IS BACTERIUM ABORTUS PRESENT IN THE URINE OF CATTLE AFFECTED WITH BANG'S ABORTION DISEASE

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THESIS.

Submitted to the Faculty of the Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

By

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THESIS

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INTRODUCTION AND HISTORY.

Since Bacterium abortus is eliminated in the milk of infected, pregnant and non-pregnant, cows, and in the uterine discharges during and shortly after parturition or abortion, the question naturally arises as to whether or not the micro-parasite is present in the excreted urine.

The fact that some animals aborted their young and that others were unable to reproduce their kind was known in earliest Bible times (Genesis XXXI, verse 38). Reference was again made to these conditions in the writings of Mascall, in 1567. It was not until 1793, however, that Eberhard and Gunther of Belgium published the first veterinary work on this subject and this was followed by numerous writings in the early nineteenth century which show that the infectiousness of abortion disease was a well known fact at that time (Lawrence 1805, Lafoose and Zündel 1807, Skellet 1808, Jonati 1837, and Barlow 1851). The "Complete Farmer" in 1807 said "It is considered certainly contagious, and when it happens the abortion should be immediately burned, and the cow kept as widely apart as possible from the herd, and not receive the bull that goes with them." St. Cyr (1875) reported the infectiousness of the disease but it remained for Frank (1876) and Lehnert and Brauer (1880) to artificially inoculate healthy pregnant cows. Nocard (1885) was the first to do

scientific investigation although he failed to isolate the causative organism. This investigation into abortion was followed by that of Woodhead, Aitken, McFadyean, and Campbell (1889) and the Royal Agricultural Society of England (1894). It was not until 1897, through the remarkable work of Bang and Striebolt, that the specific etiological agent, Bacterium abortus, was discovered. Bang's statement was not fully accepted, however, until 1906, when he reannounced his discovery before the National Veterinary Association at Liverpool. His work was confined by the investigations of Preiss (1902) and McFadyean and Stockman(1909). In America, Chester, Law, and Moore failed to isolate the organism but MacNeal, Kerr, Giltner, and Good (1910) independently confirmed Bang's discovery. Traum (1914) is the first recorded to isolate the causative organism from aborted swine. Mohler and Eichhorn in 1925 reported the pathogenicity of Brucella melitensis for sheep, goats, cattle and horses.

It is remarkable, however, that with all the research into contagious abortion by the above mentioned and many other investigators, very little has been written regarding Bacterium abortus in connection with the urinary organs, and practically nothing has been mentioned concerning the urine. Although there has been some experimental work done with the urine of man, goats, rabbits, and guinea pigs, Zeller appears to be the only one to have worked with that of cattle and his experiment will be discussed later.

REVIEW OF LITERATURE.

Although there are histo-pathological changes present in the maternal cotyledons of infected pregnant females, and those that have had recent parturition or abortion, other tissues may be invaded by the Bacterium abortus or Brucella melitensis without these changes of the normal histology of the part. This is evidenced in the udder. Notwithstanding this lack of lesions, the causative micro-parasite may be isolated from the tissues, if present, although one might encounter some difficulties in so doing.

Huddleson (1) reported the isolation of Bacterium abortus in pure cultures from diseased spleens, livers, and kidneys of guinea pigs fed forty-eight hour agar slants of virulent cultures. Meyer, Shaw, and Fleischner (2) found the porcine type of Bacterium abortus in all tissues except the heart blood or muscles. They were also able to isolate from the kidneys of a number of inoculated guinea pigs both Bacterium abortus and Brucella melitensis. Schroeder (3) observed that Bacterium abortus produced most changes in guinea pigs' livers, spleens, kidneys, testicles, bones, etc., although in a later paper (4) he claimed that no macroscopic lesions are produced in the spleen two months after injection even though the organisms can be isolated. T. C. Evans (5) was able to produce lesions in guinea pigs similar to those of Schroeder and Cotton, by feeding infected milk. Cotton (6) reported joint lesions in guinea pigs from both bovine and porcine strains with the latter appearing to be more virulent. Smillie (7) was able to recover Bacterium abortus from the kidneys, spleen,

testicles, and bones of inoculated guinea pigs regardless of the point of injection or the size of the infecting dose.

Schroeder (4) found Bacterium abortus maintaining itself in bodies of rabbits for long periods of time without causing macroscopic changes. Zeller (2) injected rabbits and guinea pigs intravenously, intraperitoneally, and subcutaneously, with large doses of Bacterium abortus and Brucella melitensis. This histo-pathological changes after varying intervals of time were mostly negative but he was able, in a few cases, to isolate the organisms from the spleen, uterus, testicles, and occasionally the kidney.

Generalized invasion of the tissues resulted when monkeys were fed virulent cultures of Bacterium abortus, as was found by Fleischner, Vecki, Shaw, and Meyer (8).

The abortion organism, according to Schroeder and Cotton (5) has been found in many more regions of the bodies of swine than in those of cattle, and Cotton (9) found that the living micro-organisms could be isolated from necrotic areas in the kidney fat and udder tissues, uterine exudates, and portions of the uterine walls of pigs that had been inoculated. Hays (10) was unable to isolate Bacterium abortus from the kidneys, spleen, liver, thyroid, and urethra of two positive barrows. Weeter (11) was unable to obtain the abortion organism from any of the internal organs of swine six months after the initial and subsequent infection, therefore concluding that the organisms were eliminated.

The reports of the Mediterranian Commission and likewise those of Mohler and Hart (12) showed that the Brucella melitensis can be isolated from the lymph nodes,

mammary glands, and spleen of goats and also indicated distinct hyperemia of the kidneys and liver, enlarged spleen and general enlargement of lymph nodes. Z. Khaled (13) and Cesari (14) were able to isolate the Brucella melitensis, in fatal goat cases, from the kidneys, enlarged mesenteric glands, spleen, liver, saliva, milk, and blood (10 per cent).

Bacterium abortus was obtained in pure cultures from the heart blood and all organs of the body of a large number of aborted lambs (15).

Out of 140 cases of pyelitis of the human female, Kidd of London (16) found one due to paramalta fever and Cesari (14) states that the kidneys constitute the avenue of elimination for Brucella melitensis.

