ISOLATION AND IDENTIFICATION STUDIES TO DETERMINE THE PERSISTENCE OF BACTERIA IN THE INTACT UTERUS OF THE POST-PARTUM COW

by

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INTRODUCTION

Delayed breeding of cattle continues to be one of the major economic problems of the cattle industry. Since infectious agents have been suggested as a possible cause of early embryo loss, it was felt that the bacterial flora of the normal bovine uterus must be known.

This study was conducted by the use of post-pregnant living cows for the following reasons: (1) Post-pregnant cows should give a fairly representative sample of cows with normal uteri. (2) The study of the empty or resting normal uterus appears to be more urgent than that of the pregnant uterus if the role of microorganisms in early embryo loss is to be known. (3) Living animals offer the advantage that the general and sexual history of the animal before, during and after sampling could be known. It would be logical to assume that the uterus could be easily contaminated at the time of parturition, so, therefore, this study was undertaken to determine if the normal bovine uterus becomes bacteria free at sometime following parturition.

Samples were taken from the lumens of the uteri of the post-partum living cows by a special instrument at various days post-partum. These were examined for presence of bacteria as were some biopsy samples of the uterine mucosa.

REVIEW OF THE LITERATURE

Literature on the flora of the uterus shows a divided opinion. The first group of workers to be mentioned reports the

presence of few, if any, organisms. Others report finding bacteria commonly present.

Early work on this subject was done by Nocard (1886), who concluded from his studies that organisms were never found in uteri of cows which had not aborted. Denzler (1904) expressed the opinion, based upon the study of genital tracts of living as well as dead animals, that the cervical canal, uterus and the uterine tubes were generally free of bacteria. He did find, however, Staphylococcus, Streptococcus and organisms from the colon-aerogenes group. In 1913 Hofstadt studied 40 non-pregnant uteri and found bacteria in the uterine cavity and in the internal os uteri in only one case. He used uteri from freshly slaughtered animals. Williams and Carpenter (1917) studied 15 animals, ten were old standing cases of sterility and five were tuberculin reactors. The cows were killed and the entire genital tract was removed and taken to the laboratory. Ten of these uteri were bacteria free and five showed bacteria. In 1919 Carpenter, after he had obtained negative cultures from 55 out of 56 maiden heifers, concluded that the normal genital tract should be free of bacteria. In 1920 Carpenter studied 18 normal genital tracts, of which five yielded no growth when cultured for the presence of bacteria. Of the 13 genital tracts giving positive cultures, six gave cultures from the uterus only. He reported the presence of Streptococcus viridans, S. hemolyticus and Staphylococcus albus in the uterus. He reported the presence of Streptococcus in the submucosa of the uterine tissue. He again reaffirmed his aforementioned hypothesis that the normal bovine uterus is essentially

bacteriologically sterile. He continued his work in 1921 and made a study of 114 abattoir slaughtered animals. These were calves, virgin heifers, non-pregnant and pregnant cows. He concluded that "it would seem that the genital tract of cattle should be normally free of bacteria." He attributed the absence of bacteria to the bactericidal action of the mucous present in the genital tract. In 1922 Beaver made a study of 19 infertile cows. He got the samples either from slaughtered animals or by inserting a sterile swab through a cervical canal into the uterus of living animals. He made bacteriological and pathological studies and concluded that in nearly all cases when no gross evidence of active inflammation exists, the genital tract is sterile and that organisms are not normally harbored as commensals in the internal genitalia of the cow. He isolated Streptococcus, B. pyogenes (Lucet) (sic) and Staphylococcus, only from the uteri that showed inflammation but not from normal ones. From studies he conducted, Beller (1927) concluded that the normal post-partum uterus eventually becomes bacteriologically sterile. He observed that organisms Bacillus pyocyaneus and B. subtilis disappear first and the cocci tend to remain longer. He stated that the uterus of domestic animals under physiological condition tolerates a latent bacterial growth without necessarily causing injury to either mother or fetus. This physiological status differentiates itself from the pathological, merely by a lack of virulence of the bacteria inhabiting the uterus or by an efficient resistance of the endometrium. He studied non-pregnant, apparently healthy, live animals and obtained his

samples by infusing into the uterine cavity physiological saline then withdrawing it. Gierke (1929) reported the study of 12 pregnant uteri that were removed from slaughtered animals and taken to the laboratory where they were cultured immediately. Five uteri showed bacteria, but he believed that in some cases the growth represented contamination. Klimmer et al., (1929) studied 57 uteri obtained from an abattoir. Thirty-seven of these uteri showed pathological lesions. They found 19 of the 57 uteri free of bacteria, and they concluded that the uterus of domestic animals is in general free of bacteria. Fitch and Bishop (1932) studied 126 slaughterhouse run cows of which 81 were pregnant and 45 non-pregnant (20 of which were virgin). They took the uterus from the freshly slaughtered cows and cultured a platinum loop inoculum obtained by exposure of the uterine mucous membrane after searing externally and incising the wall with a flamed knife. From the results obtained they considered 94% of the uteri to be bacteria free. They isolated Alcaligenes, Escherichia, Micrococcus, Staphylococcus and Corynebacterium. They concluded that the healthy bovine uterus is in general free from bacteria. Mjuljberg (1937) devised a rinsing technique in which the outer surface of the uterus obtained from a slaughtered animal was seared with hot iron and a pipette filled with nutrient broth was inserted into the lumen of the uterus. The broth was discharged, flushed back and forth several times, and the washings were used to inoculate the media. Using this technique the mucosa of 33 uteri selected at random, proved to be bacteria free, as did those from nine other animals

with protracted histories of infertility, but he was able to show that in 15% of these 42 animals some organisms were present in the thickness of the uterine wall. He reported his "wall-thickness organisms" as consisting mainly of coccal forms. Clark and Stevenson (1949) used the rinsing technique of Mjuljberg in their study of 13 non-pregnant cows and the fetal fluids from nine pregnant cows. All their cultures were negative. Smears from six uteri obtained from sterile cows slaughtered at a local abattoir were inoculated by Bradury (1949) and no bacterial growth was observed. Wulf and Dracy (1952) utilized a flush technique for obtaining their samples by placing a Koroseal tube in the uterus, the other end of which was fastened to a stoppered aspiratory flask which contained the flush solution. A tire pump was applied which would force the flush solution into the uterus. The fluid was drained from the uterus with a canula; the washing being collected in a vessel at the outside end of the canula. They found Bacillus, Corynebacterium, Staphylococcus, Actinomyces and Coccidioides to be the predominent genera isolated. Plate counts were also conducted to determine numbers, and they concluded that few organisms were present but there always seemed to be a few. In 1952, after obtaining bacteria from 20% of 38 organs he studied, Kampelmacher declared that the healthy uterus is normally bacteria free. He obtained his samples by inserting a cervical speculum 7 mm in diameter as far as it would go and a biopsy instrument of the Neilson type was passed through it into the uterus. A portion of the endometrium was biopsied and used for inoculating media.

Hagan (1916) examined pregnant uteri obtained from the slaughterhouse at all stages of pregnancy and no discrimination was made as to health. The uterus was flamed with a plumber's gasoline blowtorch, opened with a sterile knife and a swab was taken from between the uterine wall and the outer chorioallantoic membrane. Samples were also taken from fetal fluids. From 16 pregnant cows the uterochorio space gave positive cultures in 14 cases (87%). The fetal fluids gave cultures in five cases (33%). He was led to believe that the cavity of the adult bovine uterus contained bacteria. He isolated Escherichia coli, Micrococcus albus, Streptococcus and M. citreus. Day (1917) secured cultures from 56% of 18 non-pregnant uteri studied and 32% out of 25 pregnant ones. He isolated Streptococcus, Staphylococcus and a short bacillus. The cultures were made from animals selected at random in an abattoir immediately after slaughter. In 1928 Wagner studied the placenta and umbilical vessels. The placenta used for cultures was only from cases where the allantois chorion was inverted so that placental surface was inside and not exposed to contact with contaminating materials. He found bacteria in the uteri of 61% of the 35 cases he studied and concluded that "the gravid uterus of domestic animals very largely contains bacteria." He listed the presence of diplococci, colonbacilli and streptococci. Conklin and his co-workers (1931) studied 80 pregnant uteri and found 88.7% to show definite bacterial flora. They stated that 19 showed gross pathological lesions and that 18 of these had a bacterial flora. They listed 17 different genera comprised of Micrococcus (35%),

