinea pigs.

The bacteriological examination of the stomach showed no growth aerobically or anaerobically. The organism isolated from the meconium was Staphylococcus pyogenes bovis.

The blood of the guinea pigs was negative to both the complement fixation and agglutination tests for abortion. Autopsy revealed no lesions of abortion.

Case 802 was purchased September 7, 1917. The blood reaction at the time of purchase was negative.

Bred for the first time September 21, 1917 and calved June 27, 1918. She has remained negative up to the present time. She developed a uterine infection after parturition, the discharge being of a bloody nature. The microscopic examination revealed many cocci and a few short rods, also, many leucocytes and a few epithelial cells. The cervix was perforated on July 18, 1918, so no more material was collected.

The organisms isolated were:

Staphylococcus pyogenes aureus.

Staphylococcus pyogenes bovis.

Streptococcus pyogenes.

The guinea pigs did not show any lesions of abortion. The complement fixation and agglutination tests of the guinea pigs blood were negative.

Case 802A is the calf of cow 802, born June 27, 1918. The calf was strong and healthy. Meconium was obtained for examination. The organisms isolated were:

Staphylococcus pyogenes bovis.

B. coli communior.

The guinea pigs which had been injected with meconium were bled and then autopsied. Autopsy revealed no abortion.

lesions. The complement fixation and agglutination tests of the blood were negative.

Case B. S., a Brown Swiss cow from a herd near by, aborted July 5, 1918, on the 200th day of gestation. Material was collected from the uterus only once.

The organisms isolated were:

B. coli communior.

B. coli (Sub. Group I).

Bact. 212.2442014 (see table VI)

Bact. 111.2421014 (see table VI)

Bact. 122.1111032 (see table VI)

Bact. 222.2121012 (see table VI)

Case B. S.a, the fetus of the Brown Swiss cow, was aborted on the 200th day of gestation. Meconium and material from the stomach were obtained for examination and injection into guinea pigs. No organisms were found in the stomach. The blood of the guinea pigs was negative and on autopsy no lesiont of abortion could be found.

The organisms isolated from the meconium were:

B. coli communior.

B. coli (Sub Group II)

Staphylococcus pyogenes aureus.

Bact. 212.2441014 (see table VI).

A bacteriological examination of the heart blood of the fetus revealed B. coli communior.

Case 805 was purchased September 7, 1917. Blood reaction at the time of purchase was negative. She was bred November 3, 1917. At week intervals beginning May 29, 1918, she received subcutaneously 10,20,40, and 40 cubic centimeters respectively of live culture of Bact. abortus. On June 7, 1918.

she showed a partial reaction to the agglutination test; on June 21, 1918, she showed a marked positive reaction to both the complement fixation and agglutination tests and has maintained that reaction to the present time. She calved August 7, 1918. The calf was strong and apparently healthy. She cleaned in a short time, but developed a bad case of endometritis, with an abundance of dirty brown, thick discharge from the uterus. August 15, 1918, after some material was collected for examination, the uterus was treated with 2 oz. of a 25 percent Lugol's iodine solution.

Smears were made from the uterine material at the time of the first washing and many small rods and cocci were observed. There were also some lymphocytes and epithelial cells present. The organisms isolated were:

Bact. abortus.

B. pyogenes.

Streptococcus (gram negative)

Staphylococcus pyogenes albus.

Staphylococcus pyogenes bovis.

Streptococcus pyogenes.

In this case the Bact. abortus persisted in the uterus for 28 days after parturition.

Case 805A is the calf of case 805. The calf was strong and apparently healthy. Meconium was obtained for guinea pig injection and for bacteriological examination. The organisms isolated were:

Staphylococcus pyogenes aureus.

B. coli communior.

The guinea pigs were bled and then autopsied. The autopsy revealed no lesions of abortion. The blood was negative by both the complement fixation and agglutination tests.

Case 996A was born May 28, 1916. There is no record of her until November 9, 1917, at which time the complement fixation and agglutination tests of her blood were positive.

She had a very difficult parturition from an over sized calf March 31, 1918. A bad case of pyometra developed and B. coli, Streptococcus pyogenes, and B. pyogenes were isolated from the uterus by Doctor Beaver. The return of the uterus to an apparently normal condition within a few weeks was probably due to the treatment recommended by Doctor Hallman.

The latter part of July she developed a bad case of endometritis. The discharge was of a dirty brown or reddish brown color, sticky in character, adhesive to the tail and other parts of the body with which it came in contact. The uterus was washed out several times with physiological salt solution and the material was used for bacteriological examination.

The organisms isolated were:

Streptococcus pyogenes.

B. coli communior.

Bact. 222.1131032 (see table VI).

Smears of the material from the uterus revealed many rods and a few chains of streptococci, also, a few epithelial cells and lymphocytes. She was killed for beef October 7, 1918. Notes on the macroscopic examination of the uterus:

Between the body of the uterus and the rectum there was an abcess containing about three ounces of thick creamy pus.

The body of the uterus and vagina were firmly adherent to the rectum.

The left oviduct was apparently normal. The right oviduct was normal except for the presence of a small cyst about 2 m.m. in diameter situated about midway between the ovarian and uterine end of the oviduct.