Schroeder (17) found, as a result of a large number of tests, that young cattle rarely harbor abortion bacilli but they may be present in the gastro-hepatic lymph glands, liver, and stomach fluids of newly born, viable calves. Hadley (18) asserts that the germs of abortion may exist in large numbers in the stomachs or intestines of newly born calves, but shortly after birth these bacilli disappear as there are no tissues in the calves' bodies suitable for the existance of the germs. Carpenter (19) found only one fetus with kidney infection and also found (20) that the abortion bacilli invaded the lymph glands adjacent to the head as well as those along the intestines, although they did not persist in these glands upon discontinuing the feeding of infected material. Heifers rarely carry the abortion bacilli permanently in their bodies unless they have found lodgement in the udder and suprammary lymph glands. As a rule this occurs only

after the glands have actively engaged in milk secretion, although Schroeder and Cotton (21) have, in isolated cases, been able to culture the organism from udders that had never lactated. Primarily the favorite habitat of Bacterium abortus is neither in the parent nor the offspring but rather in the medium through which the two are connected. All attempts to isolate the organism from the kidneys, spleen, liver, lungs, serous membranes, synovial fluids, bone marrow, brain, spinal cord, muscle, uterus, vagina, fallopian tubes, ovaries, etc., and lymph glands from all portions of their bodies, have failed as claimed by Schroeder (17). Kavarzik (22) claimed that autopsy may reveal punctiform or streaked hemorrhages in the serous membranes of the gastro-intestional canal, and in the urinary bladder, or more or less pronounced acute swelling of the spleen and lymph glands.

Schoreder and Cotton (21), after examining hundreds of tests with milk from numerous cows, are of the opinion that the udder is the only habitat of Bacterium abortus in the bodies of non-pregnant cows, and that the abortion bacilli do not maintain themselves in the bodies of cows elsewhere than in their udders and gravid uteruses (21). Hadley (18) is of the opinion that the udder is the only organ in the body of non-pregnant cows where the bacilli can live, and Cotton (23) declares the bacillus is unique in that it requires embryonic tissue for its development and is only present in animals during this relatively short period of their existance. Huddleson (24), Mohler and Traum, Buck, Creech and Ladson, and Hart (25), and Khaled (13) claim the bacillus can be

isolated from the spleen, liver, milk, and lymph glands, as well as the cotyledons, uterus, and uterine discharges of pregnant cows.

Meyer, Shaw and Fleischner (2) were able to isolate Brucella melitensis from the urine of inoculated guinea pigs, and similar results were obtained by Durham, Eyre, and Nicolle and Conseil (2). Zeller (2), upon subcutaneous, intraperitoneal, and intravenous injections of guinea pigs and rabbits with Bacterium abortus and Brucella melitensis was able to demonstrate that the organisms were present in the urine.

Z. Khaled (13) was unable, on various occasions, to culture either Bacterium abortus or Brucella melitensis from the urine of inoculated goats although he did find the organisms in the blood. He was able, however, to demonstrate that Brucella melitensis was present in the urine of fatal cases in goats. The Reports of the Mediterranian Commission and of Mohler and Hart and Cerari (4) indicated the occasional finding of Brucella melitensis in the urine of goats.

Huddleson (26) reported the presence of Bacterium abortus and Brucella melitensis in the urine of infected humans, although only occasionly isolated and cultured from single samples. The presence of Brucella melitensis in human urine also by Cesari (14), Stitt(27), Park and Williams (28), and Buchanan (29).

As previously stated very little work appears to have been done on the urine of infected animals of the bovine species, and Zeller (30) appears to be the only one to have worked on that. He inoculated subcutaneously a cow with ten agar slant cultures in twenty mils of salt solution. Seventeen cultural

examinations, ranging from one to eighty-nine days, were made from samples of blood, milk, saliva, feces, and urine, with negative results. The cow was killed one hundred twenty-eight days after inoculation and various organs cultured with negative results. Cow number two was inoculated similarly with twenty agar slant cultures in thirty mils of salt solution and sixteen samples of the same material were examined culturally from three to sixty-seven days with negative results. This cow's organs upon autopsy proved negative culturally. Cow number three was inoculated with twenty agar slant cultures in thirty mils of salt solution and twenty-one samples of similar substances were examined culturally from one to sixty-five days and proved negative. The post mortem findings were the same. Cow number four was inoculated with ten agar slant cultures and at various intervals samples of the same material was examined previously, excepting the milk, were cultured with negative results. One hundred sixty-one days after inoculation the cow was destroyed and guinea pigs were inoculated with the materials from her spleen, uterus, ovaries, udder, and supra-mammary lymph glands with negative results. It might be well to note that since these cattle were inoculated the conditions differed from those existing where animals were infected naturally. According to Zwick and Wedemann (31) Bacterium abortus, when present in urine or dry cow manure,will die within twenty-four hours. This differs from the results obtained in the present investigation since the writer was able to grow the organism for seventy-two hours, this growth being even more luxuriant than that obtained in twenty-four hours. There was no notation as to the type, virulency, or amount of organisms that Zwick and Wedemann used in their ex-

periment. G. Ranchbar (32) was able to keep Bacterium abortus alive for nine days in the urine of mice.

HISTORY OF ANIMALS USED IN THIS EXPERIMENT.

Cow number 216 was born August 16, 1922, and is a positive reactor to the Bacterium abortus agglutination test, habing had a five plus reaction since July 1926. On November 25, 1923, she was bred and carried her fetus for two hundred sixty-eight days, with a normal parturition on August 19, 1924. She was again bred November 23, 1924, and this time carried her fetus ten days longer, having a normal parturition August 28, 1925. On her third pregnancy, she was bred November 27, 1925, and carried her fetus until August 10, 1926, two hundred fifty-six days, when she aborted. The calf died at birth and the dam cleaned in eight hours. Bacterium abortus was isolated from the fetus, placenta, and from all four quarters of the udder. The milk is positive to the agglutination test.

Cow number 236 was born April 14, 1923, and was a four plus reactor in August 1925, and has since been a partial to a five plus reactor. She was bred July 8, 1924, and carried her fetus two hundred sixty-four days to which she gave normal parturition on March 29, 1925. The second breeding was on June 27, 1925, and the fetus was carried until April 3, 1926 (two hundred eighty days), to be born normal. This dam was bred the third time, August 13, 1926, and normal parturition occurred May 12, 1927. Bacterium abortus was isolated from the colostrum on April 3, 1926, as was also streptococci. No abortion bacilli were found in the placental or fetal membranes but they were

found in one quarter of her udder.

Cow number 239 was born July 31, 1922, and since her abortion has been a positive reactor to the agglutination test. Up to this time she was negative. She was first bred July 31, 1924, and did not become pregnant so she was again bred August 22, 1924. The fetus was carried two hundred seventy-three days and the dam had a normal parturition on May 22, 1925. Her second pregnancy started August 24, 1925, and the fetus was carried two hundred twenty-nine days to be aborted April 10, 1926. She was again bred July 7, 1926, with a normal parturition April 14, 1927 (two hundred twenty-seven days). There has been a partial agglutination of her milk to the Bacterium abortus.