Bacillus (26.2%), Escherichia (23.7%), Streptococcus (21%), Alcaligenes (17%), Staphylococcus (10%), Eberthella (7.5%), Bacteroides, Aerobacter, Sarcina, Salmonella, Actinomyces, Rhodococcus, Proteus, Serratia, Corynebacterium, and Mycobacterium. After culturing samples obtained by a swab and glass speculum from the cervical canal of 53 cows, Hatch et al., (1949) found 26 to be negative for presence of bacteria and 27 to show bacteria. Sixteen of the 53 cows showed abnormal discharge in the vagina and several of these were cases of purulent endometritis. They concluded that bacterial infection either clinically apparent or not, may be responsible for many cases of infertility. A set of 19 cows sent to the abattoir with normal breeding histories was studied by Dawson (1950), six of these cows were pregnant, the rest represented different phases of oestrus cycle. Eight yielded bacteria that were identified as Pseudomonas, Neisseria catarrhalis, Proteus, Staphylococcus and coli-type organisms. Easley et al., (1951) obtained samples from 148 normal cows and 40 repeat breeder cows near the time of oestrus by using a glass speculum 15 inches long by 8 mm in diameter and a glass pipette 16 inches by 4 mm with a 2.0 c.c. glass syringe attached. The pipette was inserted through the speculum placed in the cervical canal and the plunger of the syringe withdrawn, aspirating a small amount of the uterine contents into the sterile pipette. Sterile samples were taken from 39% of the 146 normal cows in contrast to 20% of the 40 repeat breeder cows sampled. They reported the identification of Neisseria, Micrococcus, Streptococcus, Pseudomonas, Corynebacterium, Bacillus,

Flavobacterium, Sarcina, coli-aerogenes, Actinomyces, Proteus, Bacterium, Gaffkya, Bacteroides, yeast, Mycobacterium and Salmonella. M. Pyogenes was found in 26% of uteri from normal animals from which bacteria were isolated and 45% of positive uteri of the repeat breeders. Observations on the bacterial flora of infertile dairy cows were made by Lindley and Hatfield (1952). They obtained their samples by introducing a sterile fiber speculum eight inches long into the vagina and a sterile glass pipette was passed through it into the uterus, then a sterile glass syringe containing 50-100 ml of physiological saline was connected to the pipette and the saline injected into the uterus. The saline was massaged slightly into the uterus and as much of the solution as possible was then withdrawn, and these uterine washings were used for culturing. They isolated 26 separate species of bacteria. The most prominent of these were micrococci and members of genus Neisseria. Although they did not list the number of cows cultured or the number that were positive or negative for bacteria, they concluded that "low grade bacterial infection appears to be a cause of infertility". Gunter et al., (1955) made a detailed bacterial study of 260 uterine and cervical swab samples obtained from 106 dairy cows at successive heat periods beginning at the second or third oestrus after calving and continuing until the cow was settled. Ninety-two cows were normal breeders and the remaining 14 repeat breeders. Thirty-three percent of the 201 samples from the normal cows were bacteria free while only 5% of 59 samples obtained from the repeat breeders were free of bacteria. They obtained samples by an

instrument that was patterned after the one designed by Frank and Bishop (1952). The latter's instrument is composed of a tube within a speculum (6cm in diameter). The tube can be used either to pass a sterile swab through a rubber diaphragm or as a receptacle into which to suck mucous, through a mouth piece of a rubber tubing fixed to the end of the speculum. Gunter et al., adapted this instrument by omitting the rubber tube and mouth piece and all samples were taken by the means of a swab. The rubber diaphragm over the end of the tube was replaced by a plug of 2% agar-agar. Martinez (1955) took cervical mucous from the neck of the uterus of 100 pregnant normal cows by inserting a glass speculum into the neck of the uterus and passing a sterile pipette through the speculum then sucking mucous into the pipette. He reported finding bacteria in 83 of the samples. The bacteria were identified as M. epidermidis, M. auranticus, S. acidominimus, Bacillus pumilis, M. luteus, B. firmis, M. flavum, M. caseolytius, M. candidus, Flavobacterium suaveolens, Bacteroides fundibuliformis and Achromobacter lipoliticum. Gibbon et al., (1959) took samples from the cervix of 207 post-partum cows. The cervical mucous was obtained by passage of a cotton swab through a glass speculum. Of the 207 cows cultured, 40.1% showed the presence of bacteria. They isolated: Streptococcus, Staphylococcus, diptheroids, Proteus, Gaffkya tetragena, Bacillus, E. coli, A. aerogenes and Vibrio fetus.

Elliott (1961) examined 110 post-partum uteri obtained from the slaughterhouse. Out of the 110 uteri he cultured, 67

(60.9%) showed microflora. He reported the presence of a distinct correlation between the length of post-partum period and the presence of microorganisms in the uterus. From 0-15 days post-partum, approximately 85% of the uteri examined contained bacteria, and this percentage regressed until at 60 days only about 10% of the uteri contained bacteria. He concluded from his results that "the normal bovine uterus becomes essentially free from bacteria at 60 days post-partum". He isolated: Proteus, Staphylococcus, Micrococcus, Streptococcus, Escherichia, Aerobacter, Pseudomonas, Corynebacterium, Microbacterium, Actinomyces, Alcaligenes, Arthrobacter, Clostridium, a non-spore forming anaerobe, and Vibrio. He also isolated a mold, Aspergillus.

Due to this considerable divergence of opinion regarding the incidence of bacteria in the normal bovine uterus, it was felt that further research was needed to clarify this point and also to see if the normal uterus becomes bacteria free at sometime after parturition.

MATERIALS AND METHODS

Sample Collection

Samples were collected during the period of October 1961 to May, 1962. Cows used were from the herd of the Dairy Science Department at Kansas State University. These are registered cows with normal breeding history. Nineteen of these cows calved normally; three showed a retained placenta. Of these 22 cows,

ten were Holsteins, five Ayrshires, four Jerseys and two Guernseys. Fourteen cows were multiparous and seven uniparous.

Samples were taken at various days after calving from 20 cows. From the first six cows, samples were taken from each cow on the 5th, 10th, 15th, 23rd, 35th, and 50th day following parturition. From the remaining 14 cows, samples were taken on the 8th, 15th, 23rd, 35th, and 50th day following parturition. Only one sample was taken from one cow eight days after calving, another two samples were taken from another cow on the 8th and 15th day after parturition, and all were clinically healthy at the time of sampling. None of them were treated with antibiotics or sulfa drugs after calving. The samples were taken by a special instrument designed by Minocha (1961) who proved that this instrument was efficient in getting uncontaminated samples. The instrument is composed of an aluminum speculum, two telescopic stainless steel tubes and a rod. (See Plate I, page 15.) Speculum A is made of aluminum, 15 inches long by 5/8 inch I.D. Its front end was spun into a taper that is approximately 3/4 inch long reducing the diameter from 5/8 inch to 5/16 inch. The first tube "B" is made of stainless steel. It is 19 inches long, 1/4 inch O.D. and 3/16 inch I.D. It goes into the speculum that has at its back end a locking device. This locking device is made of two pieces, the first is partially pressed into the back end of the speculum and its diameter is drilled to receive the first tube, outer end was turned and threaded with a 1/8 inch pipe die and in turn receives the second piece which is a knurled handle tapped with a 1/8 inch pipe tap. The second tube "C"

goes into the first tube "B". It is 22 inches long by 3/16 inches O.D. The final member of the instrument "D" is a rod 3/32 inch in diameter and 30 inches long, it is inserted into the second tube"C" and carries a swab at its front end and its back end forms an L 1-1 1/2 inches long.