The left ovary was covered by peritoneum and showed a few immature cystic follicles on the surface. The right ovary was completely ensheathed in fibrous tissue that was a continuation of the abscess capsule. The cervical canal was six centimeters long. The body of uterus was two centimeters long, uterine cavity contained apparently no exudate; the mucosa was moist and glistening. The color was not entirely uniform, but particularly around some of the cotyledons, including the cotyledons of the left horn, the mucosa was more vascular. The cotyledons varied in size from one quarter to three quarters of a centimeter in diameter. The apex of the first cotyledon in the horn which was about one and one half centimeters in diameter, was distinctly vascular or like a recent hemorrhage (bright red). The gland mucosa otherwise was of a grayish chocolate color. On the tissues of the broad ligament there were several small abscesses varying in size up to the size of a hazelnut. These contained a thick creamy pus.

Unfortunately, it was not possible to make a bacteriological examination of the pus as the case was available at the time the use of the laboratory and assistants (Mr. Welsh, Mr. Kidmans and myself) were given over to the bacteriological work in connection with the influenza epidemic in the U. A. T. C.

Case 808 was purchased September 7, 1917. Blood reaction at the time of purchase was negative.

September 23, 1917, she was injected subcutaneously with 25 cubic centimeters of live culture of Bact. abortus. October 5, 1917, she developed a marked positive reaction th the complement fixation and agglutination tests. This positive reaction was maintained until she was killed.

She was bred November 1, 1917 for the first time and on several occasions afterwards, but failed to conceive. She was a nymphomaniac and was, therefore, killed August 12, 1918. The reproductive organs were removed and taken to the laboratory for a bacteriological as well as for a macroscopic examination. Swabs were taken from the uterus; a bacteriological examination of these failed to show any organisms.

An examination of a cystic ovary revealed two organisms.

B. coli communior.

B. 121.4442012 (see table VI).

Notes on the macroscopic examination; The vagina contained a considerable quantity of clear mucus. The cervix was contracted. There was a slight amount of adhesive mucus in the cervical canal. The body of the uterus which was four centimeters long was very much thickened and apparently fibrous. The mucosa of the horns was yellowish gray and moist. The cotyledons of the horns were one quarter to one half centimeter long and one quarter centimeter wide. Ovarian tubes were apparently normal.

Right ovary which was three centimeters long and oval,

contained a cyst about the size of a hazelnut. No corpus luteum was present.

Left ovary was slightly smaller; it presented a cicatrix extending around its dorsal border. No corpus luteum was present.

Case 13, was born August 30, 1915.

Calved in 1917.

Calved July 10, 1918.

There was no history of abortion, retained placenta, or difficulty in getting with calf. She has shown no signs of estrum since calving. This cow was killed for beef August 9, 1918, and the reproductive organs saved for bacteriological and macroscopic examination.

Notes taken on the macroscopic examination are as follows:

Vagina contained considerable mucus. External os closed. Cervical canal about nine centimeters long. Body of uterus five centimeters long.

Uterine mucosa a light chocolate color. Cotyledons one quarter to one half centimeter long. Color was more yellowish gray than the gland mucosa. Mucosa of the uterine horns about the same color as that of the body. Cotyledons average a little larger. Oviducts apparently normal.

Right ovary about three centimeters in diameter. There was a cystic corpus luteum present; also about 20 small cysts.

Left ovary three centimeters long and one and one half centimeter in diameter. Showed no regressed corpus luteum.

Bacteriological examination: No growth from uterus. Cystic ovary contained *B. coli communior*.

Case 14., was a cow from a beef herd that reacted to the tuberculin test without showing lesions post mortum.

The reproductive organs were removed and taken to the laboratory. The bacteriological examination of the uterus gave negative results.

Notes on the macroscopic examination are as follows:

The vagina was apparently normal. External os, which was closed, was about three centimeters in diameter. Cervix about fourteen centimeters long. Mucosa was light chocolate color. Cotyledons were one to one half centimeter long and showed a depressed center. Mucosa of the horns was light chocolate color but not uniform. Some areas showed a slight congestion. Cotyledons about one half to one and one half centimeters. Oviducts were apparently normal.

Right ovary was about five centimeters long. It showed a protruding corpus luteum and either a cyst or a graafian follicle.

Left ovary was about five centimeters long.

Case L. M. was a cow from a neighboring dairy herd. Her first calf was aborted, after which she had three normal calves.

March 12, 1917 was the last time she calved. She was bred four times since the last calving but failed to conceive. She went from May 29, 1917 to December 20, 1917 without occurrence of estrum. The last estrual period was July 8, 1918.

An examination was made by Doctor Hallman for sterility July 24, 1918. The vagina showed a small amount of almost clear mucus. The os uteri was contracted and apparently normal. The uterus was not enlarged and was apparently normal. The

right ovary was normal but the left ovary showed a protruding corpus luteum. The uterus was douched with physiological salt solution. The vagina and cervix were douched with salt-soda solution.

Bacteriological examination of the uterus was negative.

The vaginal secretion was very slightly alkaline. A few rods and cocci were present, also, a few epithelial cells but no lencocytes. Cervix was slightly alkaline. Smears from the cervix showed considerable mucus, but only a few cocci and small rods were present. There were a few epithelial cells and numerous lymphocytes.

September 10, 1918, she was bred and conceived.

Case D. B. was examined by Doctor Hallman for sterility, July 16, 1918. The date of the last calving was January 15, 1917. She had been served at irregular intervals for more than a year since last calving. Her general condition was good and state of lactation was dry at the time of examination. Os uteri was comparatively dry, cicatrix on lower left side. Uterus was not enlarged and tone was firm. Right ovary was about two centimeters long and left ovary was the same size with small cysts about the size of a pea. Secretions from the vagina and cervix were acid.