Cow number 147 was born October 18, 1921, and is a positive reactor. There is no record of retained placental membranes but she aborted on her first and second pregnancies. This, her fifth, resulted in abortion on December 1, 1926. She has been bred four different times since the above date, the last being April 4, 1927.

Cow number 191 was born May 7, 1924, and is a positive reactor. On her first pregnancy there was an abortion and on August 7, 1926, she aborted in the pasture. There have been four different breeding dates, the last June 25, 1927.

Cow number 193 was born May 18, 1924, and is a positive reactor. There was no previous history of abortion. She aborted a dead calf on August 5, 1926, and the placenta was retained. Bacterium abortus was isolated from the placental membranes, and the milk is positive to the agglutination test.

Cow number 95 was born March 12, 1920, and is a positive reactor. Her history shows abortion on her first and fourth pregnancies. On her fifth pregnancy, the third abortion occurred while in the pasture, November 17, 1926. The placental membranes were retained, and the calf was dead at delivery. Bacterium abortus has been isolated from the membranes and milk.

Cow number 84 is a positive reactor and a chronic spreader. Her previous pregnancy resulted in an abortion as did the ninth on February 14, 1926, with death of the calf on delivery. The same occurred October 5, 1926, and this time Bacterium abortus was isolated from the fetal membranes. The milk was positive to the Bacterium abortus agglutination test. Later this animal became very emaciated and was killed May 17, 1927. Upon autopsy, it was found that a hay wire had penetrated through the diaphragm at three different points. There were three fistulous tracts with slight pleurisy, traumatic pneumonia, and some pericarditis.

Cow number 171 was born February 7, 1923, and is a positive reactor. She was first bred on July 7, 1924, and carried her fetus two hundred eighty-three days until April 15, 1925, with a normal parturition. She was twice bred again July 20, 1925, and August 16, 1925 when she became pregnant. This time the fetus was carried two hundred forty-three days and aborted April 15, 1926. The calf was dead on delivery with fetal pneumonia. After this abortion, she was bred three times, May 31, 1926, June 24, 1926, and August 15, 1926. This time she carried her fetus two hundred sixty-one days to abort a dead calf May 6, 1927. Bacterium abortus was isolated from the fetus and fetal membranes.

Cow number 1665 is ten years old and is a positive reactor. March 17, 1920, she gave birth to a normal calf and the same occurred July 4, 1921. On July 27, 1921, she was bred and on December 28, 1921, there was an abortion. Again she aborted on November 17, 1922, losing a seven months old fetus, and retained the placental membranes, which required manual removal. Metritis was present in this case. The fetus, placenta, and milk showed Bacterium abortus. On March 26, 1923, she was again bred but aborted an eight and one-half months old fetus and retained the placenta which required manual removal. This occurred December 15, 1923. There were symptoms of severe metritis which continued for a long time. On May 30, 1925, this dam had a normal parturition and cleaned properly. The placenta showed no indications of Bacterium abortus. Again on August 1, 1926, she had another normal parturition. No Bacterium abortus was present in the placental membranes although she has had the organisms in all four quarters since March 23, 1922.

Cow number 33 was born February 8, 1925, and is a positive reactor to the agglutination test for Bacterium abortus infection. She was artificially fed cultures of the abortion bacilli on September 7, 1926, and reacted to the agglutination test on September 29, 1927. This animal was bred November 30, 1926, but aborted an eight months old fetus. The placenta came through normal involution. Bacterium abortus was isolated from the fetal membranes, lungs, liver, kidneys, and abdominal fluid.

Cow number 175 was born June 15, 1923, and is a positive reactor. Her first pregnancy resulted in an abortion

which took place on June 12, 1926, two hundred fifty-three days after being bred. The placenta was retained and had to be removed manually. Bacterium abortus was isolated from the placenta. This animal failed to conceive upon subsequent breedings and died March 21, 1927, from traumatic pericarditis.

Cow number 99 was born April 14, 1922, and is a positive reactor. There is no history of previous retained placentas although she aborted on her second pregnancy. On her third pregnancy she aborted, April 6, 1926 and retained her placenta. Bacterium abortus was isolated from her placental membranes and her milk was positive to the Bacterium abortus agglutination test. Normal calving occurred March 17, 1927.

Cow number 93 was born February 16, 1920, and is a positive reactor. Her first two calves born June 15, 1927, and December 12, 1923, were dead at birth. After this there were eight breeding dates before conception took place that resulted in a normal parturition on March 18, 1925. Her calf born February 23, 1927, died shortly after birth with navel ill. The last breeding date is May 10, 1927.

Cow number 1659 is about eleven years old, and is a positive reactor. She was naturally infected in 1922 and Bacterium abortus has been located in her udder ever since. This animal has never aborted nor have the organisms been found in her placental membranes. She has had four or five calves since being in the experimental herd.

Cow number 161 was born March 13, 1920, and is a positive reactor. Her first pregnancy, on November 19, 1922, terminated with a favorable parturition. She was bred May 22,1923

and as there were no signs of pregnancy, she was again bred June 8, 1923. The fetus was carried one hundred eighty-six days to be aborted dead on December 14, 1923. Bacterium abortus was isolated at this time from the placental membranes. The next pregnancy required two breedings, April 11, 1924, and June 22, 1924. The fetus was carried until February 24, 1925 (two hundred seventy-nine days), and terminated with a favorable parturition. On June 1, 1925, she was bred and this time the fetus was carried until February 10, 1926 (two hundred sixty-five days), but was dead at delivery. May 13, 1926, she was bred and one hundred eighty-seven days later (February 18, 1927), normal parturition took place. Since the latter date, she has not been in heat and became very poor, walking with a stiff gait. After her death, June 6, 1927, post mortem revealed multiple abscesses of the liver and a chronic diffused nephritis.

Cow number 75 was born April 28, 1921, and is a positive reactor. Her first conception took place November 19, 1922, and in one hundred ninety-two days (May 20, 1923), she delivered a dead calf. Her third pregnancy required three servings, July 24, 1923, August 14, 1923, and September 23, 1923. There was a normal parturition after two hundred seventy-four days (June 23, 1924). Her next pregnancy from August 6, 1924, to May 19, 1925, terminated nromally at the end of two hundred eighty-seven days. Again it required the three services of the sire (August 24, 1925, September 29, 1925, and November 21, 1925) before normal pregnancy occurred. This took place two hundred sixty-one

days (July 12, 1926) after the last breeding. The calf died one month later. The next pregnancy required three servings, September 28, 1926, January 2, 1927, and February 5, 1927. After carrying the fetus one hundred seventy-five days, she aborted (June 21, 1927) and the calf was removed manually. She died June 22, 1927 from Bacillus coli septicemia.