Before sterilization, the instrument is assembled by locking the first tube "B" in the speculum "A", then inserting the second tube "C" through the first tube "B" and finally the rod "C" carrying a cotton swab is inserted into the second tube "C". A piece of plastic tubing one inch long by 1/4 inch in diameter was tied at the back end of the second tube "C" to protect it from contamination while taking the sample and consequently protecting the swab while withdrawing the rod after getting the sample. The assembled instrument was wrapped in paper and sterilized in the autoclave at 121°C for 15 minutes. After sterilization, it was allowed to cool, a sterile gelatin capsule was put on the anterior end of each of the first and second tubes under aseptic conditions, and then the instrument was wrapped again to be ready for use. To obtain the samples, the vulva of the cow was wiped clean with a paper towel and washed with a diluted solution of Roccal. The end of the assembled instrument was passed through the parted lips of the vulva into the vagina by the right hand while the left hand was in the rectum. An assistant then released the knurled handle and pushed the first tube "B" so that it extruded about three inches from beyond the end of speculum "A", then the handle is fixed tightly on the

first tube so that it does not move. While holding the cervix by the left hand and holding the instrument in the right hand, the end of the first tube "B" is inserted into the cervical canal, then the assistant pushes the second tube forward beyond the end of the first tube till its end enters the uterus guided by rectal palpation. Finally the rod is pushed forward until the cotton swab becomes two inches or more beyond the end of the second tube. By this manner the end of speculum "A" will be at the anterior portion of the vagina, the end of the first tube "B" will be in the cervix, while the end of the second tube "C" will be in the posterior part of the uterus and the end of the rod "D", the swab, will be forward in the uterus. Plate II, page 17, shows the instrument with the approximate position of each part while taking the sample. While keeping the speculum and the two tubes in their positions, the uterus is then massaged against the swab and the swab is rotated against the inner surface of the uteri so that enough mucous can stick to the swab. The swab was also moved to different parts of the uterus. To remove the instrument, the rod is withdrawn first inside the second tube, then the latter is withdrawn into the first one and finally the whole instrument is withdrawn from the animal and taken to a clean room in the barn. Before taking the rod out of the instrument, the back end of the second tube was heated on a spirit lamb to sterilize it. After withdrawing the rod, the swab was rinsed immediately in 1 ml of sterile phosphate buffered glucose broth. This served as the source of inoculum for the various media employed. After rinsing the swab,

EXPLANATION OF PLATE I

A. Speculum

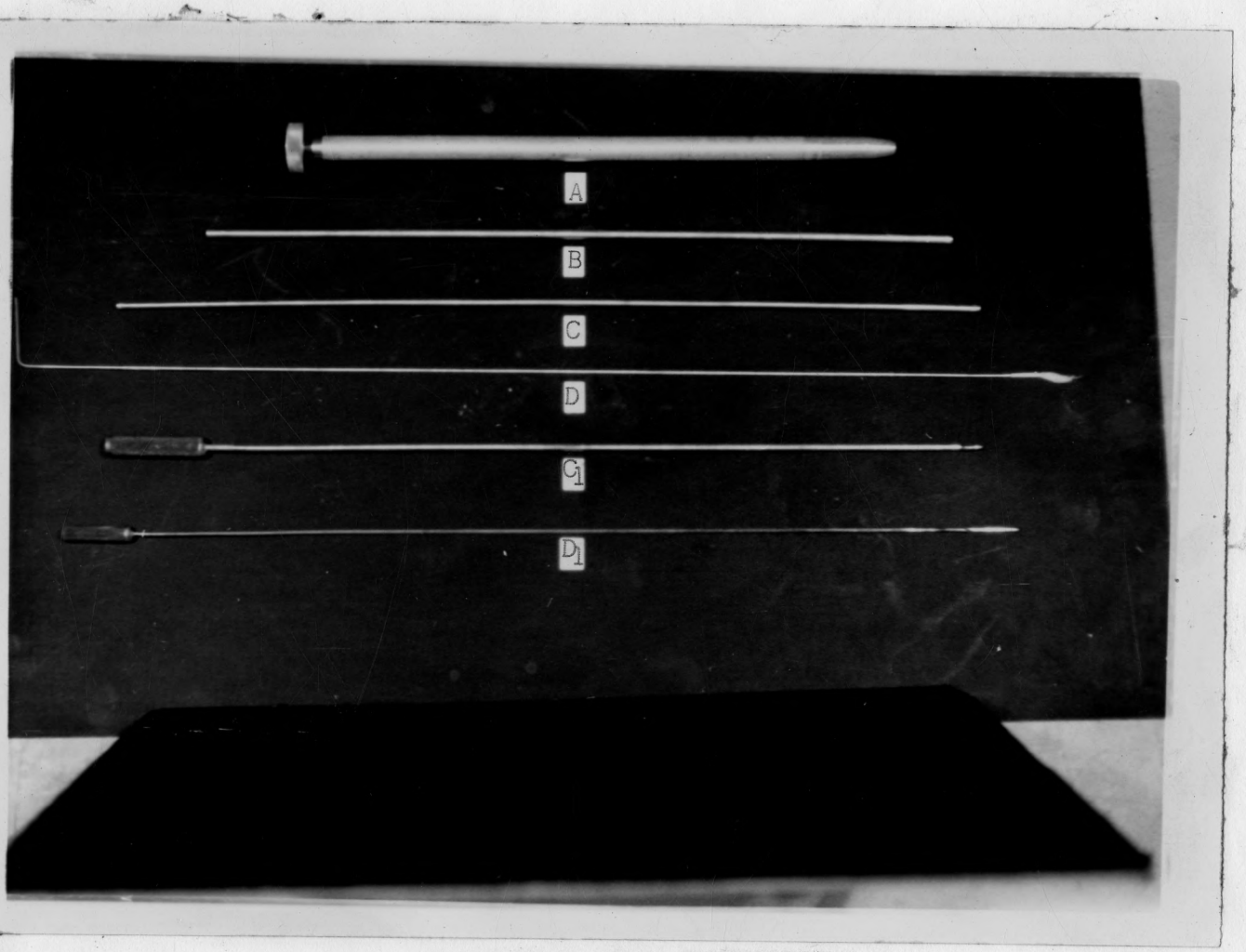
B. First tube

C. Second tube

D. Rod

C_1. Second tube of the biopsy instrument

D_1. Rod of the biopsy instrument


A
B
C
D
C1
D1

EXPLANATION OF PLATE II

A. Speculum

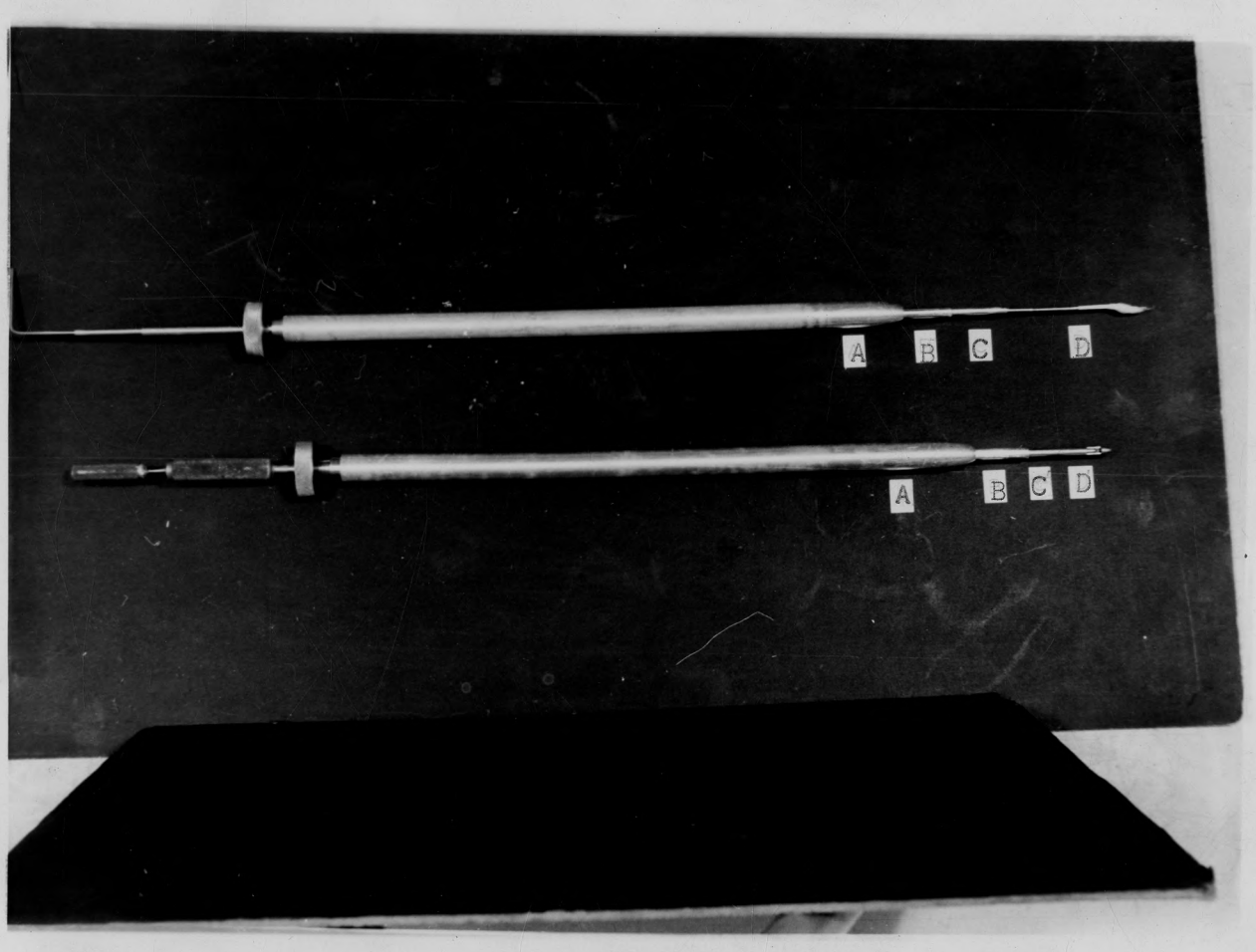
B. First tube

C. Second tube

D. Rod

C_1. Second tube of the biopsy instrument

D_1. Rod of the biopsy instrument



it was streaked over a blood agar plate. This plate and the tube in which the swab was rinsed were taken to the laboratory. The plate was incubated at 37°C and the different media were inoculated with the broth in which the swab had been rinsed. The time between taking the sample and inoculating it ranged between 20-40 minutes.