Smears from the vagina revealed quite a few epithelial cells and an occasional lymphocyte. A few rods usually in clumps and a few cocci were present. Some of the epithelial cells showed a few cocci in the process of phagocytosis.

Smears from the cervix showed numerous epithelial cells a few lymphocytes and a few cocci.

The cervix was flushed with salt-soda solution. The uterus was flushed with sterile physiological salt solution. Some of this salt solution was collected in a sterile flask for

an examination. Then the uterus was flushed with a salt-soda solution.

One organism was isolated from the uterus; Bact. 211.1421012 (see table VI).

August 8, 1918, the vagina and cervix were flushed with salt-soda solution and then the cow was bred and conceived.

Case M. C. never had a normal calf. She had two distinct abortions one of which was her last calving on June 16, 1917. A third one was suspected of abortion. This cow was served four times at irregular intervals since last calving and on July 16, 1918, an examination was made by Doctor Hallman for sterility. She was in heat July 12, 1918, but was not in heat for three months previous to this. The general condition of the cow was abnormally beefy. She never gave any milk. The vagina contained a small quantity of clear mucus with streaks of cloudiness. Os uteri was almost completely contracted. Uterus was not enlarged. Right ovary was about three centimeters long. Left ovary was about three centimeters long and showed a protruding corpus luteum. Secretions of the vagina and cervix were acid to phenolphthalein.

Smears from the vagina showed a few epithelial cells of the squamous type and an occasional cell of columnar type. There were a few lymphocytes present, but only a few rods and cocci.

Smears from the cervix show numerous lymphocytes, epithelial cells, a few cocci and oval organisms.

The uterus was flushed with physiological salt solution; a sample was collected in a sterile flask and taken to the laboratory for bacteriological examination. The examination revealed *B. coli communior*.

After flushing the vagina, cervix, and uterus with one

percent sodium bicarbonate in salt solution, the cow was bred.

Case H. L. aborted December 25, 1917 with retained placenta. From this time, until July 24, 1918, the day of examination for sterility by Doctor Hallman, she has had two services and only two estrual periods. Her general condition was good and the state of lactation was far advanced at the time of examination. Examination showed a large amount of mucus with streaks of pus in the vagina. Os uteri was contracted and very moist.

Left horn of uterus was a little larger than the right; walls were more flaccid. Right ovary was two centimeters long and apparently normal. Left ovary contained a cyst about the size of a hazelnut.

Secretions of the vagina and cervix were very slightly alkaline. The cell content of the vaginal and cervical smears were much greater than in the former three cases observed. In addition, there were quite a few polynuclear cells, apparently more of the latter in the vagina than in the cervical smears. The number of microorganisms occurring was apparently few considering the cell content. There were a few short rods and numerous clumps of staphylococci which appeared to be encased in mucus.

The uterus was flushed with salt solution and a sample collected for bacteriological examination. After flushing the cervix and vagina with one percent soda-salt solution, the cow was bred July 29, 1918. At this time the cervix and vagina contained considerable mucus with a few streaks of pus. The cow was in heat.

The bacteriological examination of the uterus did not reveal any organisms.

Case 19; a calf five weeks old, that died of gastro-intestional intoxication, was autopsied and found to contain a

cystic ovary. The ovary was removed and taken to the laboratory for examination.

The organisms isolated were:

B. coli communior.

Streptococcus.

Case 32 was born August 30, 1910.

She was bred on the following dates:

Dec. 21, 1912; Calved Oct. 2, 1913.

April 18, 1914; Calved Feb. 1, 1915.

March 16, 1915; Aborted May 30, 1915.

Aug. 22, 1915.

Oct. 26, 1915.

Dec. 10, 1915.

April 2, 1916; Aborted Nov. 4, 1916.

Jan. 12, 1917.

Feb. 2, 1917; (Yeast treatment)

Feb. 26, 1917.

March 18, 1917.

April 9, 1917.

May 6, 1917.

Nov. 6, 1917.

There were no records of abortion beside those recorded above.

On December 14, 1918, this cow was examined by Doctor Hallman and the following notes made:

Cervix much congested; considerable cloudy mucus around external os. Cervix slightly dilated. Uterus not enlarged but walls flaccid.

Diagnosis: catarrhal cervicitis and endometritis. The animal was slaughtered for beef on February 11, 1919. The uterus

was taken to the laboratory where swabs were made: A bacteriological examination was made. The uterus was sterile.

Notes on the macroscopic examination are as follows:

Posterior end of the cervix was about five centimeters in diameter. External os was slightly dilated. Mucous membrane was covered with considerable quantity of slightly cloudy, adhesive mucus. Color of mucous membrane was not uniform, but there were numerous small echymotic-like areas. Whether these were local hemorrhages or highly vascular areas was hard to tell. Cervical canal was about nine centimeters long. The color was grayish drab with few echymotic-like areas. Body of uterus was six centimeters long. Uterine mucosa was darker drab than the cervical mucosa and fairly uniform. Mucous membrane of the left horn was similar in appearance to the body, some portions were a little more vascular. Cotyledons were about one centimeter in diameter and were of a yellowish gray color. Mucous membrane of the right horn was similar to that of the left horn. Mucous membrane was moist and glistening but no apparent exudate. The right oviduct apparently normal. Right ovary three centimeters in diameter, spherical in shape, contained a protruding corpus luteum one centimeter in diameter and several immature follicles. Left ovary about three centimeters by two centimeters. Contained a protruding corpus luteum one centimeter in diameter and a cyst like structure one and one half centimeter in diameter.