Cow number 232 was born November 11, 1924, and is a positive reactor. There was no history of abortion up to the one that took place January 27, 1927. At this time Bacterium abortus was isolated from the placental membranes.

Cow number 47 was born October 18, 1921, and is a positive reactor. The history shows there was an abortion on her first and second pregnancies but with no record of retained placentas. She again aborted upon her fifth pregnancy, December 1, 1926. The Bacterium abortus agglutination test was positive for her milk. Seven breeding dates have occurred since the last abortion, the last being on May 4, 1926.

METHOD OF STUDY.

Urine of cattle infected with Bang's abortion disease was caught in sterile one liter flasks upon the animals' urinating. From these flasks, thirty-five cubic centimeters was centrifugalized at about 2500 revolutions per minute for one hour and the sediment then smeared over the surface of three plates consisting of beef liver infusion agar (26) and a saturated aqueous solution of gentian violet to make a 1:10,000 dilution. This inhibited the Gram positive organisms especially the fast growing ones. After plating, the inoculated plates were incubated at 37°C. under ten per cent CO_2 conditions for seventy-two hours and then examined for typical Bacterium abortus colonies.

Inasmuch as my purpose was to try to isolate Bacterium abortus from the urine of infected cattle and since contamination from other organisms would naturally result from the urine passing over the floor and inferior commissure of the vulva, no attempts to identify the other organisms found growing on the plates were made.

The first eight trips to the barn where these animals were stalled were made in the evening, and as result were negative it was decided to make all future trips in the morning so as to procure the night's urine upon the animals' first morning micturition.

Table I.

Number of different: Individual tests	Series of tests	Date of collection	Hour of Same	Number of animal	Result of examinations.
1	1	August 9,1926	5:00 P.M.	84	Negative.
2		" " "	" "	171	"
3		" " "	" "	1665	"
4	2	August 11,1926	5:00 P.M.	171	"
5		" " "	" "	1659	"
6		" " "	" "	1665	Question.
7	3	August 16,1926	5:00 P.M.	84	Negative.
8		" " "	" "	1659	"
9		" " "	" "	1665	"
10	4	August 19,1926	5:00 P.M.	84	"
11		" " "	" "	171	"
12		" " "	" "	1659	"
13		" " "	" "	1665	"
14	5	August 21,1926	5:00 P.M.	84	Question
15		" " "	" "	171	Negative
16		" " "	" "	1659	"
17		" " "	" "	1665	"
18	6	August 23,1926	5:30 P.M.	171	"
19		" " "	" "	1659	"
20		" " "	" "	1665	"
21	7	August 25,1926	4:30 P.M.	84	"
22		" " "	" "	171	"
23		" " "	" "	1659	"
24		" " "	" "	1665	"
25	8	August 30,1926	4:30 P.M.	84	"
26		" " "	" "	1659	"
27		" " "	" "	1665	"
28	9	Sept. 1, 1926	4:00 A.M.	171	"
29		" " "	" "	1659	"
30		" " "	" "	1665	"
31	10	Sept. 4, 1926	4:00 A.M.	84	"
32		" " "	" "	171	"
33		" " "	" "	1659	"
34		" " "	" "	1665	"

Table I (continued).

Number of different: Individual tests	Series of tests	Date of collection	Hour of same	Number of animal	Result of examinations.
34	11	Sept. 8, 1926	4:30 A.M.	84	Negative.
35		" " "	" "	171	"
36		" " "	" "	1659	"
37		" " "	" "	1665	"
38	12	Sept.18, 1926	5:00 A.M.	1659	"
39		" " "	" "	1665	"
40	13	Sept.29, 1926	5:00 A.M.	84	"
41		" " "	" "	1659	"
42		" " "	" "	1665	"
43	14	Oct. 9, 1926	4:30 A.M.	171	"
44		" " "	" "	1659	"
45		" " "	" "	1665	"

DISCUSSION OF TABLE I.

Table number I shows the results of forty-five different attempts to isolate the Bacterium abortus from four individual cows positive to the agglutination test, and kept in the same barn during a period of eight weeks. Eight separate urine samples were collected from cow number 84, ten samples from cow number 171, and thirteen samples from cows number 1659 and 1665, respectively. Individual urine sample number six was not cultured since the cotton stopper of the urine flask became contaminated when knocked from the investigator's hands into the gutter. The table also shows a question as to the results with urine sample number fourteen. Only about fifty cubic centimeters were collected at the time of urination since the first to be passed was missed.

Negative results were obtained throughout this experiment.

METHOD OF STUDY.

Since the results, as indicated in Table I, were negative certain questions arose in the investigator's mind: (1) Were the abortion bacilli in the urine too few to isolate? (2) Would they grow sufficiently to isolate if allowed to stand for varying periods of time at 23°C.? (3) If urine were inoculated with abortion bacilli would they grow?

Along with the individual inoculated urine samples plated out as explained for the previous table, samples of urine from the same one liter flask were inoculated and incubated as follows: after the thirty-five cubic centimeters of urine were removed from the one liter flask for centrifugalization, ten cubic centimeters were pipetted into each of eight different sterile test tubes and inoculated with one cubic centimeter of a suspension of Bacterium abortus.* This bacterial suspension was prepared by washing off the surface growth from a seventy-two hour beef liver infusion agar slant with ten cubic centimeters of physiological saline solution. The eight tubes were divided into five series of two tubes each and incubated at 23°C. for varying periods of time: the first set for twelve hours, second set for twenty-four hours, third set for forty-eight hours, and the fifth set for seventy-two hours. Upon the expiration of the first period of incubation one tube of the first set (twelve hours) was centrifugalized at about 2500 revolutions per minute for thirty minutes before plating the sediment on three beef liver infusion gentian violet plates. The other tube of the set containing the in-

oculated whole urine was plated out on three separate plates, similarly. The contents, sediment and whole urine, of set two (twenty-four hours) was plated as above upon the completion of twenty-four hours incubation, set three upon forty-eight hours incubation, and set four upon seventy-two hours incubation. After each set was plated out, it was incubated for seventy-two hours under ten per cent CO_2 conditions. Beef liver infusion agar slants were inoculated upon the appearance of typical transparent dew drop colonies on any of the plates. Those slants that showed typical Bacterium abortus subcultures were washed off with a solution containing nine-tenths per cent sodium chloride and five-tenths per cent phenol. This suspension was standardized with the Gate's nephelometer (33) to a density of seven centimeters, after which the pH was adjusted to 6.8. This suspension then comprised the antigen for the agglutination test to be run against a four plus blood serum. Into each of five precipitation tubes were pipetted two cubic centimeters of the standardized antigen and eight-hundredths, four-hundredths, two-hundredths, one-hundredths, and five-thousandths cubic centimeters of four plus blood serum, respectively, into each tube. This made dilutions of one to twenty-five, one to fifty, one to one hundred, one to two hundred, and one to five hundred.