The uteri of five cows (35-45 days after calving) that were found to be bacteria free when samples were taken by the swab, were biopsied to see if organisms are present in the mucous membrane itself while absent from the lumen. The biopsy instrument was similar to the swab instrument in being composed of a speculum, two tubes and a rod, but the end of each of the second tube and the rod were modified so that both ends acted as a cutter. The end of the second tube "C_1" (Plate I, page 15) is closed and rounded in a bullet shape, then a section about 3/8 inch long and 1/2 the diameter of the tube was filed about 1/4 inch back from the end. The back end of the tube carries a handle. The rod "D_1" does not carry a swab but instead a small tube 1 1/2 inch long joined to its front end, and the anterior end of this tube was sharpened so that it acts with the filed section as a cutter. The instrument was sterilized and prepared as with the swab instrument. To get the biopsy sample the whole assembled instrument was introduced into the vagina, then the first tube pushed into the cervix followed by the second one further into the uterus and finally the rod was pushed to the end of the second tube (see Plate II, page 17) by the right hand while pressing over the uterine wall with the left hand. After

cutting a piece of the uterine mucosa, the rod "D_1" and the second tube "C_1" were withdrawn into the first tube, then the whole instrument was withdrawn. The cut piece was taken out of the instrument aseptically and put in a tube containing 1 ml of phosphate buffered glucose broth and taken to the laboratory where it was transferred from the 1 ml of the transport medium into a sterile tissue grinder and macerated. When the maceration was complete, the suspension was transferred to a sterile test tube and used for inoculating the same media used in culturing the samples taken by swab from the lumen.

Media Employed, Incubation Techniques and Incubation Methods

Each sample was streaked over the surface of three blood agar plates, one Sabouraud dextrose agar plate and one tryptose agar plate containing crystal violet at a concentration of 1:700,000. Two tubes of thiol media and a tube of thioglycollate broth were inoculated with 0.1 ml of each sample. The blood agar plate that was streaked by the swab and another one of three streaked at the laboratory, the Sabouraud dextrose agar plate, the thioglycollate broth tube and one thiol medium tube were incubated at 37°C under aerobic conditions ten days. The second blood agar plate, the tryptose agar with crystal violet plate and the second thiol medium tube were incubated in a candle jar with increased CO_2 at 37°C ten days. The remaining blood agar plate was placed in Brewer's jar under anaerobic condition at 37°C one week.

To check for sterility, all media employed were incubated 24 hours at 37°C before use. The blood agar contained 5% sterile defibrinated ovine blood. The media employed are listed in Difco Manual 9th edition, 1953.

Methods of Isolating Organisms

Every two days during and after the appropriate incubation time, the plates and tubes were observed for growth; individual colonies appearing alone on the plates were considered as contaminants. Colonies following the streak were isolated and placed on the respective medium, and each different colony type was considered to be a different isolate until proven otherwise. The isolates were then incubated under the respective conditions and placed in stock.

Different methods were utilized for the thiol medium and thioglycollate broth. The growth from each tube was examined microscopically to check for organism types. Each tube of thiol medium was streaked on two blood agar plates, one to be incubated aerobically and one to be incubated in a candle jar. After a five-day incubation period, each plate was observed and different colony types were isolated and placed in stock. The thioglycollate broth was streaked onto two blood agar plates per tube, one being incubated aerobically and one anaerobically. Different colony types were isolated after a five-day incubation period.

Identification of Bacteria

The optimum growth temperature, morphology and Gram staining characteristics were first determined for each organism. These characteristics served as a basis for further classification.

The separation into Micrococcaceae and streptococci was determined by morphology, catalase and nitrate reduction ability. The staphylococci were separated from the micrococci on the basis of the oxygen relationship and the anaerobic fermentation of glucose by the former (Bergey's Manual of Determinative Bacteriology, 7th ed. 1957). All streptococci were typed with Lancefield A, B, C, and D antisera that were obtained from Difco Laboratories. The Streptococcus antigen was prepared by the Rantz and Randal method (1955).

All Gram negative rods were flagella stained by Leifson's stain and technique as described by the Manual of Microbiological Methods, (1957).

The Gram positive rods were observed for pleomorphism and stained for the presence of metachromatic granules. These cultures were also pasteurized by heating to 85°C for ten minutes to check for the presence of heat resistant spores.

After these initial tests had been completed, each group was subjected to the indicated biochemical tests. The biochemical tests and media employed are described in Appendix I, page 38. All reactions of the organisms isolated are shown in detail in Appendix II, page 46.

EXPERIMENTAL RESULTS

Table 1 shows organisms isolated from each cow on the different days post-partum.

From Table 1 it is observed that with three exceptions, all uteri examined contained bacteria on the 8th, 15th, 23rd or 35th day post-partum. No organism was isolated on the 15th, 23rd or 35th day that was not isolated on the 8th day. Corynebacteria, streptococci and S. aureus disappeared slowly from the uterus.

Table 2 shows the name of each organism, number of cows from which it was isolated and percent of total isolations.

Micrococcaceae were present in the uteri of 58.5% of the cows examined, and this family constituted 31.35% of the total isolations. Streptococci, corynebacteria and pseudomonads each constituted 11.4% of the total isolations.

All biopsy samples that were taken from five cows 35-45 days after calving, were bacteria free.

Table 3 shows the results obtained when the uteri are divided into the groups indicating days post-partum. From these data the proportion of uteri containing bacteria expressed as precent of total uteri examined is calculated.

The results of Table 3 are represented by plotting percent of uteri containing bacteria vs. days post-partum. (Figure 1, page 28) It can be seen from Figure 1 that after 23 days the curve is almost a linear function.

Table 4 shows the last positive sample, number of services

Table 1. Organisms isolated from each cow on the different days post-partum.

Cow Number	8th day* Sample	15th day Sample	23rd day Sample	35th day Sample	50th day Sample
156C	Streptococcus agalactiae Micrococcus roseus Pseudomonas cruciviae	S. agalactiae	None	None	None
223C	Escherichia coli	None	None	None	None
360C	Corynebacterium renale	None	None	None	None
176B	M. candidus E. coli	M. candidus	None	None	None
145C	Alcaligenes metalcaligenes Staphylococcus epidermidis	A. metalcaligenes	A. metalcaligenes	None	None
159C	S. epidermidis C. bovis	S. epidermidis	None	None	None
5R	S. aureus	S. aureus	S. aureus	None	None
160C	None	None	None	None	None
107B	M. freudenreichii Serratia marcescens	M. freudenreichii	M. freudenreichii	None	None
374C	S. faecalis Achromobacter guttatus	S. faecalis	S. faecalis	None	None
378C	Flavobacterium rigense Gaffkya tetragena	F. rigense	None	None	None
157C	None	None	None	None	None

Table 1 (concl.).

Cow Number	8th day* Sample	15th day Sample	23rd day Sample	35th day Sample	50th day Sample
203C	M. roseus S. equisimilis	M. roseus S. equisimilis	None	None	None
239C	C. pyogenes P. arvilla	C. pyogenes P. arvilla	C. pyogenes	C. pyogenes	None
299B	S. epidermidis Proteus rettgeri	S. epidermidis P. rettgeri	None	None	None
153C	None	None	None	None	None
491B	S. aureus Pseudomonas sp.	S. aureus Pseudomonas sp.	S. aureus	None	None
379C	Aerobacter cloacae	A. cloacae			
101C	S. epidermidis A. aerogenes	S. epidermidis A. aerogenes	None	None	None
235C	S. faecalis Sarcina flava	S. faecalis S. flava	S. faecalis	None	None
194C	P. aeruginosa (achromogenic variety)	P. aeruginosa (achromogenic variety)	Sample not taken	Sample not taken	Sample not taken
6C	S. epidermidis Bacillus subtilis	Sample not taken	Sample not taken	Sample not taken	Sample not taken

*This represents in the first six cows, the 5th and 10th day samples as no difference was found between the two samples in each cow.

