THE BACTERIAL FLORA OF THE UTERUS OF THE POST-PARTUM COW

by

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INTRODUCTION

Each year the repeat breeder cow costs the animal industry about 250 million dollars. This large economic loss stimulated the initiation of a project at Kansas State University which was designed to investigate the possible role of microorganisms and antigen-antibody reaction in early embryo loss in cattle. Due to the fact that the study was designed to be an intensive long range investigation dealing with the causes of infertility, it was felt that before a program dealing with bacterial infection as a possible cause of embryo loss could be formulated, the bacterial flora of the normal bovine uterus must be known. It would be logical to assume that the uterus could be easily contaminated at the time of parturition, so therefore it was also undertaken to determine if the normal uterus becomes bacteria free at some time following parturition.

The study of the normal uterus was conducted with the use of post-partum cows, because it would seem that past pregnant cows should give a fairly representative sample of cows with normal uteri. The uterine horn was taken aseptically from the cow at various days post-partum and dated as such. These horns were then examined for the presence of bacteria to determine if the apparently normal bovine uterus is bacteriologically sterile.

REVIEW OF THE LITERATURE

As a result of the work that has been conducted on the normal uterus, there are two avenues of thought. The first group to be mentioned contends that there are few if any organisms present in the normal bovine uterus and the second group contends that there are many.

Research on this subject dates back to Denzler (1904), who stated that the normal reproductive tract could not support the growth of microorganisms. He did find, however, Staphylococcus, Streptococcus, and organisms from the colon-aerogenes group. In 1919 Carpenter (1919-1920), after he had obtained negative cultures from five out of six uteri taken from maiden heifers, said the normal genital tract should be free of bacteria. Carpenter (1920-1921), continued his work and 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 Strep. viridans, Strep. hemolyticus, and S. albus in the uterus. Carpenter states an interesting observation, in that he reported he found the presence of Streptococcus in the submucosa of the uterine tissue. He again reaffirms his aforementioned hypothesis that the normal bovine reproductive tract is essentially bacteriologically sterile. From studies he conducted, Beller (1927) concluded that the normal post-partum uterus eventually becomes sterile. He observed that organisms such as Bacillus pyocyaneous and B. subtilis disappear first and the cocci tend to remain longer. Beller obtained his samples from the uterus by inserting a glass speculum through which a catheter was passed to obtain a mucus sample. He also said:

> "The uterus of our domestic animals, under wholly physiological conditions tolerates a latent bacterial growth without necessarily causing injury to either mother or fetus. The physiological,

> differentiates itself from the pathological status merely by a want of virulence of the bacteria inhabiting the uterus or by an efficient resistance of the endometrium."

Klimmer et.al. (1929) studied 22 normal uteri and found 72 per cent to be bacteria free; therefore, they concluded that the normal bovine uterus is bacteriologically sterile. After some work in 1930, Haupt (1930) said that the healthy uterus lacks the presence of bacteria. Fitch and Bishop (1932) studied 126 slaughterhouse run cows of which there were 81 gravid and 45 non-gravid uteri. They took the uterus directly from the freshly slaughtered cows, washed the area to be opened and seared this area with a hot spatula to get rid of any possible contamination. The seared area was then opened with a sterile knife, the mucosa was stroked with a platinum loop, and this loop with the mucoid material was used to inoculate the culture media utilized. From the results obtained using these techniques, they considered 91 per cent of the uteri to be bacteriologically sterile. The organisms they did isolate were Alcaligenes, Escherichia, Micrococcus, Staphylococcus, and Corynebacterium. Twenty-two slaughterhouse run uteri, of which nine were gravid and 13 were non-gravid, were examined by Clark and Stevenson (1949). They obtained only one isolate from the 22 uteri examined and the one bacterium reported was considered a contaminant. Kampelmacher (1952) studied 38 normal uteri and considered 70-80 per cent to be free from bacteria. He inserted a cervical speculum into the uterus and then passed a biopsy instrument of the Neilson type through the speculum to obtain a sample of the uterine mucosa. Utilizing a flush technique, Wulf and Dracy (1952) determined the numbers and types of organisms present in the normal uterus. They obtained their bacteriological 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 then attached to the side arm and pressure was applied which would force the flush solution into the uterus. The fluid was drained from the uterus with a canula; the washings being collected in a vessel at the outside end of the canula. They found Bacillus, Corynebacterium, Actinomyces, Staphylococcus, and Coccidioides to be the predominant genera isolated. Plate counts were also conducted to determine numbers, and they concluded that very few organisms were present but there always seemed to be a few.

The contention held by the first group that the normal uterus of the bovine is essentially lacking the presence of bacteria would seem to be very well established, but as will be seen, the second group has equally well expressed their point.

