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IS BACTERIUM ABORTUS PRESENT IN THE URINE OF
CATTLE AFFECTED WITH BANG'S ABORTION DISEASE
THESIS FOR DEGREE OF M. S.

L. E. LONG

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AFFECTED WITH BANG'S ABORTION DISEASE ?

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THESIS.

Submitted to the Faculty of the Michigan State College in
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By

L. E. Long

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THESIS

CONTENTS.

INTRODUCTION AND HISTORY.

REVIEW OF LITERATURE.

HISTORY OF ANIMALS USED IN THIS EXPERIMENT.

EXPERIMENTAL WORK.

METHOD OF STUDY.

TABLE I.

DISCUSSION OF TABLE I.

METHOD OF STUDY.

TABLE II.

DISCUSSION OF TABLE II.

METHOD OF STUDY.

TABLE III.

DISCUSSION OF TABLE III.

GENERAL DISCUSSION.

SUMMARY.

ACKNOWLEDGMENT.

BIBLIOGRAPHY.

IS BACTERIUM ABORTUS PRESENT IN THE URINE OF CATTLE
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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 Zundel 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 confirmed 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, Veckl, 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 Mediterranean 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 existence 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-intestinal canal, and in the urinary bladder, or more or less pronounced acute swelling of the spleen and lymph glands.

Schroeder 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 existence. 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 Mediterranean 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 occasionally 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 mls 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 mls 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 mls 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, having 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

1. The first step is to identify the problem or question that needs to be answered.

2. The second step is to gather relevant information and data to address the problem.

3. The third step is to analyze the information and data to identify patterns and trends.

4. The fourth step is to develop a hypothesis or a proposed solution.

5. The fifth step is to test the hypothesis or solution through experimentation or observation.

6. The sixth step is to evaluate the results of the test and determine if the hypothesis is supported.

7. The seventh step is to draw conclusions based on the results of the test.

8. The eighth step is to communicate the findings of the study to others.

9. The ninth step is to reflect on the process and identify areas for improvement.

10. The tenth step is to apply the knowledge gained to other situations.

11. The eleventh step is to continue to learn and grow from the experience.

12. The twelfth step is to share the knowledge with others to help them learn.

13. The thirteenth step is to stay curious and open to new ideas.

14. The fourteenth step is to be persistent and not give up when faced with challenges.

15. The fifteenth step is to be collaborative and work with others to achieve common goals.

16. The sixteenth step is to be adaptable and able to change direction when necessary.

17. The seventeenth step is to be resilient and able to bounce back from setbacks.

18. The eighteenth step is to be proactive and take initiative in solving problems.

19. The nineteenth step is to be organized and manage time effectively.

20. The twentieth step is to be a lifelong learner and always seeking to improve.

21. The twenty-first step is to be a team player and support others.

22. The twenty-second step is to be a leader and inspire others.

23. The twenty-third step is to be a mentor and guide others.

24. The twenty-fourth step is to be a role model and set a good example.

25. The twenty-fifth step is to be a positive influence and bring joy to others.

26. The twenty-sixth step is to be a good listener and understand others.

27. The twenty-seventh step is to be a good communicator and express ideas clearly.

28. The twenty-eighth step is to be a good negotiator and find win-win solutions.

29. The twenty-ninth step is to be a good decision maker and choose wisely.

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 normally 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 CO₂ 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 exami- nations.	
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:						Result of	
Individual:		Date		Hour		Number:	
Series :		of		of		of :	
tests	of tests:	collection		same		animal:	exami-
							nations.
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 CO₂ 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.