* The culture, number 44, of Bacterium abortus used to inoculate the eight different sets of urine was isolated about two years ago.

Table II.

Number of different Individual tests.	Series of tests	Number of animal	Date of collection and hour of same.	Culture growth. 12 hour CU.	12 hour UU.	24 hour CU.	24 hour UU.	48 hour CU.	48 hour UU.	72 hour CU.	72 hour UU.	Uninoculated urinary sediment.
1	1	216	Oct.16,1926.4:00A.M.	-	-	+	-	+	+	-	-	Negative
2		239	" " " " "	+	+	+	-	-	-	-	-	"
3	2	216	Oct.23,1926.4:00A.M.	-	-	+	-	+	+	+	-	"
4		232	" " " " "	-	-	-	-	-	-	-	-	"
5		236	" " " " "	+	-	+	-	+	+	+	+	"
6		239	" " " " "	+	-	-	-	-	-	-	-	"
7	3	216	Oct.30,1926.4:15A.M.	-	-	+	+	+	+	-	-	"
8		236	" " " " "	-	-	+	+	-	-	-	-	"
9		239	" " " " "	?	?	?	?	?	?	?	?	Question
10	4	75	Nov.13,1926.4:30A.M.	-	-	+	+	+	+	+	+	Negative
11		95	" " " " "	-	+	+	-	-	-	-	-	"
12		147	" " " " "	-	+	+	+	+	-	-	-	"
13	5	75	Nov.20,1926.4:00A.M.	-	-	+	+	+	+	+	+	"
14		147	" " " " "	-	-	-	-	-	-	-	-	"
15		161	" " " " "	+	+	-	+	+	-	-	-	"
16		193	" " " " "	+	-	-	-	-	-	-	-	"
17	6	75	Dec.11,1926.4:30A.M.	-	-	+	-	+	+	+	+	"
18		193	" " " " "	?	?	?	?	?	?	?	?	Question
19		191	" " " " "	-	-	+	-	+	-	-	-	Negative
20	7	99	Dec.18,1926.4:00A.M.	-	-	-	-	-	-	-	-	"
21		161	" " " " "	+	-	+	-	-	-	-	-	"
22		191	" " " " "	+	+	-	-	+	-	-	-	"

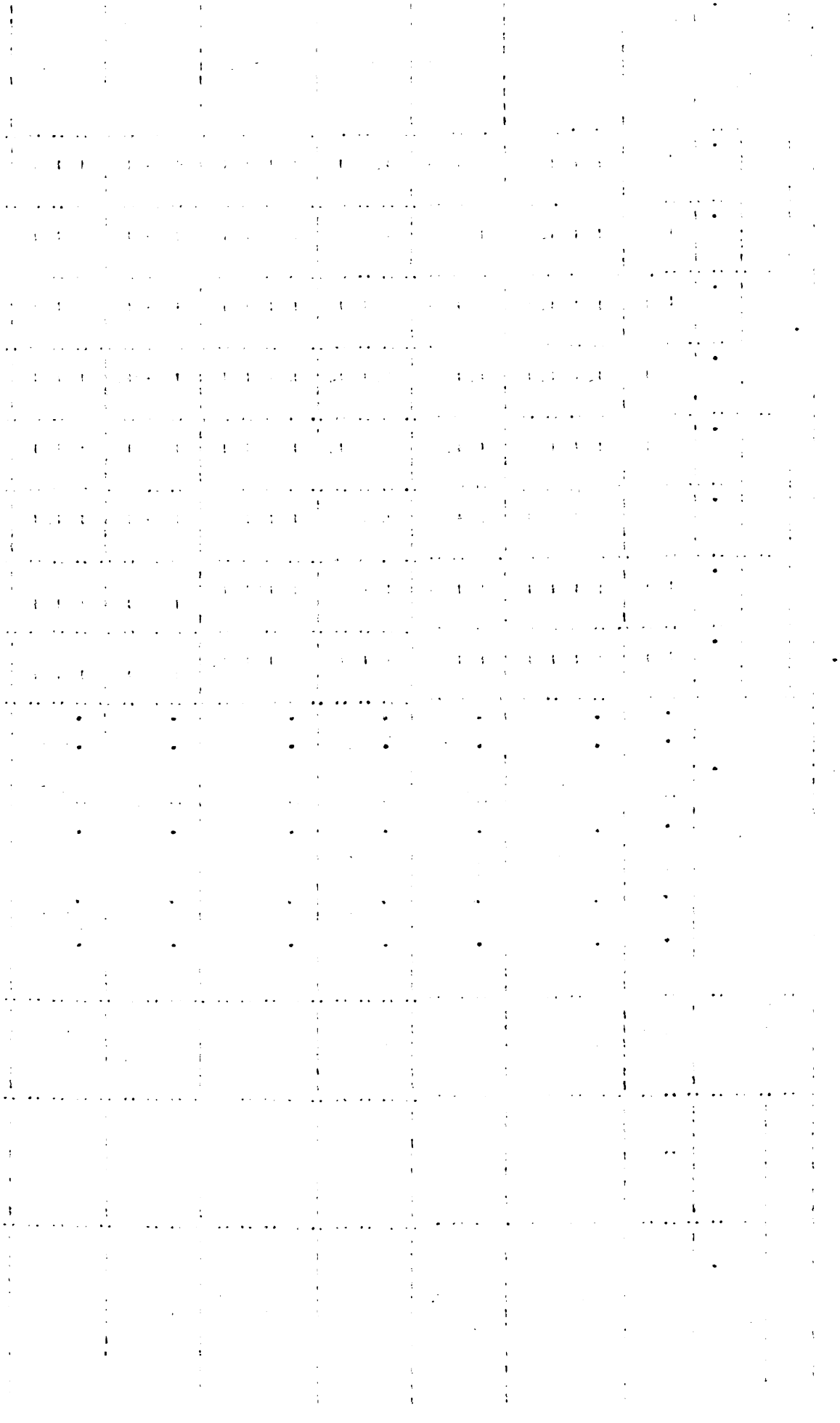


Table II. (continued).