Case 17, a Red Polled heifer three years old that had never calved, was bred in April 1918. There was no history of breeding since April 1918. Cow was in pasture during the summer. The first week in December 1918, the cow aborted a fetus about nine

inches (approximately four months old). This was probably from service in the pasture during the summer.

This cow was killed for beef January 21, 1919. Swabs were made from the uterus. Cystic fluid was collected for examination. There were no organisms found in the uterus or cystic ovary.

Notes taken on the macroscopic examination are as follows:

Uterus not enlarged. Right ovary normal. Left ovary contained a full sized corpus luteum, one cyst one centimeter in diameter, and three or four small ones. Both oviducts normal. External cervix three centimeters in diameter. External os closed. Small quantity light chocolate colored, slightly adhesive substance in os. Hypertrophy of second and third folds. Body four centimeters long. Mucous a light drab, fairly uniform in color. Mucus of right horn was not uniform in color; a portion was light drab while other parts were dark drab (mottled). Cotyledons one half to one centimeter long; quite uniform in color except in mottled areas. Mucous membrane was moist and glistening with no exudate. Mucous membrane of left horn was of lighter and more uniform color than that of the right horn.

Case 18 was born May 18, 1913.

Bred August 20, 1914;

Calved May 21, 1915.

After calving she had some trouble in getting with calf and was treated for sterility by Doctor Hallman.

She was bred January 27, 1916; Calved October 28, 1916. There is no record of treatment after second calving.

She was bred on the following dates:

Sept. 17, 1917.

Jan. 14, 1918.

Feb. 14, 1918.

March 10, 1918.

Aug. 9, 1918.

There is no record of abortion during this time.

Cow was slaughtered for beef January 27, 1919. Swabs were made from the uterus. No organisms were found in the uterus.

Notes on macroscopic examination of the uterus are as follows:

Cyst four centimeters long and one centimeter in diameter, projecting above the surface of vaginal mucosa, was about twelve centimeters posterior to the os. External cervix was six centimeters in the largest diameter. On opening the cervical canal, the external folds at its lower border were found to be considerably enlarged. Left lateral portion of third fold was not quite so enlarged as the first. Body of uterus two centimeters in length. Small quantity of clear, slightly adhesive exudate in the cervical canal. Mucosa of the left horn was of a light chocolate color; not uniform, some streaks showing more blood pigment than others. Cotyledons three millimeters in diameter showing depressed area in center. Walls of the right horn apparently a little thicker than those of the left horn. Adjacent to some of the cotyledons of the right horn, the gland mucosa was decidedly more vascular than other portions. Mucosa of both horns was moist and glistening, with no inflammatory exudate. Both oviducts apparently normal. Right ovary contained a corpus luteum one and one half centimeters in diameter, projecting well above the surface. In the center, there was a cyst

one centimeter in diameter containing clear fluid, also, several small cysts spherical in shape. Left ovary three and one half centimeters long, slightly flattened; cyst one centimeter in diameter.

One organism, B. coli communior was isolated from the cyst.

Case 997B was born February 4, 1916. The cow was negative to the complement fixation and agglutination tests. January 16, 1917, she received 25 c.c. of a live culture of Bact. abortus. October 12, 1918 and October 13, 1918, she received 5 c.c. of a live culture of Bact abortus on feed. She calved November 15,1918 and has remained negative to abortion up to the present time.

Amniotic fluid was collected at the time of parturition. Washings from the uterus were collected three times. Following this, the os was closed so no more washings were obtained.

The amniotic fluid was sterile.

All the uterine washings were sterile. The blood of the guinea pigs was negative to abortion.

Case 997B1, a strong healthy calf from cow 997B, was born November 15, 1918.

The meconium was examined and 2 c.c. injected into each of two guinea pigs. The blood of the guinea pigs was negative to abortion.

The meconium was sterile.

Case 999 was purchased at the age of one year. Blood reaction at the time of purchase was negative to abortion.

October 10, 1913 and November 8, 1913, she received 10 and 10 c.c. of a live culture of Bact. abortus subcutaneously. She developed a reaction October 29, 1913 and remained positive until May 29, 1914.

She calved February 3, 1916.

December 13, 1916 she received 10 c.c. of a live culture of Bact. abortus intravenously.

Calved December 31, 1916; premature.

January 5, 1917 she developed a positive reaction to abortion.

Calved November 5, 1917.

Bred February 15, 1918; calved November 26, 1918.

This cow cleaned in a short time. Three uterine washings were examined and then the cow was sold. Staphylococcus pyogenes aureus was found. The blood of the guinea pigs was negative to the complement fixation and agglutination tests for abortion.

Case 999 D., a strong calf from cow 999 was born November 16, 1918. The meconium was examined and 2 c.c. was injected into each of two guinea pigs. The blood of the guinea pigs remained negative and no lesions of abortion were found.

Staphylococcus pyogenes aureus was found in the meconium.

Case 806 was purchased September 7, 1917. The reaction at the time of purchase was negative to abortion. On September 25, 1917, she received intravenously 10 c.c. of a killed culture of Bact. abortus. October 5, 1917, she received intravenously 30 c.c. of a live culture of Bact. abortus. October 5, 1917, she developed a full positive reaction to abortion.

This cow had not been in heat up to January 3, 1918. Observations had shown that after an electric dissociation treatment cows would come in heat. On January 3, 1918, she was given an electric dissociation treatment for sterility by Doctor Hallman.