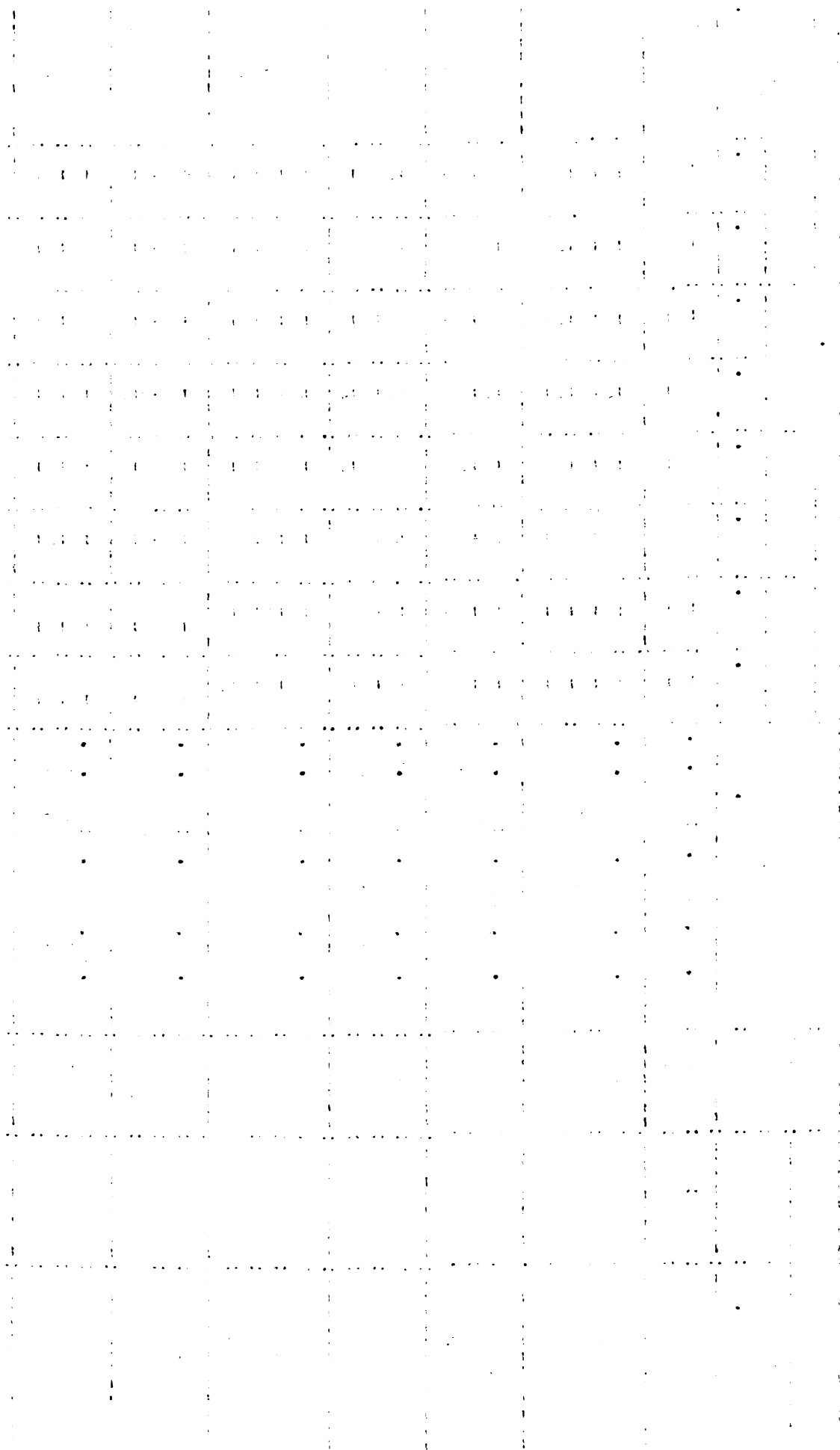


Table II. (continued).

Number of different:		Number:		Date of collection:		12 hour:		24 hour:		48 hour:		72 hour:		Uninocu-	
Individual:		Series:		of:		Date of collection:		12 hour:		24 hour:		48 hour:		72 hour:	
tests		of tests:		animal:		hour of same.		CU.		UU.		CU.		UU.	
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	" " " "	" " " "	+	+	-	-	-	-	-	-	-	-	"