Table 2. Percent of total isolations each organism was obtained and of total number of cows examined.

Name of organism	No. of cows from which it was isolated	Percent of total number of cows	Percent of total organisms isolated
Staphylococcus epidermidis	5	22.5	14.25
S. aureus	2	9	5.7
Micrococcus roseus	2	9	5.7
Streptococcus faecalis	2	9	5.7
Corynebacterium bovis	2	9	5.7
Escherichia coli	2	9	5.7
C. renale	1	4.5	2.85
C. pyogenes	1	4.5	2.85
M. candidus	1	4.5	2.85
M. freudenreichii	1	4.5	2.85
Sarcina flava	1	4.5	2.85
Gaffkya tetragena	1	4.5	2.85
S. equisimilis	1	4.5	2.85
S. agalactiae	1	4.5	2.85
Pseudomonas arvilla	1	4.5	2.85
P. cruciviae	1	4.5	2.85
P. aeruginosa (achromogenic variety)	1	4.5	2.85
Pseudomonas species	1	4.5	2.85
Alcaligenes metalcaligenes	1	4.5	2.85
Flavobacterium rigense	1	4.5	2.85
Achromobacter guttatus	1	4.5	2.85
Aerobacter aerogenes	1	4.5	2.85
A. cloacae	1	4.5	2.85
Serratia marcescens	1	4.5	2.85
Bacillus subtilis	1	4.5	2.85
Proteus rettgeri	1	4.5	2.85

Total number of organisms isolated--35.
Total number of cows examined--22.

Table 3. A comparison of uteri containing bacteria with those lacking bacteria.

Days post partum	Total No. of cows per group	No. of uteri containing bacteria	No. of uteri lacking bacteria	Percent containing bacteria	Percent lacking bacteria
8	22	19	3	86.4	13.6
15	21	16	5	74.3	25.7
23	20	7	13	35	65
35	20	1	19	5	95
50	20	0	20	0	100

per conception and the uterine regression interval of each cow. It can be observed that all uteri were bacteria free at the time of service and no apparent relationship existed between number of services per conception and the length of time the bacteria persisted in the uterus. It also appears that uterine regression interval days did not influence the length of time the bacteria persisted in the uterus. The three cows with retained placentas did not carry the bacteria in their uteri for a longer time than the other cows.

Table 4. The association of uterine regression interval, number of services per conception, and persistence of bacteria.

Cow Number	Last sample showing bacteria	Services*** per conception	Uterine regression interval days
156C	15th day	1	35
223C	10th day	1	34
360C	10th day	1	41
176B*	15th day	3	39
145C	23rd day	4	37
159C	15th day	1	42
5R	23rd day	3	39
160C	all samples were negative	1	40
107B*	23rd day	6	49
374C	23rd day	1	36
378C	15th day	1	30
157C	all samples were negative	1	33
203C	15th day	4	45
239C	35th day	3	34
299B**	15th day		
153C	all samples were negative	2	41
491B	23rd day	4	37
379C	15th day	3	30
101C	15th day	2	39
235C*	23rd day	2	35

* Retained placenta.
** Due to rectal abscess rate of uterine regression and services per conception were not determined.
*** All these cows were serviced 55-60 days after calving.

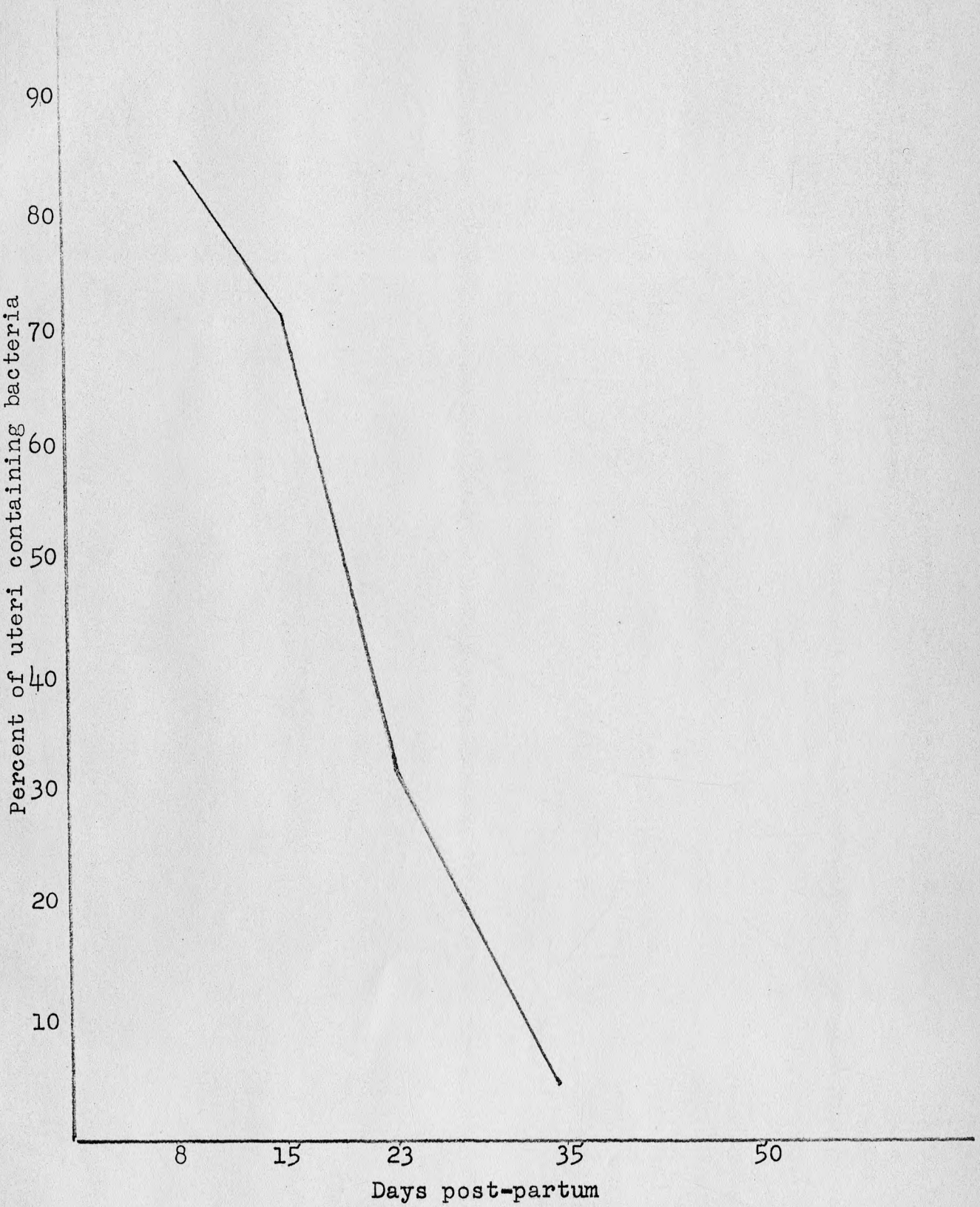

Percent of uteri containing bacteria
90
80
70
60
50
40
30
20
10
8
15
23
35
50
Days post-partum

Figure 1

DISCUSSION

From the results of this investigation it is seen that bacteria were present in most of the uteri in the first week after calving. This microflora gradually disappeared so that on the 35th day post-partum only very few contained bacteria and on the 50th day all the uteri were bacteria free. These results indicate that the normal bovine uterus (lumen and mucosa) is bacteria free, but it becomes easily contaminated at the time of parturition, and again becomes bacteria free within 50 days post-partum. A similar conclusion was stated by Beller (1927) and Elliott(1961). A difference is noticed between the results obtained in this work and those obtained by Elliot (1961); the latter found that most of the uteri examined were bacteria free at about 60 days post-partum, while in this work it was found that most of the uteri were bacteria free by the 35th day post-partum. This difference may be due to the fact that Elliot examined uteri obtained from the slaughterhouse without any known history. It is possible that many of the cows were not in good health and did not represent normal cows.

Nocard (1886), Denzler (1904), Williams and Carpenter (1917), Carpenter (1920 and 1921), Beaver (1922), Beller (1927), Gierke (1929), Klimmer et al., (1929), Fitch and Bishop (1932), Mjuljberg (1937), Clark and Stevenson (1949), Bradury (1949), Wulf and Dracy (1952) and Kampelmacher (1952) would seem to agree with the conclusion stated above as they all concluded that the normal bovine uterus is generally free of bacteria.