Number of different Individual tests	Series of tests	Number of animal	Date of collection and hour of same.	Culture growth: 12 hour CU.	12 hour UU.	24 hour CU.	24 hour UU.	48 hour CU.	48 hour UU.	72 hour CU.	72 hour UU.	Uninoculated urinary sediment
23	8	84	Jan. 8,1927,4:00A.M.	-	/	-	-	-	-	-	-	Negative
24		33	" " " " "	/	-	-	/	-	-	-	-	"
25		171	" " " " "	-	-	-	-	-	-	-	-	"
26		1665	" " " " "	-	/	-	-	/	-	/	-	"
27	9	84	Jan.15,1927.4:00A.M.	-	-	-	-	-	-	-	-	"
28		33	" " " " "	?	?	??	?	?	?	?	?	"
29		171	" " " " "	-	/	/	/	-	-	-	-	"
30		1665	" " " " "									"
31	10	161	Jan.22,1927.4:00A.M.	/	/	/	-	/	-	-	-	"
32		175	" " " " "	-	-	-	-	-	-	-	-	"
33		232	" " " " "	-	-	/	/	-	-	-	-	"
34		236	" " " " "	/	-	-	-	-	-	/	-	"

Key - CU. centrifugalized inoculated urine.
UU. inoculated whole urine.
? see discussion of chart.
/ colonies appeared on plates.
- no Bacterium abortus colonies on plates.

DISCUSSION OF TABLE II.

Thirty-four different urine samples were cultured and studied, from October 16, 1926, until January 22, 1927, using sixteen different cows. The urine of cows number 75, 161, 236, and 1665 was examined culturally three times; that of cows number 33, 84, 147, 171, 191, 216, 232, and 239 was cultured twice; and that of cows number 95, 99, 175, and 193 was examined only once. Only forty cubic centimeters of urine were collected on sample number twenty-eight, so it was centrifugalized and the sediment examined. There was a question concerning the results of urine samples number nine and eighteen (cows 239 and 193 respectively) as they were both lost; the former flask was tipped over and the latter broken. Results showed that Bacterium abortus existed and appeared to multiply in inoculated urine, both centrifugalized and whole. There seemed to be more colonies, as a general thing, in the twenty-four and forty-eight hour growths than in the twelve hour growth, while the colonies seemed to become fewer during the seventy-second hour. At no time, though, were the colonies profuse on any of the plates. Bacterium abortus seemed more likely to be found and the colonies greater in number in the urine samples that had been centrifugalized.

All thirty-four samples of the uninoculated urinary sediment failed to show colonies.

METHOD OF STUDY.

To insure the finding of the microorganism, Bacterium abortus, in non-inoculated cows' urine, having found that the pathogen would grow and to some extent multiply in inoculated urine, samples of urine were mixed with sterile whipping cream and held for twenty-four, forty-eight, and seventy-two hours at 23°C. The purpose of this was that the rising fat globules and body cells would carry to the surface the abortion bacilli if present (26). From each one thousand cubic centimeters of cows' urine sufficient amount was placed in a five hundred cubic centimeter Florence flask as to fill it to the neck and to this was added ten cubic centimeters of sterile whipping cream. This mixture was thoroughly shaken and then allowed to stand at 23°C. for seventy-two hours. In twenty-four hours three cubic centimeters of the cream in the neck of the flask were plated, respectively, on each of three beef liver infusion gentian agar plates. These three plates were then incubated at 37°C. for seventy-two hours under ten per cent CO_2 conditions. This procedure was repeated in forty-eight and seventy-two hours.

In conjunction with this "urine-cream" method, non-inoculated samples of urine alone were held for the same periods of time, centrifugalized, and similarly plated.

As there were no typical colonies on any of the plates in either of these two tests, no agar slants and agglutination tests were made.

Table III.

Number of different Individual tests	Series of tests	Number of animal urine examined	Date of collection and hour of same.	Cream / Urine on standing. 24 hour	48 hour	72 hour	Centrifuged Urine after standing. 24 hour	48 hour	72 hour
1	1	47	Feb. 5,1927.4:00A.M.	-	-	-	-	-	-
2		93	" " " " "	-	-	-	-	-	-
3		95	" " " " "	-	-	-	-	-	-
4		99	" " " " "	-	-	-	-	-	-
5	2	95	Feb.19,1927.4:00A.M.	-	-	-	-	-	-
6		99	" " " " "	-	-	-	-	-	-
7		232	" " " " "	-	-	-	-	-	-
8		236	" " " " "	-	-	-	-	-	-
9	3	147	Feb.26,1927.4:00A.M.	-	-	-	-	-	-
10		161	" " " " "	-	-	-	-	-	-
11		175	" " " " "	-	-	-	-	-	-
12	4	84	Mar.12,1927.4:00A.M.	-	-	-	-	-	-
13		33	" " " " "	-	-	-	?	?	?
14		1665	" " " " "	-	-	-	-	-	-
15	5	47	Mar.19,1927.4:00A.M.	-	-	-	-	-	-
16		93	" " " " "	-	-	-	-	-	-
17		99	" " " " "	-	-	-	-	-	-
18		193	" " " " "	-	-	-	-	-	-