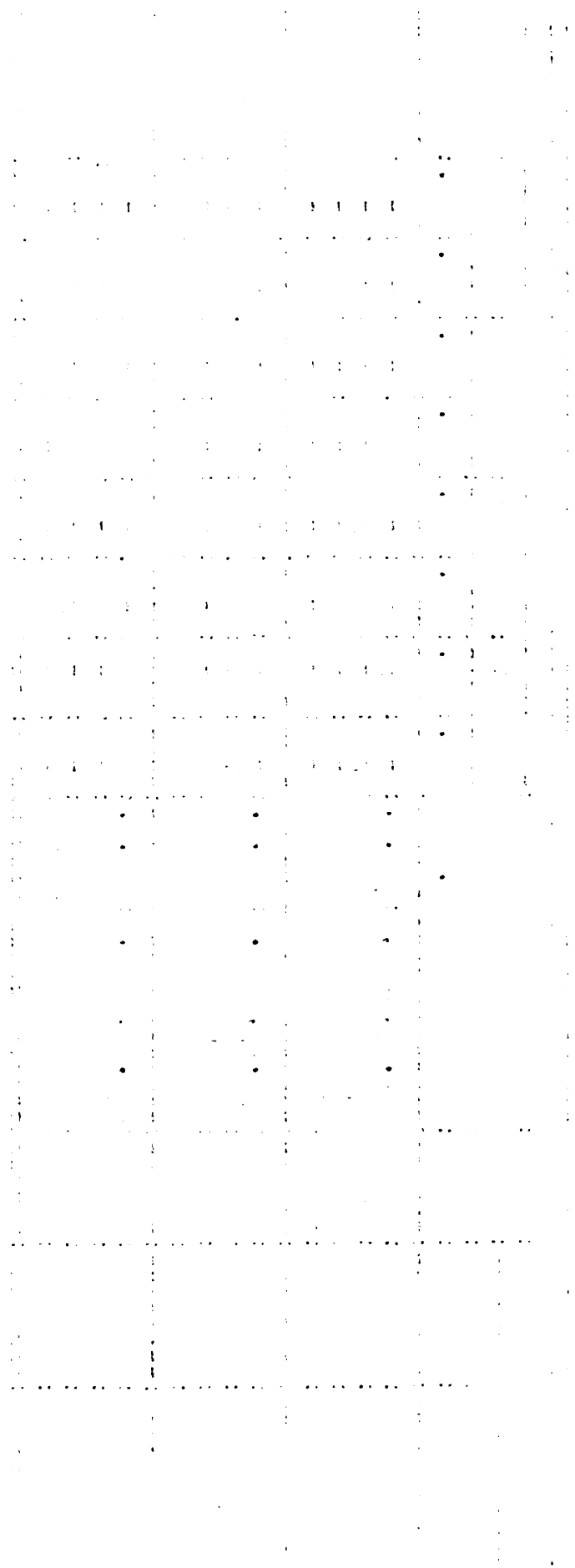
Key - CU. centrifugalized inoculated urine.

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.

• 100 •

• 100 •

• 100 •

• 100 •

• 100 •

• 100 •

• 100 •

• 100 •

• 100 •

• 100 •

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• 100 •

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 CO₂ 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.

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1. The first step in the process of creating a new product is to identify a market need. This involves conducting market research to determine what consumers want and what problems they are trying to solve.

2. Once a market need has been identified, the next step is to develop a concept for a product that addresses that need. This involves brainstorming ideas and selecting the most promising one.

3. The third step is to create a prototype of the product. This allows the designer to test the product's functionality and make any necessary adjustments before moving forward with production.

4. After the prototype has been tested and approved, the next step is to develop a detailed design for the product. This includes creating technical drawings and specifications that will guide the manufacturing process.

5. The final step in the process is to manufacture the product. This involves sourcing materials, setting up a production line, and overseeing the manufacturing process to ensure that the product is produced to the highest quality standards.

6. Once the product has been manufactured, the next step is to distribute it to the market. This involves finding a distribution channel, such as a retailer or distributor, and promoting the product to potential customers.

7. The final step in the process is to monitor the product's performance in the market. This involves tracking sales, customer feedback, and any issues that may arise, in order to make improvements and ensure the product's long-term success.

8. In addition to the steps outlined above, there are several other factors that can influence the success of a new product. These include the timing of the product's launch, the quality of the manufacturing process, and the effectiveness of the marketing strategy.

9. Finally, it is important to note that the process of creating a new product is often iterative. Designers may need to go back and forth between different steps, making adjustments as they learn more about the product and the market.