Beginning February 22, 1918, she was given live culture of Bact. abortus on feed every day for one week.

She was bred February 27, 1918 and calved November 25, 1918.

On the date of parturition a washing was taken from the uterus. November 27, 1918, there was a discharge of pus from the uterus. It diminished until by the fourth week it was entirely absent.

The organisms isolated were:

B. coli communior.

Streptococcus pyogenes.

The guinea pigs that were injected with uterine washings were killed but no lesions of abortion were present. The blood was negative to both complement fixation and agglutination tests for abortion.

Case 806 A., the calf of cow 806 was born November 25, 1918. Meconium was obtained for a bacteriological examination and for injection into guinea pigs.

B. coli communior was isolated from the meconium.

The guinea pigs were bled and then autopsied after the incubation period.

The blood of the pigs was negative to abortion tests. All organs were apparently normal.

Case 11A1A was purchased January 1917 at the age of two years. Reaction at the time of purchase was positive. She aborted her first calf January 1917. January 19, 1918, she gave birth to an apparently normal calf.

She was bred April 7, 1918 and calved January 13, 1919.

She cleaned in a few hours after calving. Washings were taken from the uterus for examination and for injecting into guinea pigs.

There was no discharge from the uterus for two weeks, when there appeared a dirty yellowish discharge which contained epithelial cells, cocci and a few leucocytes.

There were no organisms grown from the contents of the first three washings. A streptococcus was isolated from the next three washings.

The results of the guinea pig inoculation were negative

Case 11A1A2, the calf from 11A1A was born January 13, 1919. The meconium was collected and 2 c.c. of a suspension of it was injected into each of two guinea pigs.

March 24, 1919 the pigs were killed. All the organs were apparently normal. The blood was negative to abortion tests.

The meconium was sterile.

Case 50 was a clinic case of Doctor Hallman. The cow calved the last week in November 1918. No attention was given her until December 24, 1918. On this date the diagnosis of pyometra was made. The uterus was irrigated with physiological salt solution and then 2 oz. of 50 percent Lugol's solution was introduced into the uterus. Frequent treatments were given.

January 31, 1919, a 5 percent formalin solution was introduced into the uterus. The same treatment was given on February 7, 1919.

On February 10, 1919, Doctor Hallman collected some of the discharge from the uterus and sent it to the laboratory for examination. Streptococcus pyogenes was isolated. It will be noted that the streptococcus was isolated after the drastic treatment which had been given every few days since December 24, 1918, and especially after the formalin treatment.

Case 999 A was born February 3, 1916, She gave a negative reaction to abortion tests.

She calved January 23, 1918.

Was bred March 16, 1918, and calved again December 24 1918.

She cleaned in about two hours but developed a metritis. Uterine washings were taken for examination. Two guinea pigs were injected with 2 c.c. each of the various washings.

The cow remained negative to the abortion tests before and after parturition.

The organisms isolated were:

B. coli communior.

Streptococcus.

The streptococcus failed to grow on the media during the first two days of incubation at 37 degrees C., but appeared as tiny pin point blue colonies at the end of the third day of incubation in the Novy jar.

A stain made from the colonies taken from the Novy jar showed the organisms to be short chain, gram negative streptococci. This streptococcus was isolated twice out of the five washings.

The guinea pigs injected with the washings of 999 A were all negative to abortion tests. There were no macroscopic lesions of abortion.

Case 999 B., the calf of 999 A, was born December 24, 1918. Meconium was obtained for examination. Guinea pigs were injected.

The blood of the guinea pigs was negative to the

abortion tests.

No macroscopic lesions of abortion were present.

B. coli communior was isolated from the meconium.

Case 997 was purchased at the age of one year. On October 10, 1913 and on November 8, 1913, she received 10 c.c. of a live culture of Bact. abortus. She developed a positive reaction to the abortion tests and maintained it until May 29, 1914.

She calved February 4, 1916.

On December 18, 1916, she received 10, c.c. of a live culture of Bact. abortus. She developed a positive reaction January 5, 1917, and maintained it.

She calved December 21, 1916.

Was bred April 8, 1918, and calved again January 18, 1919.

Uterine washings were examined and 2 c.c. were injected into guinea pigs.

There were no organisms isolated from the uterine washings.

The blood of the guinea pigs was negative to the abortion tests.

There were no macroscopic lesions.

Case 997 C., the calf of 997 was born January 18, 1919. The calf was strong and apparently in a healthy condition.

Meconium was plated out but gave negative results. The guinea pigs that were injected gave negative results to the abortion test.

Case 807 was purchased September 7, 1917. The blood reaction at the time of purchase was negative to the abortion

tests.

September 13, 1917, she was given 10 c.c. of a culture of killed Bact. abortus subcutaneously. She developed a positive reaction September 20, 1918, which lasted until November 16, 1917. Since this time she has maintained a negative reaction to the abortion tests.

September 23, 1917, she was given 25 c.c. of a culture of live Bact. abortus subcutaneously.

Observations have shown that an animal will come in heat shortly after an electric dissociation treatment. Since this cow would not come in heat, she was given an electric treatment for sterility by Doctor Hallman. (January 2, 1918).

On April 2, 1918, she received 5 c.c. culture of live Bact. abortus intravaginally and was immediately bred but failed to conceive. She was given the same treatment April 22, 1918. May 31, 1918, and on June 9, 1918. On June 10, 1918, she conceived.