Hatch (1949), Easley et al., (1951), Lindley and Hatfield (1952), Gunter et al., (1955), and Gibbon et al., (1959) examined non-gravid living animals and found bacteria in most of the uteri. The great percentage of the uteri containing bacteria found by them might have been due to either contamination or sampling in the early post-partum days. These two factors would account for the presence of bacteria in a great many of the uteri. These workers obtained their samples by employing a speculum and a swab or pipette. It is not unfair to assume that the end of the swab or pipette could have been contaminated as it passed through the vagina. Gibbon mentioned that he took samples from post-partum cows, but he did not mention how many days post-partum. Easley et al., and Lindley did not indicate whether the cows were post-partum or not. Martinez (1955) examined the cervical mucous of living pregnant cows by inserting a pipette into the cervix and sucking the mucous. He found that 83% contained bacteria. This high percentage could be due to contamination during inserting the pipette and sucking the mucous. Hagan (1916), Wagner (1928), Conklin et al., (1931), and Dawson (1950) examined pregnant uteri obtained from the slaughterhouse and reported the presence of bacteria in most of the uteri. An explanation to this high percentage could be contamination. Conklin lists 88.7% of the uteri he examined to contain bacteria, and out of this 88.7% they recovered the genus Bacillus 26.2% of the time. This fact would leave some doubt about his technique due to the fact that Bacillus is a common airborne organism, and throughout this study and the studies cited the genus Bacillus

is rarely reported.

These criticisms would point out that there is a great need for caution during taking the sample. This worker has faith in the methods employed during this study, especially the technique of getting the samples from the uterus, as very few contaminants were found on the streaked plates after their incubation. Massaging the uterus and rotating of the swab against the uterine wall at different places helped in getting the swab loaded with mucous each time and in no time was it obtained free of mucous. It was felt that the time elapsing between collecting and inoculating the sample (20 to 40 minutes) would not give a drastic reduction in number of bacteria. The buffer and the peptone in the transport medium made it a satisfactory one (Straka and Stokes 1957). The blood agar plate streaked by the swab at the barn also served as added protection. At no time was there a growth on this blood agar plate while none on all the other media inoculated at the laboratory. It was felt that the media employed would isolate a very broad spectrum of bacteria. The thiol medium and thioglycollate broth proved especially valuable for isolating not only Vibrio (Huddleson 1948) and anaerobes but also facultatively anaerobic bacteria and aerobes. The 37°C was utilized due to the fact that the organism would have to be able to multiply at that temperature in order to survive in the uterus. Incubation times were selected in accordance with the length of time required for development of visible colonies.

Most of the organisms listed could be reasonably classified as saprophytes such as: Micrococcus, Pseudomonas, coagulase

negative staphylococci, enterics, members of family Achromobacteriaceae, some corynebacteria and some streptococci. Others isolated including certain corynebacteria, certain streptococci and coagulase positive staphylococci could be considered as pathogens. The cows examined had normal breeding histories and were in good health during their last pregnancy and after calving. So, this group of animals appeared to be a fairly representative sample of cows with normal uteri.

SUMMARY

The uteri of 20 normal healthy registered post-partum cows were examined for the presence of bacteria by taking swab samples from the uterine lumen of each on the 8th, 15th, 23rd, 35th and 50th day post-partum. The uterine mucosae of five cows were biopsied 35-45 days post-partum and examined for the presence of bacteria.

A distinct correlation was found between the length of the post-partum period and the presence of microorganisms in the uterus of the cow. Bacteria were isolated from 86.4% of the cows examined on the 8th day post-partum. By the 50th day all the uteri examined were bacteria free. After 35-45 days post-partum, none of the uterine mucosae of five cows contained bacteria.

Bacteria isolated were members of the following genera: Staphylococcus, Micrococcus, Streptococcus, Corynebacterium, Pseudomonas, Sarcina, Gaffkya, Aerobacter, Escherichia, Alcaligenes, Achromobacter, Flavobacterium, Serratia and Bacillus.

The results of this research indicate that the normal bovine uterus (lumen and mucosa) is bacteria free. Apparently microorganisms enter the uterus at the time of parturition, but they are eliminated within 50 days.

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APPENDIX I

Common Biochemical Tests and Stains Employed

Coagulase test. Small agglutination tubes were used to hold 1 ml quantities of fresh rabbit plasma and this plasma was then inoculated with a big loop full of the culture to be tested. The tubes were incubated in a water bath set at 37°C and observed for coagulation at 1/2, 1, 2, 3, and 24 hour intervals. Positive controls were run with the test.

Starch hydrolysis test. Starch agar was utilized for the test. The plates were inoculated and when good growth appeared, they were flooded with Gram's iodine and read for starch hydrolysis.

Indole test. Tryptone broth tubes were inoculated and incubated until heavy growth appeared, then tested for presence of indole by the Gore test as follows: The cotton stopper was removed and the underneath side was moistened with several drops of Ehrlich's reagent #1 and then with an equal amount of Ehrlich's reagent #2, the stopper was then replaced in the tube and pushed down until within one inch of the medium. The tube was then placed erect in a boiling water bath for ten minutes. Formation of red color on the stopper indicates the presence of indole.

Hydrogen sulfide test. Peptone iron agar was inoculated by stabbing. H_2S production was indicated by blackening in the medium along the line of inoculation. Triple Sugar Iron agar was used instead in studying members of Enterobacteriaceae for H_2S production.

Test for aerobic or anaerobic fermentation of glucose and

mannitol. Tubes of glucose and mannitol B.C.P. agar were utilized. The tubes were stabbed and incubated. Yellowing progressing from the bottom was read as anaerobic utilization, whereas if it progressed from the top it was read as aerobic.

Sodium hippurate hydrolysis test. The medium (1% sod. hippurate broth) was inoculated and incubated until good growth appeared. Hippurate hydrolysis was checked as follows: to 0.8 ml of culture, 0.2 ml of ferric chloride reagent was added, mixed and observed after 15 minutes. A permanent precipitate indicated positive test.

Oxygen relationship determination. Shake cultures of 0.1% glucose yeast extract agar were steamed to the boiling point and then cooled to 45°C in a water bath. A tube was stabbed with the culture and immediately chilled in cold water until solidification occurred, then incubated at optimum growth temperature for 48 hours. Organisms were classified as: 1) Aerobes, those which grew only on surface. 2) Anaerobes, those which grew only on lower part of the medium. 3) Facultative, those which grew both on surface and throughout depth of medium.

Optimum temperature determination. Stroke cultures on slants of a suitable agar medium were made in quadruplicate of each organism, then one culture was incubated at each of the following temperatures: 8°C, 20°C, 25°C, and 37°C. Then all cultures were observed at the end of 1, 2, 4, and 7 days incubation for growth. The lowest temperature of incubation giving the most abundant growth was considered the optimum growth temperature.

Heat test for spores. An old stroke culture (at least one week old) was selected and a suspension of the organism was made in 3 ml sterile water. The tube of the suspension was immersed in a water bath at 85°C ± 1°C so that the level of the water in the bath was within one inch of the top of the tube. The suspension was heated for ten minutes then chilled by placing in cold water for five minutes. Aseptically a tube of N. broth or tryptose phosphate broth was poured into the tube of heated suspension, then incubated at the optimum temperature. Any growth appearing within a week was an indication of a positive test.

Catalase test. Cultures 48 hours old on either nutrient agar or tryptose phosphate agar slants were used for this test. A small quantity of three percent hydrogen peroxide was poured over the slant and observed for bubbles.

Nitrate reduction test. Cultures were allowed to incubate until a turbid broth was noted and checked at intervals thereafter. The medium employed was a 0.1% potassium nitrate broth. A 0.5 ml quantity of sulfanilic acid reagent and a 0.5 ml quantity of dimethyl alpha naphthylamine reagent were added to 5 ml of the broth culture which was then observed for a red color. If no color appeared, a small quantity of zinc dust was added to determine if a red color would develop.

Gelatin hydrolysis test. Modified Frazier's gelatin agar (Lord's Manual 1959) was utilized for the gelatin hydrolysis test. The plates were inoculated, and when good growth appeared, they were flooded with Frazier's gelatin developer and observed for hydrolysis of the gelatin.

A 15% nutrient gelatin medium contained in test tubes were utilized to check for different forms of liquefaction.