10. Overall, the process of creating a new product is a complex and challenging one, but it is also a rewarding one. By following these steps and staying focused on the customer's needs, designers can create products that truly make a difference in the world.

11. One of the key challenges in the product development process is managing the timeline. It is important to set realistic deadlines and to communicate clearly with all stakeholders to ensure that the project stays on track.

12. Another challenge is managing the budget. It is important to allocate resources wisely and to avoid unnecessary costs, in order to ensure that the product is profitable and sustainable.

13. Finally, it is important to stay up-to-date on the latest trends and technologies in the industry. This allows designers to create products that are innovative and competitive in the market.

Table III.

Number of different:		Number of:	Date of collection:		Cream / Urine		Centrifuged Urine	
Individual:		Series :	animal :	and :	on standing.	after standing.		
tests :	of tests:	examined :	hour :	hour :	hour :	hour :	hour :	hour :
1	47	Feb. 5, 1927.	4:00A.M.	-	-	-	-	-
2	93	" "	" "	-	-	-	-	-
3	95	" "	" "	-	-	-	-	-
4	99	" "	" "	-	-	-	-	-
5	95	Feb. 19, 1927.	4:00A.M.	-	-	-	-	-
6	99	" "	" "	-	-	-	-	-
7	232	" "	" "	-	-	-	-	-
8	236	" "	" "	-	-	-	-	-
9	147	Feb. 26, 1927.	4:00A.M.	-	-	-	-	-
10	161	" "	" "	-	-	-	-	-
11	175	" "	" "	-	-	-	-	-
12	84	Mar. 12, 1927.	4:00A.M.	-	-	-	-	-
13	33	" "	" "	-	-	-	-	-
14	1665	" "	" "	-	-	-	-	-
15	47	Mar. 19, 1927.	4:00A.M.	-	-	-	-	-
16	93	" "	" "	-	-	-	-	-
17	99	" "	" "	-	-	-	-	-
18	193	" "	" "	-	-	-	-	-