The writer noticed that she was discharging shreds of tissue and a dirty yellowish putrid pus about November 20, 1918. This continued until December 27, 1918, when she aborted. The placenta came away in small pieces mixed with an abundance of dirty yellowish putrid pus. The discharge contained numerous small rods, cocci, epithelial cells and leucocytes. Washings were taken from the uterus every week until February 5, 1919.

The organisms isolated were:

B. coli communior.

Streptococcus.

The guinea pigs injected with 2 c.c. of the various washings of the uterus failed to show any lesions of abortion. The blood of the guinea pigs was negative to the abortion tests.

The streptococcus failed to grow the first two days of incubation of the plates at 37 degrees C. On the third day of incubation in the Novy jar, the colonies appeared as very small, pin point, bluish colonies. This organism was isolated six times in this case.

April 3, 1919, a 24 hour growth of this organism on veal agar slant was washed off with physiological salt solution and 1 c.c. of the suspension injected subcutaneously into a pregnant guinea pig. April 6, 1919, the pig aborted. On April 7, 1919 the pig was autopsied. The spleen was once and a half its normal size. The streptococcus was isolated from the spleen, blood, and uterus of the guinea pig.

Case 807 A, the fetus of cow 807, was aborted on the 200th day of gestation. The fetus was taken to the laboratory for examination. A streptococcus which grew the same as the one isolated from the uterus of case 807 was isolated from the blood, liver, spleen, stomach and meconium. A gram stain of the blood, liver, and spleen showed short chains of a cocci which were gram negative.

The liver and spleen were very friable. The male guinea pigs injected with 2 c.c. of a suspension of meconium did not show any lesions of abortion and the blood was negative to the abortion tests. The pigs used for the injection of the stomach contents were apparently normal.

The organism caused abortion in a pregnant guinea pig in about three days.

Case 20, a grade shorthorn, was about six years old when her last calf was born in January 1917.

The cow was bred in late spring or early summer and was

thought to be with calf during the summer. There was no evidence of abortion but the animal began to show signs of estrum, at first irregularly but during the last summer and fall quite regularly. She was bred persistently for some time with failure to conceive.

Cow was killed for beef February 11, 1919.

The reproductive organs were sent to the laboratory where a bacteriological and a macroscopic examination was made.

Results of the bacteriological examination were:

The uterus was sterile. The cystic ovary contained B. coli communior.

Notes on the macroscopic examination:

External cervix was four centimeters in diameter. External os was closed. There was quite a quantity of clear only slightly adhesive mucus in the external os and covering the posterior end of the cervix. Cervical canal was seven centimeters long. Transverse folds were apparently normal but there was an appreciable quantity of rather adhesive exudate present. The body of the uterus was five centimeters long. Mucous membrane was of a light gray uniform color except two small areas averaging about two centimeters by one half centimeter near internal os. These were apparently more vascular than that surrounding the gland mucosa. The cotyledons were about one half centimeter in diameter; and were depressed in center. Left horn was similiar to the body, fairly uniform with few vascular areas. Color of the horns was similiar to that of a heifer more than that of a cow that had calved. Left oviduct was normal. Left ovary was four centimeters in diameter, spherical in shape and contained a protruding corpus luteum two centimeters in diameter. The right ovary was four

centimeters long; contained one cyst-like structure two centimeters in diameter and several small ones.

Case 9, was born May 16, 1911.

The animal was bred on the following dates:

Feb. 29-13.

May 30-13.

Dec. 20-13.

Jan. 12-14.

Feb. 3-14.

March 10-14.

April 10-14.

April 27-14.

May 5-14.

June 30-14.

Aug. 9-14. Calved May 15-15.

There was no record of abortion at any time nor did the records indicate the appearance of estrum between May 30, 1913 and December 20, 1913.

Artificial impregnation was attempted on the following dates:

Dec. 20-13.

June 12-14.

Feb. 2-14.

March 10-14.

March 30-14.

April 10-14.

April 27-14.

May 5-14.

The yeast treatment was given June 30, 1914 and August 9, 1914.

This animal was bred again on the following dates:

July 20-15.

Sept. 9-15.

Sept. 18-15.

Oct. 4-15.

Oct. 21-15.

Dec. 21-15.

Dec. 31-15.

Jan. 20-16.

Feb. 24-16.

March 31-16 Yeast treatment was given.

May 19-16 " " " "

June 7-16. " " " "

June 23-16. " " " "

July 11-16.

Sept. 9-16.

Oct. 12-16.

Nov. 8-16. After washing out vagina with salt solution

Jan. 20-17.

Sept. 22-17. After using soda solution.

Dec. 6-17.

March 25-18.

Sometime during the spring of 1918, (date not recorded) Doctor Hallman made an examination and pronounced the animal pregnant, apparently about seventy or eighty days advanced. A few weeks later, another examination was made and it was found that the animal was not pregnant. Apparently she had aborted but had been unnoticed by the herdsman. No record was made of the clinical condition at this time. In a short time the animal was

turned out to pasture without further service by bull and remained in the pasture until October 13, 1918. On this date the following notes were made by Doctor Hallman:

"There is a small quantity of clear mucus in vagina in which are seen a few flakes of pus. On manipulation of cervix, a tablespoonful or more of an adhesive light mud colored mucus is forced out of cervical canal. The external os and posterior end of cervix is open (can insert three fingers), but internal os is closed."

"On rectal examination, it is found that the uterus is distended. The walls are very tense simulating pregnancy of the fourth to fifth month, though a fetus cannot be felt. A diagnosis of uterine dropsy was made and we proceeded to draw off the fluid. Fourteen to sixteen quarts of a straw colored fluid, in which there is considerable coagulated blood, is drawn from the uterus."