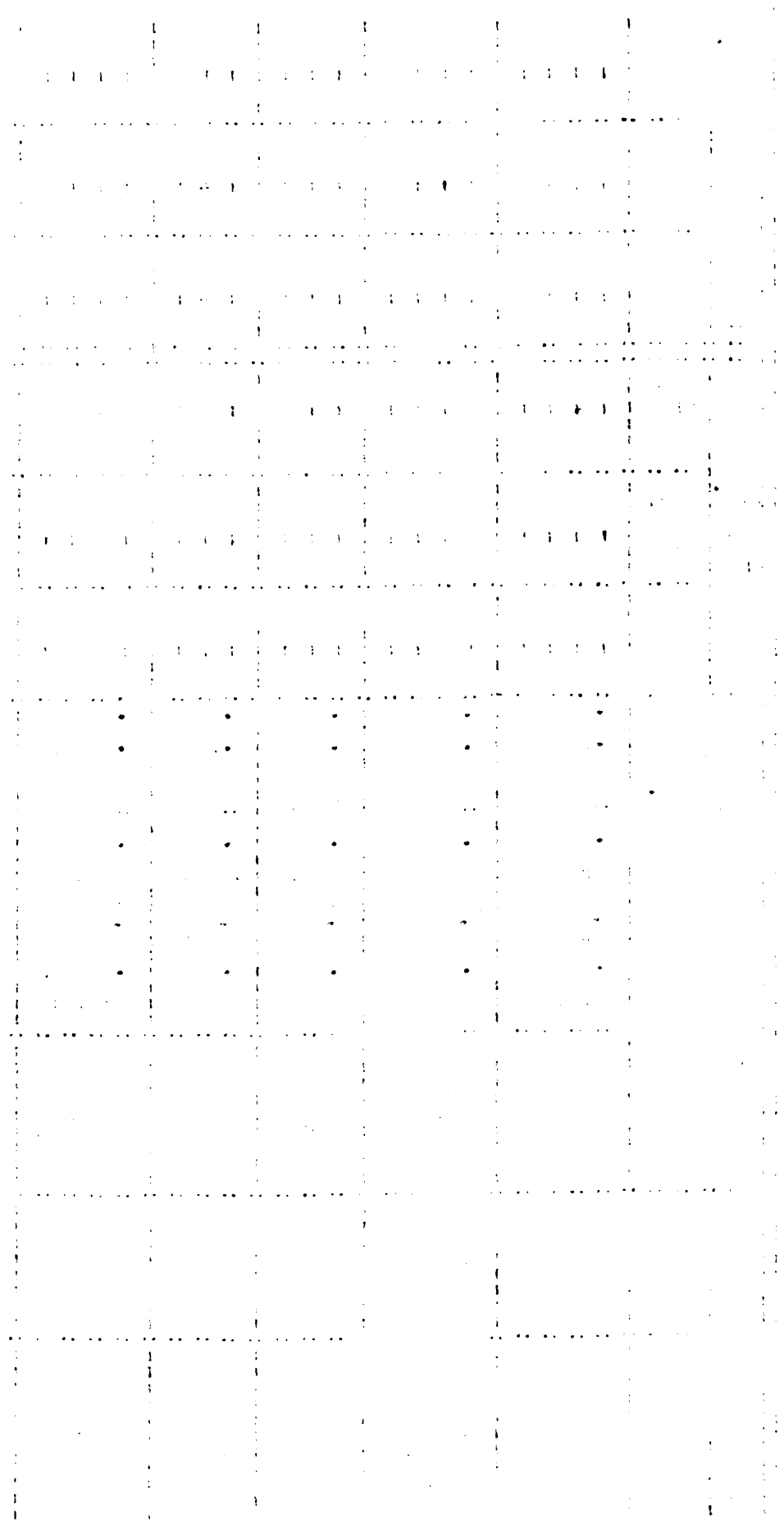
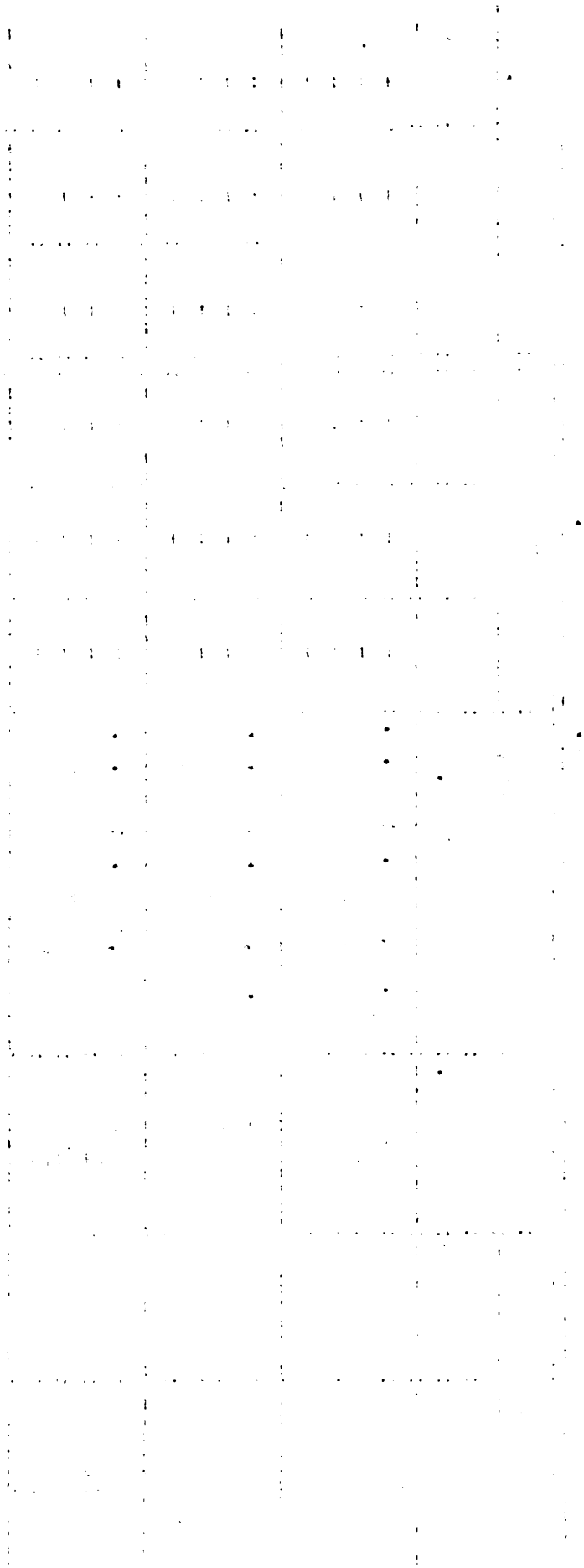


Table III. (Continued).

Number of different Individual tests	Series of tests	Number of animal urine examined.	Date of collection and hour of same.	Cream / Urine on standing 24 hour	48 hour	72 hour	Centrifuged urine after standing. 24 hour	48 hour	72 hour
19	6	99	Apr. 9,1927.4:00A.M.	-	-	-	-	-	-
20		147	" " " " "	-	-	-	-	-	-
21		191	" " " " "	-	-	-	-	-	-
22		193	" " " " "	-	-	-	-	-	-
23	7	95	Apr.23,1927.4:00A.M.	-	-	-	-	-	-
24		99	" " " " "	-	-	-	-	-	-
25		175	" " " " "	-	-	-	-	-	-
26		232	" " " " "	-	-	-	-	-	-
27	8	75	May 14,1927.4:00A.M.	-	-	-	-	-	-
28		147	" " " " "	-	-	-	-	-	-
29		193	" " " " "	-	-	-	-	-	-
30		196	" " " " "	-	-	-	-	-	-



DISCUSSION OF TABLE III.

Eight different series of urine cultures were made from February 5, 1927 until May 14, 1927, covering urine samples from sixteen different cows. These eight series consisted of thirty different individual urine cultures; five from cow 99; three from cows 95, 147, and 193; two from cows 47, 93, 175, and 232; and one from cows 33, 75, 84, 161, 191, 236, and 1665.