M. R. - V. P. test. M. R. - V. P. medium was inoculated and incubated until slight turbidity was observed. To 1.0 ml of the culture, 0.6 ml of 5% alcoholic alpha naphthol solution was added and mixed. Then 0.4 ml of 40% aqueous potassium hydroxide was added and mixed. The development of a red color formed at the top of the culture within 30 minutes was considered a positive V. P. test.

To the remaining portion of the cultures a few drops of methyl red indicator were added, and a red color was considered a positive M. R. test. Negative tests were rerun at two-day intervals.

Test for the utilization of urea as a nitrogen source. Slants of urea glucose agar were used for this test. Growth on the slant indicated the use of urea as a nitrogen source, and yellowing indicated the production of acid from glucose.

Test for the utilization of ammonia as a nitrogen source. This was checked by the use of ammonium phosphate glucose agar slants. The presence of growth on the slants was proof of the utilization of ammonia as a nitrogen source. The production of acid from glucose could also be checked by the presence of a yellow color on the slant.

Urea hydrolysis test. Tubes of urea broth were prepared and inoculated. The presence of a red color after incubation showed the hydrolysis of urea. The red color in the tube was due to the presence of ammonium hydroxide which caused an

alkaline pH.

Action on milk test. Litmus milk was utilized for most milk tests. It was inoculated and then incubated for 14 days, being observed the 1st, 2nd, 4th, 7th, 10th, and 14th day. The litmus milk was observed for acid curd, rennet curd, peptonization, reduction of litmus, gas production, and pigment production.

One-tenth percent methylene blue milk was used to determine the reduction of methylene blue.

Tests for motility. Hanging drop preparations of 20-hour cultures were used to observe motility. Motility test agar was also utilized. The agar was stabbed and observed for motility at 8, 24, and 48 hours.

Sodium chloride tolerance test. This test was conducted with the use of sodium chloride agar and broth containing varying percentages of sodium chloride. The agar slants and broth tubes were inoculated and observed for growth.

Potato slant test. Potato slants were prepared and inoculated. They were then observed for growth and pigment production.

Hemolysis of red blood cells. Cultures were streaked on blood agar plates prepared with blood agar base plus the addition of 5% sterile ovine blood. Alpha or beta hemolysis, if present, was read after good growth of the culture.

Colony and agar streak characteristics. Cultures were streaked on nutrient agar slants and nutrient agar plates. Characteristics and pigmentation were observed and recorded

after 24 to 48 hours incubation.

Sugar fermentation test. Brom thymol blue fermentation broth base was prepared and 0.5% of desired sugar was added to the base and tubed with a Durham tube in each tube of culture media. It was then autoclaved 15 minutes at 121°C. The inoculated tubes were observed after the 1st, 2nd, 4th, 7th, 10th, 14th, and 21st day of incubation. During the interval acid production, gas production and alkalinity were observed.

Leifson's flagella stain. This stain was prepared according to the Manual of Microbiological Methods (1957) and was used on all Gram negative organisms. Eighteen to 20 hour culture slants were washed with 2-3 ml of sterile water. This suspension was then placed in a small tube for a ten minute incubation period. Smears prepared by allowing the suspension to wash down a cleaned slide, were air dried and stained by Leifson's method.

Cellulose utilization test. Cellulose salts medium containing filter paper strips was inoculated, incubated at the optimum temperature and observed for 14 days. Damage to the filter paper was considered a positive test.

Spore stain. The Snyder modification of the Dorner's spore stain was employed, using cultures 2-7 days old.

Metrachromatic granule stain. Forty-eight hour cultures were stained with Loeffler's alkaline methylene blue and observed for the presence of metachromatic granules.

Lancefield typing. The antigen was prepared from 18 hour cultures of streptococci grown in tryptose phosphate broth by the method described by Rantz and Randall (1955). The capillary

tube method precipitin test was carried out for identification as described by Difco Laboratories.

All media employed for biochemical tests, techniques utilized and methods are listed in Determinative Bacteriology (Lord 1959).

APPENDIX II

STREPTOCOCCUS

Source and days post-partum	Name of organism	Morphology	Oxygen relationship	Catalase test	Optimum temperature	Motility test agar	NO_3 reduction test	Gelatin hydrolysis	Starch agar	Litmus milk	Bile solubility	2% NaCl broth	6.5% NaCl broth	pH 9.6 N. broth	0.1% Methylene blue milk	1% hippurate broth	5% sucrose broth	Xylose	Glucose	Lactose	Sucrose	Maltose	Trehalose	Raffinose	Inulin	Glycerol	Sorbitol	Hemolysis	Lancefield type
156C (5th, 10th, & 15th day)	Streptococcus agalactiae	Gram + cocci	Facultative	-	37°C	-	-	-	-	Acid-curd reduction	-	+	-	-	-	+	growth	-	+	+	+	+	+	-	-	+	-	Beta	B
374C (8th, 15th, & 23rd day)	S. faecalis	Gram + cocci	Facultative	-	37°C	-	-	+	-	Acid	-	+	+	+	+	+	growth	-	+	+	+	+	-	-	-	+	+	Alpha	D
203C (8th & 15th day)	S. equisimilis	Gram + cocci	Facultative	-	37°C	-	-	-	+	Acid-curd reduction	-	+	-	-	-	-	growth	-	+	+	+	+	+	-	-	+	-	Beta	C
235C (8th, 15th, & 23rd day)	S. faecalis	Gram + cocci	Facultative	-	37°C	-	-	+	-	Acid and curd	-	+	+	+	+	+	growth	-	+	+	+	+	+	-	-	+	+	Alpha	D

MICROCOCCACEAE

Source of culture and days post-partum	Name of organism	Morphology	Oxygen relationship	Optimum temperature	Motility test agar	Catalase test	Litmus milk	Gelatin hydrolysis	NO_3 reduction test	$(NH_4)_3PO_4$ glucose agar	Urea glucose agar	Glucose agar (BCP)	Mannitol agar (BCP)	Glucose broth (BCB)	Lactose	Urea broth	7% NaCl agar	12% NaCl agar	Potato slant	Coagulase test
176B (5th, 10th, & 15th day)	Micrococcus candidus	Gram + cocci	aerobic	28°C	-	+	No change	-	-	No growth	No growth	-	-	-	-	-	-	-	whitish grey growth	-
145C (5th & 10th day)	Staphylococcus epidermidis	Gram + cocci	Facultative	37°C	-	+	Acid	+	+	growth and acid	growth only	Fermented anaerobically	-	+	+	-	+	+	white growth	-
159C (5th, 10th, & 15th day)	S. epidermidis	Gram + cocci	Facultative	37°C	-	+	Acid	+	+	No growth	No growth	Fermented anaerobically	-	+	+	-	+	+	whitish growth	-
156C (5th & 10th day)	M. roseus	Gram + cocci	aerobic	25°C	-	+	No change	slow +		growth and acid	growth only	-	-	-	-	-	+	+	rose colored growth	-
5R (8th, 15th, & 23rd day)	S. aureus	Gram + cocci	Facultative	37°C	-	+	acid and curd	+	+	growth and acid	No growth	Fermented anaerobically	Fermented anaerobically	+	+	-	+	+	white growth	+
107B (8th, 15th, & 23rd day)	M. freudenreichii	Gram + cocci	aerobic	28°C	-	+	No change	-	-	growth and acid	No growth	-	-	-	-	-	+	-	white growth	-
378C (8th day)	Gaffkya tetragena	Gram + cocci	Facultative	37°C	-	+	Acid	-	+	growth and acid	No growth	Fermented anaerobically	-	+	+	-	-	-	greyish white growth	-
203C (8th & 15th day)	M. roseus	Gram + cocci	aerobic	28°C	-	+	Alkaline	+ slow	+	growth and acid	No growth	-	-	-	-	-	+	-	rose colored growth	-
299B (8th & 15th day)	S. epidermidis	Gram + cocci	Facultative	37°C	-	+	Acid	+	+	growth and acid	No growth	Fermented anaerobically	-	+	+	-	+	+	whitish growth	-
491B (8th, 15th, & 23rd day)	S. aureus	Gram + cocci	Facultative	37°C	-	+	acid and curd	+	+	No growth	No growth	Fermented anaerobically	Fermented anaerobically	+	+	-	+	+	whitish growth	+
101C (8th & 15th day)	S. epidermidis	Gram + cocci	Facultative	37°C	-	+	Acid	+	+	growth and acid	growth only	Fermented anaerobically	-	+	+	-	+	+	whitish growth	+
235C (8th & 15th day)	Sarcina flava	Gram + cocci	aerobic	25°C	-	+	Alkaline	+	-	growth only	growth only	-	-	-	-	+	+	-	yellow growth	+
6C (8th day)	S. epidermidis	Gram + cocci	Facultative	37°C	-	+	Acid	+	+	No growth	No growth	Fermented anaerobically	-	+	+	-	+	+	whitish grey growth	-