"After removal of this fluid, a foetus about twenty five or thirty centimeters long can be palpated per rectum. There is no record of breeding this animal after March 25. It is positively known that she was not bred to the herd bull before turning to pasture. The only explanation of pregnancy is that a bull of some neighbors must have broken into the pasture during the summer and served the cow. This assumption is verified by the fact that on this same date a heifer, which had not been bred to the herd bull and which was put on pasture at the same time as the animal under consideration, was examined and found to be pregnant."

"No effort was made to remove the fetus at this time, believing that it would be delivered in a few days".

On November 8th this animal was again examined and the following notes were made:

"There is an adhesive muco-purulent discharge in the anterior part of vagina and cervical canal. The uterus is very little if any enlarged. Tone is fair. There is a small cyst in right ovary. The cervix was cleansed and one ounce of 50 percent Lugol's solution was injected into the uterus."

"February 1, 1919 considerable muco-purulent discharge in the vagina. Cervical canal is open. External cervix is congested. No marked enlargement of the uterus. Tone is fair."

With the record of this animal, an unfavorable prognosis was made and it was decided to slaughter her for beef.

She was killed February 11, 1919.

The reproductive organs were sent to the laboratory where a bacteriological and a macroscopic examination was made.

Results of the bacteriological examination were:

B. coli communior was found in the uterus. The cystic ovary was sterile.

Notes on the macroscopic examination were:

External os was dilated; there was a slight hypertrophy of the external fold at the lower and right lateral border. There was also a small quantity of a clear slightly adhesive secretion of the external os. The cervical canal was nine centimeters long and contained a small quantity of slightly adhesive exudate. Mucous membrane was light drab color. The body of the uterus was two centimeters long and of slightly mottled pinkish color. In the mucous membrane small areas of a lighter grayish color were observed.

The mucous membrane of the posterior end of the left horn was similiar in appearance to that of the body of the uterus. The cotyledons were three milimeters in diameter and were of a

grayish white color, contrasting strikingly with gland mucosa. In proximity to one cotyledon near the posterior end of the horn was a small nodule one centimeter in diameter, and one centimeter high, which from superficial examination appeared to be a small tumor. The mucous membranes of the middle and anterior portions were not uniform in color; some portions appeared more vascular, other portions were light drab in color. There was a small quantity of turbid cream-like exudate.

The mucous membrane of the right horn was a little more uniform in color and a little lighter drab than the left horn. Same character of exudate was present. In the anterior end, a small piece of a flat bone five centimeters long and about one centimeter wide was found. Looked like it might be a flat piece of a shaft or long bone. B. coli communior and an abundance of leucocytes were in the exudate which surrounded this piece of bone.

The right oviduct was apparently normal except for the presence of a cyst about one and one half centimeters in diameter at the fenestral end.

The right ovary was five centimeters by four centimeters and contained a cyst-like structure two centimeters in diameter. The left ovary was two and one half by four centimeters and contained a large cystic (?) corpus luteum four centimeters in diameter.

Left oviduct was apparently normal.

7-20-18	Bact. abortus		
	Streptococcus pyogenes		
	Staph. pyogenes bovis		
	B. coli communior		
7-26-18	Staph. pyogenes bovis		
	Streptococcus pyogenes		

807A	12-27-18		Liver, Spleen, Blood, Streptococcus	Streptococcus
11A1A	1-13-19	No growth		
	1-14-19	No growth		
	1-21-19	No growth		
	1-29-19	Streptococcus pyogenes		
	2-5-19	Streptococcus pyogenes		
	2-12-19	No growth		
11A1A2	1-13-19		No growth	
997	1-18-19	No growth		
	1-21-19	No growth		
	1-28-19	No growth		
997C	1-18-19		No growth	

TABLE III

A Study of Uteri and Ovarus from the Abattoir.

Case	Date	Uterus	Cystic ovaries
19* (calf)	7-24-18		B. coli communior Streptococcus
13	8-9-18	Sterile	B. coli communior
14	8-9-18	Sterile	
808		Sterile	B. coli communior B. 121.4442012
17	1-21-19	Sterile	Sterile
18	1-27-19	Sterile	B. coli communior
32	2-11-19	Sterile	No examination
20	2-11-19	Sterile	B. coli communior
9	2-11-19	B. coli communior	Sterile

* Calf five weeks old that died of gastro-intestional intoxication.

The above cases (except 19) will appear in an article to be published by Doctor Hallman on the Histopathological lesions of the uteri.

TABLE IV.

Bacteriological Examination of the Uterus of Sterile Cows.

Case	Date	Uterus	Remarks
L.M.	6-16-18	Sterile	Physical exam. by Dr. Hallman
D.B.	6-16-18	Bact. 211.1421012	" " " " "
M.C.	6-16-18	B. coli communior	" " " " "
H.L.	7-24-18	Sterile	" " " " "
996A*	8-1-18	Bact. 222.1131032	" " " " "
		B. coli communior	
	8-22-18	Bact. 222.1131032	
		B. coli communior	
		Streptococcus pyogenes	
50**	2-10-19	Streptococcus pyogenes	Physical exam. by Dr. Hallman

* Bad case of pyometra.

** Bad case of pyometra.

	2-5-19	Yes		-	-	-	-	
11A1	2- 1-13-19		Yes	-	-	-	-	
997	1-18-19	Yes		-	-	-	-	
	1-28-19	Yes		-	-	-	-	
9970	1-18-19		Yes	-	-	-	-	

Note: dash (-) negative results.