The results obtained in both the uninoculated "cream-urine" mixture and the sediment of uninoculated urine were negative. Individual urine sample number thirteen was not sufficient to run both tests so the "cream-urine" method was used alone. Twice in the "cream-urine" mixture of cow number 33, once in twenty-four hours standing and again in forty-eight hours, colonies were present that had the appearance of Bacterium abortus but on transplanting to beef liver infusion agar slants the characteristic appearance disappeared.

GENERAL DISCUSSION.

In order that Bacterium abortus be present in the urine, it would have to gain admission by one or more of three routes, namely, the urethra, blood stream, or lymph channels.

To summarize from the writings of various investigators, Bacterium abortus is found in the urine of guinea pigs and humans with histopathological lesions apparent only in the kidney of the guinea pig. Although these lesions are also present in the kidneys of rabbits and monkeys, no mention, apparently, has been made of isolating the bacillus from the urine. Neither lesions nor bacilli are found in the kidneys or urine of goats. In regard to Brucella melitensis, the organism is found in the urine of guinea pigs, goats, and humans, and accompanied by lesions of the kidneys. Rabbits and lambs, although they show lesions in the kidneys, appear not to have the Malta fever bacillus in their urine. With these comparisons, excepting Bacterium abortus in the human, every case of urine contamination appears to be associated with pathological conditions of the kidney. These results bring up two questions: (1) Is Brucella melitensis more virulent than Bacterium abortus? (2) Are the kidneys of small animals more susceptable to pathogens than those of large animals? Khaled (13) states that from his experiments with Bacterium abortus and Brucella melitensis he found the latter is six times more virulent for guinea pigs. Pfenninger (34) claims that "certain experimental infections of small animals have a tendency to localize in the kidneys".

In order that Bacterium abortus be present in the kidney, there has to be either a haematogenous or an ascending type of infection. Although Bacterium abortus when introduced into the circulatory system of cattle will localize in the udder that has functioned or in the pregnant uterus, post-mortem lesions show that they do not localize in the kidneys. They appear to have developed an "organ virulence". Since the typhoid bacilli pass through the human kidney into the urine, and Bacterium abortus does the same thing in certain animals, possibly through a lesion of some sort, one would be led to surmise that it would be possible for the same to take place if Bacterium abortus were present in the kidneys of cows. Hemholz and Millikin (35), on introducing into the circulation avirulent and virulent staphylococcus, avirulent and virulent streptococcus hemolyticus, and avirulent and virulent colon bacilli, found that there was no secretion of these organisms in the urine excepting where there was damage to the renal structures. They also believe that the endothelial cells of the large capillary net work aid the kidneys in being actively phagocytic so that they are able to dispose of large numbers of bacteria. It would be possible for the ascending type of infection to take place during a urinary stasis, but during normal conditions, with the continuous flushing of the ureters with urine it would be impossible for a non-motile organism such as Bacterium abortus, to ascend the lumen of the ureters. The amount of urine excreted daily by the cow is considerably more than that of any of the animals, including man, that has been so far discussed in connection

with Bacterium abortus and Brucella melitensis. Sampson (16) demonstrated that it was not possible to force fluid into the ureters from the bladder. He removed a bladder, cut off both ureters, and then filled the bladder with a fluid, but was unable to force any of the fluid through the severed ureters.

The bladder would have to receive Bacterium abortus from the kidneys, blood, or the urethra. It is far more probable, in view of the foregoing discussion, that the infection should arise from the latter than the two former. One naturally would be led to suppose that in the non-pregnant cow Bacterium abortus either succumbs to some unknown factor upon leaving the udder in the lymph system, since there are a lack of lesions in the lymph glands excepting in the supramammary one, or that they do not leave the udder at all since there are no embryonic tissues in the non-pregnant cow. Williams (36) believes that Bacterium abortus plays a negligible part as the cause of cystitis.

One would suspect that Bacterium abortus, if present in the urethra, would reach the lumen of that vessel by way of the meatus urinarus, although it could as readily do so by means of the blood and lymph systems. Sanderson and Rettger (37) were able to produce infection by inoculating the urethra of guinea pigs, and mice. Traum (25) out of eight heifers, produced abortion in two and permanent reaction in three by superficial intra-urethral injection of cultures. According to Embleton and Thiel (16), bacteria, other than Bacterium abortus and Brucella melitensis, when placed on the urethral mucosa passed into the lymphatics of the wall of the bladder and urethra and collected beneath the capsule of the kidney.

They also stated that pathogens did not pass into the kidney or enter the urine but passed rapidly into the lumbar glands and thoracic duct and from there into the blood stream. Inasmuch as this was merely experimental work Lubberkusen and Fitch (38) added, from their experimental evidence, that heifers are not readily infected with Bacterium abortus by way of the urethra. It is the common opinion of many investigators that the bull acts in the minor role in regard to implanting abortion bacilli in the vagina upon coition. Granting though that the male acts mechanically in placing the pathogens in that organ their habitation there would be very short. Carpenter (39) quotes Danzler and Berthold as finding that the mucous secretion of the vagina has a marked bacterial action and is a barrier against invading bacteria. It is true that infected fetal membranes and discharges from the uterus of abortive cattle do pass over the floor of the vagina and vulva, but the meatus urinarus is protected by being more or less covered by the anterior wall of the suburethral diverticulum.

In these series of tests the writer examined culturally the urine of nineteen different cattle affected with Bang's abortion disease, over a period of ten months, considering of thirty-two different series of examinations of one hundred nine individual urine samples. The urine of five cows, numbers 47, 93, 216, 236, and 239, was cultured two different times; three cows, 33, 175, and 191, three different times; four cows, 75, 95, 161, and 193, four times; cow 147, five times; cows 99 and 232, six different times; cow 84, eleven times; cow 171, twelve times; cow 1659, fifteen times; and cow 1665, six teen times without finding a single Bacterium abortus colony.

SUMMARY.

Bacterium abortus can live and propogate in cows' urine for seventy-two hours.

Bacterium abortus can be isolated from cows' urine that has been inoculated and incubated at 37°C. for seventy-two hours under ten per cent CO_2.

Bacterium abortus can be isolated more easily from cows' urinary sediment than from whole urine, after the urine has been inoculated and incubated.

Bacterium abortus at no time produced profuse colonies on any of the beef liver infusion gentian-violet agar plates.

Bacterium abortus was not found in uninoculated urine of cattle infected with Bang's abortion disease.

ACKNOWLEDGMENT.

In conclusion I wish to acknowledge my indebtedness to Doctors I. Forest Huddleson and Ward Giltner for assistance and suggestions received during this investigation.

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