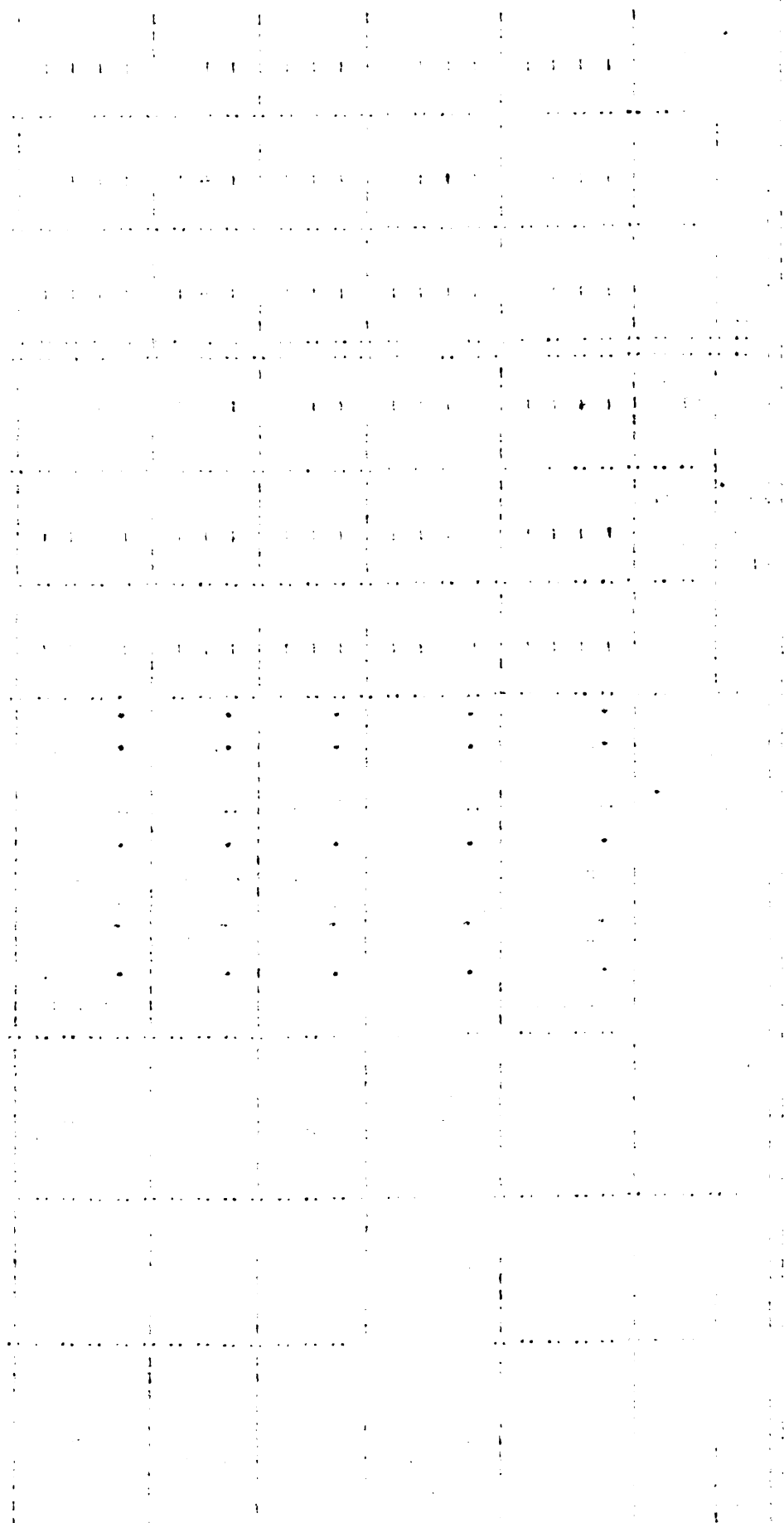


Table III. (Continued).

Number of different:		Number of:	Date of collection:	Cream / Urine		Centrifuged urine	
Individual:	Series:			on standing	after standing	after standing	after standing
tests	of tests:	urine	hour of same.	24 : hour	48 : hour	72 : hour	48 : 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	" " " "	-	-	-	-

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	1222	1223	1224	1225	1226	1227	1228	1229	1230	1231	1232	1233	1234	1235	1236	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246	1247	1248	1249	1250	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260	1261	1262	1263	1264	1265	1266	1267	1268	1269	1270	1271	1272	1273	1274	1275	1276	1277	1278	1279	1280	1281	1282	1283	1284	1285	1286	1287	1288	1289	1290	1291	1292	1293	1294	1295	1296	1297	1298	1299	1300	1301	1302	1303	1304	1305	1306	1307	1308	1309	1310	1311	1312	1313	1314	1315	1316	1317	1318	1319	1320	1321	1322	1323	1324	1325	1326	1327	1328	1329	1330	1331	1332	1333	1334	1335	1336	1337	1338	1339	1340	1341	1342	1343	1344	1345	1346	1347	1348	1349	1350	1351	1352	1353	1354	1355	1356	1357	1358	1359	1360	1361	1362	1363	1364	1365	1366	1367	1368	1369	1370	1371	1372	1373	1374	1375	1376	1377	1378	1379	1380	1381	1382	1383	1384	1385	1386	1387	1388	1389	1390	1391	1392	1393	1394	1395	1396	1397	1398	1399	1400	1401	1402	1403	1404	1405	1406	1407	1408	1409	1410	1411	1412	1413	1414	1415	1416	1417	1418	1419	1420	1421	1422	1423	1424	1425	1426	1427	1428	1429	1430	1431	1432	1433	1434	1435	1436	1437	1438	1439	1440	1441	1442	1443	1444	1445	1446	1447	1448	1449	1450	1451	1452	1453	1454	1455	1456	1457	1458	1459	1460	1461	1462	1463	1464	1465	1466	1467	1468	1469	1470	1471	1472	1473	1474	1475	1476	1477	1478	1479	1480	1481	1482	1483	1484	1485	1486	1487	1488	1489	1490	1491	1492	1493	1494	1495	14
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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 susceptible 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 Lubberkussen 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, sixteen times without finding a single Bacterium abortus colony.

SUMMARY.

Bacterium abortus can live and propagate 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 CO₂.

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.

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