PSEUDOMONAS

Source and days post-partum	Name of organism	Morphology	Motility test agar	Flagellation	Oxygen relationship	Optimum temperature	Growth at 42°	Catalase	Litmus milk	NO_3 reduction test	Starch hydrolysis	Gelatin hydrolysis (Krazier)	Gelatin hydrolysis (15% Gelatin)	Indole production	H_2S production	Glucose	Lactose	Sucrose	N. broth	5% NaCl broth	Napthalene salts medium	Cellulose salts medium	Chromogenesis on ordinary agar
156C (5th & 10th day)	Pseudomonas cruciviae	Gram (-) rods	+	Polar	Facultative	25°C	Scanty	+	Alkaline	-	-	-	-	-	-	-	-	-	Turbid	+	-	-	whitish
239C (8th & 15th day)	P. arvilla	Gram (-) rods	+	Polar	Facultative	25°C	Scanty	+	Alkaline	-	-	-	-	-	-	+	-	-	Turbid	-	+	-	whitish
491B (8th & 15th day)	Pseudomonas sp.	Gram (-) rods	+	Polar	Facultative	25°C	-	+	Alkaline	+	-	+	+	-	-	-	-	-	Turbid	+ slight	-	-	-
194C (8th & 15th day)	P. aeruginosa (achromogenic variety)	Gram (-) rods	+	Polar	Facultative	25°C	+	+	Alkaline	+	-	+	+	-	-	+	-	-	Turbid	+	-	-	whitish

CORYNEBACTERIUM

Source and days post-partum	Name of organism	Morphology	Heat test result	Acid fast stain	Granule stain	Motility test agar	Oxygen relationship	Optimum temperature	Growth on ordinary agar	Litmus milk	Gelatin hydrolysis	NO_2 reduction test	Indole production	Glucose	Lactose	Sucrose	Mannitol	Loeffler's blood serum slant	Potato slant	Cellulose salts medium	Catalase
360C (5th & 10th day)	Coryne-bacterium renale	Gram (+) rods	-	-	Banded	-	Facultative	37°C	+	Acid and reduction	-	+	-	+	-	-	-	No liquefaction	greyish growth	-	+
159C (5th & 10th day)	C. bovis	Gram (+) rods	-	-	Banded	-	Facultative	37°C	+	Alkaline	-	-	-	-	-	-	-	No liquefaction	No growth	-	+
239C (8th, 15th, 23rd, & 35th day)	C. pyo-genes	Gram (+) rods	-	-	Banded	-	Facultative	37°C	-	Acid and curd	+	-	-	-	-	-	-	Liquefaction	No growth	-	+
192C (8th & 15th day)	C. bovis	Gram (+) rods	-	-	Banded	-	Facultative	37°C	+	No change	-	-	-	-	-	-	-	No liquefaction	No growth	-	+

ACHROMOBACTERIACEAE

Source and days post-partum	Name of organism	Morphology	Motility test agar	Flagellation	Oxygen relationship	Optimum temperature	Chromogenesis on ordinary agar	Gelatin hydrolysis	NO_3 reduction test	Litmus milk	Glucose	Lactose	Sucrose	Indole production	N. broth	6% NaCl broth	Naphthalene salts medium	0.1% phenol mineral salts medium	Potato slant
145C (5th, 10th, 15th, & 23rd day)	Alcaligenes metalcaligenes	Gram (-) rods	-	-	Facultative	28°C	-	+	+	Alkaline	-	-	-	-	Turbid	+ slight	-	-	greyish growth
374C (8th day)	Achromobacter guttatus	Gram (-) rods	+	Peritrichous	Facultative	28°C	-	-	-	No change	+	-	-	-	Turbid	+	+	-	dirty creamish growth
378C (8th & 15th day)	Flavobacterium rigense	Gram (-) rods	+	Peritrichous	Facultative	28°C	Pale yellow	+	-	No change	-	-	-	-	Turbic and pellicle	+	-	-	yellow growth

ENTEROBACTERIACEAE

Source and days post-partum	Name of organism	Morphology	Motility	Flagellation	Chromogenesis on ordinary agar	Oxygen relationship	Optimum temperature	Catalase	NO_3 reduction test	Gelatin hydrolysis (Frazier)	15% gelatin	Litmus milk	Indole production	Urea hydrolysis	H_2S production on (T.S.I. agar)	Acetyl methyl carbinol production	Methyl red test	Koser's citrate	Pot. cyanide broth	Sod. malonate broth	Glucose	Lactose	Cellobiose	Rhamnose	Sorbitol	Inositol	Dulcitol	Sucrose	Salicin	Glycerol	Mannitol	Maltose
223C (5th day)	Escherichia coli	Gr (-) rods	-	-	whitish	Facultative	37°C	+	+	-		Acid-curd reduction	-	-	-	-	+	-	+	-	(+)	(+)	-	(+)	-	-	(+)	(+)	(+)			
176B (5th & 10th day)	E. coli	Gr (-) rods	+	Peritrichous	whitish	Facultative	37°C	+	+	-		Acid-curd reduction peptonization	-	-	-	-	+	-	+	-	(+)	(+)	-	(+)	-	-	(+)	(+)	(+)			
107B (8th & 15th day)	Serratia marcesens	Gr (-) rods	+	Peritrichous	red	Facultative	25°C	-	+	+	+ No pellicle	Acid-curd	-	-	-	+	-	+	+	-	+	+	+	+	-	+	-					
299B (8th & 15th day)	Proteus rettgeri	Gr (-) rods	+	Peritrichous	-	Facultative	37°C	+	+	+ slight		Alkaline	+	+	-	-	-	+	+	-	+	-	-	+	+	-	-				+	-
379C (8th & 15th day)	Aerobacter cloacae	Gr (-) rods	+	Peritrichous	whitish	Facultative	37°C	+	+	+		Acid-curd peptonization reduction & gas	-	-	-	+	-	+	+	-	(+)	(+)	(+)	-	-	+	-			+		
101C (8th, 15th, & 23rd day)	A. aerogenes	Gr (-) rods	-	-	whitish	Facultative	37°C	+	+	-		Acid-curd	-	-	-	+	-	+	+	-	(+)	(+)	(+)	-	-	+	-			(+)		

( ) = gas

ISOLATION AND IDENTIFICATION STUDIES TO DETERMINE THE PERSISTENCE OF BACTERIA IN THE INTACT UTERUS OF THE POST-PARTUM COW

by

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Delayed breeding of cattle continues to be one of the major economic problems of the cattle industry. Since low grade bacterial infection has been suggested as a possible cause of early embryo loss, it was felt that the bacterial flora of the normal bovine uterus must be known. The uteri of living post-pregnant cows were examined for the presence of bacteria. Post-pregnant cows should give fairly representative samples of cows with normal uteri. Living animals offer the advantage that the general and sexual history of the animal will be known. It would be logical to assume that the uterus could be easily contaminated at the time of parturition, so, therefore, this study was undertaken to determine if the normal bovine uterus becomes bacteria free at sometime following parturition.

A group of 20 normal healthy registered post-partum cows was examined for the presence of bacteria in their uteri by taking six swab samples from each of six cows on the 5th, 10th, 15th, 23rd, 35th, and 50th day after calving, and five swab samples from each of the remaining 14 on the 8th, 15th, 23rd, 35th, and 50th day post-partum. The uterine mucosae of five cows were biopsied 35-45 days post-partum and examined for the presence of bacteria. Special instruments were used in getting the samples.

A distinct correlation was found between the length of post-partum period and the presence of microorganisms in the uterus of the cow. From 0-10 days post-partum 86.4% of the cows examined showed microorganisms in their uteri. This percentage gradually regressed until by the 35th day bacteria were found in only 5% of the uteri, and on the 50th day post-partum, none of

the uteri of the 20 cows examined contained bacteria. After 35-45 days post-partum, none of the uterine mucosae of five cows contained bacteria.

Bacteria isolated from the uteri of the examined post-partum cows were: Staphylococcus, Micrococcus, Streptococcus, Corynebacterium, Pseudomonas, Sarcina, Gaffkya, Aerobacter, Escherichia, Alcaligenes, Achromobacter, Flavobacterium, Serratia, and Bacillus.

The results of this investigation indicate that the normal bovine uterus (lumen and mucosa) is bacteria free, but it becomes contaminated at parturition. It again becomes free of bacteria within 50 days post-partum.