Bacteriological - spleen was the only organ examined.

TABLE VI

A Numerical System of Recording the Salient Characters of an Organism.

(Descriptive Chart-Society of American Bacteriologists).

100.	Endospores produced.
200.	Endospores not produced.
10.	Aerobic (strict).
20.	Facultative anaerobe.
30.	Anaerobic (strict).
1.	Gelatin liquefied.
2.	Gelatin not liquefied.
0.1	Acid and gas from dextrose.
0.2	Acid without gas from dextrose.
0.3	No acid from dextrose.
0.4	No growth with dextrose.
.01	Acid and gas from lactose.
.02	Acid without gas from lactose.
.03	No acid from lactose.
.04	No growth with lactose.
.001	Acid and gas from saccharose.
.002	Acid without gas from saccharose.
.003	No acid from saccharose.
.004	No growth from saccharose.
.0001	Nitrates reduced with evolution of gas.
.0002	Nitrates not reduced.
.0003	Nitrates reduced without gas formation.
.00001	Fluorescent.
.00002	Violet chromogens.
.00003	Blue chromogens.
.00004	Green chromogens.
.00005	Yellow chromogens.
.00006	Orange chromogens.
.00007	Red chromogens.
.00008	Brown chromogens.
.00009	Pink chromogens.
.00000	Non-chromogenic.
.000001	Diastasic action on potato starch (strong)
.000002	Diastasic action on potato starch (feeble)
.000003	Diastasic action on potato starch (absent)
.0000001	Acid and gas from glycerin.
.0000002	Acid without gas from glycerin.
.0000003	No acid from glycerin.
.0000004	No growth with glycerin.

In Table V will be found the results of the inoculations of the guinea pigs with uterine washings and with meconia.

Past investigators claim that it requires from twelve to fourteen weeks after injection for the lesions of abortion to develop.

In December 1918, Smillie, of Rockefeller Institute, published in the Journal of Experimental Medicine an article entitled, "An Improvement in the Method of Isolating and Recovering the Bacillus of Cattle Abortion through Guinea Pigs." Smillie autopsied the pigs in four weeks after they had been injected. The lesions found in these pigs were not so extensive as those found in pigs which were left for twelve weeks but more organisms could be grown from the spleen of these pigs than from those which were given a longer incubation period.

The following method was used in isolating the organisms

A piece of the spleen was rubbed across a veal agar slant and then deposited in the water of condensation in the tube. The tubes were sealed with sealing wax and placed in an incubator at 37 degrees C. for eight to ten days.

At Ohio University this method of Smillie was tried out with stock cultures of Bact. abortus. Negative results were obtained at each trial.

All inoculated guinea pigs used in the process of this investigation for the isolation of Bact. abortus were autopsied after an incubation period of three months.

Before completing this article, the method of Smillie was tried out with a stock culture of Bact. abortus. Each of two agar slants was inoculated with a strain of Bact. abortus

that had b en in the laboratory for four years. When sufficient growth had developed, it was washed off the agar with eight cubic centimeters of physiological salt solution. On March 25, 1919, a guinea pig was injected with two cubic centimeters of this suspension; a second pig was injected with one cubic centimeter of the suspension. On April 25, 1919, the pigs were autopsied and samples of the blood were taken for examination. The blood in each case gave a strong positive test to abortion.

The spleen of the pig that received the two cubic centimeters of suspension of Bact. abortus was two and one half times its normal size and contained small nodules. The spleen of the pig that received one cubic centimeter of the suspension was twice its normal size and contained small nodules. The method of inoculating the veal agar slants was the same as that of Smillie. Instead of sealing the tubes with sealing wax, they were placed in a Novy jar. The air was then pumped out of the jar.

A piece of the spleen was also streaked across plates of veal agar. These plates were put in Novy jars with B. Subtilis The Novy jars containing the test tubes and plates were incubated for four days at 37 degrees C. At the end of this time, there were many isolated colonies of Bact. abortus on the tubes and also on the plates. There was no noticeable difference in the number of colonies from the two pigs.

Summary.

The twenty four cases (Table II) studied for a comparison of the flora of the uterus and meconium showed that the twelve uteri gave cultures in 83 1/3 percent. The uterus con-

tained more organisms than the meconium. The meconium was found to be infected with B. coli communior, staphylococcus and streptococcus. These organisms were also found in the uterus. Bact. abortus was found to persist in the uterus for twenty eight days. In no case was Bact. abortus found in the meconium. In all cases except Case 805A, whenever the meconium was infected, the uterus was found to harbor the same organism. In some cases the meconium was found to be sterile, while the uterus harbored organisms. B. coli and certain cocci were found in the sealed uteri of apparently normal cows. The results obtained in this article will be of interest to investigators, of white scours of calves, who believe that the calf is infected before birth. A large percentage of calves are born with infected meconium. The uterus is first infected, probably followed by the fluids, and lastly, the meconium. The infection probably reaches the uterus by passing through the cervix from the vagina before the seal is formed and persisting there throughout pregnancy.

I wish to acknowledge my indebtedness to Doctor L. T. Hallman under whose direction this work was begun; to Doctor Ward Giltner for his suggestions and for the use of his laboratory; to Doctor H. J. Stafseth for his suggestions during the last few weeks of the investigation; to Doctor D. J. Lamoureaux * for his aid in collecting material; to Nellie L. Bandeen and Mark F. Welsh for their assistance in the laboratory.

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