One of the first men to isolate organisms from a large percentage of his samples taken from the normal uterus was Hagan (1916) who examined 20 pregnant uteri obtained from the slaughterhouse. The uterus was flamed with a plumber's gasoline blowtorch, opened with a sterile knife and a swab sample taken from between the uterine wall and the outer chorioallantoic membrane. He found 87 per cent to contain microorganisms and identified Bacterium coli, Micrococcus albus, Streptococcus, and M. citreus. Day (1917) reported the presence of Streptococcus, Staphylococcus, and a short bacillus in the normal uterus. He cultured pregnant and non-pregnant uteri he obtained directly from the slaughterhouse and found organisms present in 56 per cent of the non-pregnant uteri cultured and in 32 per cent of the pregnant uteri cultured. Having conducted a survey on 26 cows shortly after parturition, Wagner (1928) said, "the gravid uterus of domestic mammals very largely contains bacteria." He listed the presence of diplococci, colon-bacilli, and diplo-streptococci. Conklin et.al. (1931)

studied 80 pregnant uteri and found 88.7 per cent to contain a bacterial flora which was comprised of Micrococcus, Bacillus (26.2 per cent of total number of organisms isolated), Esherichia, Streptococcus, Alcaligenes, Staphylococcus, Eberthella, Bacteroides, Aerobacter, Sarcina, Salmonella, Actinomyces, Rhodococcus, Proteus, Corynebacterium, and Mycobacterium. They also reported the presence of gross pathological lesions in 19 of the uteri taken for sampling purposes. In 1950 Dawson (1950) studied 19 uteri, six of which were pregnant, from cows with normal breeding histories and found 58 per cent to be devoid of bacteria. He identified Pseudomonas, Neisseria catarrhalis, and Proteus. Eastly et.al. (1951) obtained samples from 148 normal cows by insertion of a sterile pipette through a tubular speculum placed in the cervical canal, the mucus being sucked into the sterile pipette. The cultured samples yielded growth 61 per cent of the time. They reported the identification of Neisseria, Micrococcus, Streptococcus, Pseudomonas, Corynebacterium, Flavobacterium, Esherichia, Aerobacter, Bacillus, Sarcina, Actinomyces, Proteus, Bacterium, Gaffkya, molds, Alcaligenes, Bacteroides, a yeast, Mycobacterium, and Salmonella, of which M. pyogenes was found in 26 per cent of the uteri from which bacteria were isolated. Alford et.al. (1955) determined the presence of group A Streptococcus in the normal breeding bovine uterus and reported the presence of this group in seven per cent of 92 animals studied. In a recent survey Gunter et.al. (1955) found only 33 per cent of their uterine swab samples to be sterile. For sample collection, they utilized a sterile tube, one end of which was plugged with a sterile agar plug that contained a sterile swab. The vulvas were washed and the instrument was inserted into the uterus with the agar plugged end forward; the agar plug could then be forced out with the swab, and the swab sample could be taken. They reported the presence of diptheroids of which C. bovis and

Microbacterium lacticum were the predominant species. Micrococcus and Streptococcus were also present of which 15 per cent of the micrococci were coagulase positive and five per cent of the streptococci were listed as being potential pathogens. Martinez (1955) took cervical mucus from the neck of the gravid uterus of 100 normal cows and examined the mucus samples for the presence of bacteria and found bacteria in 83 of the samples. He inserted a sterile glass speculum into the neck of the uterus and passed a sterile pipette through the speculum and sucked mucus into the pipette. He isolated M. epidermidis, M. aurantiacus, Strep. acidominimus, B. pumilus, M. luteus, B. firmus, M. flavus, M. caseolyticus, M. candidus, Flavobacterium suaveolens, Bacteroides fundibuliformis, and Achromobacter lipolyticum. He classified the 100 animals he studied as normal by virtue of the fact they settled by the third insemination. Gibbons et.al. (1959) took 207 samples of cervical mucus from normal breeding cows, one month before calving and 30 to 60 days following parturition, and found 59.9 per cent of their samples to be negative for bacteria. They isolated Streptococcus, Staphylococcus, diptheroids, Proteus, Gaffkya tetragena, Bacillus, E. coli, Aerobacter aerogenes, and Vibrio fetus.

Due to the differences in normal uterine microflora reported by other workers, it was felt that further research was needed to clarify this point.

MATERIALS AND METHODS

Sample Collection

This survey was conducted throughout the months of June to August of 1960. A total of 110 uterine horns were examined from 110 different cows. The reproductive organs were gathered from the eviscerating table at Armour and Company located at Kansas City, Kansas, and the uterus was taken directly from the

eviscerating table and prepared for sampling. One of the uterine horns was tied off at the base with a strong piece of twine, and about 3 centimeters below this twine, the horn was severed from the rest of the reproductive organs. The remaining horn was then opened by either Dr. H. T. Gier, Department of Zoology, or Dr. G. B. Marion, Department of Dairy Husbandry, and dated according to days post-partum. The dating was by observation of the size of the caruncle, the size of the regressing horn, the presence of the corpus luteum and the replacement of uterine epithelium.

The other uterine horn, reserved for bacteriological samples, was swabbed at a central point with tincture of iodine. The uterus was held by one worker while another worker using a needle and syringe injected 10 ml of peptone-buffered water, into the uterine cavity. The solution was gently agitated back and forth in the uterine central canal without withdrawing the syringe. After the agitation was completed, as much as possible of the solution was pulled back into the syringe and 0.1 ml of the solution was expelled into a tube of thiol medium plus one per cent supplement B and 0.1 ml expelled into a tube of thioglycollate broth; the remaining portion of the solution was expelled into a sterile screw cap test tube.

At the onset of the study duplicate tubes of the uterine wash solution, plus inoculated tubes of thiol medium, and thioglycollate broth were transported to the laboratory at 0° C. (ice chest) and ambient temperatures. The difference in transport temperature apparently did not influence the viability of the microorganisms. Therefore, the tubes of thiol medium and thioglycollate broth were transported at surrounding temperatures and the uterine wash sample was transported at 0° C. The uterus reserved for further bacteriological sampling

was placed in a plastic bag which was tied shut, placed in a waterproof jar, and transported in the ice chest.

The time between sampling and culturing ranged from four to nine hours, with most of the samples being cultured at approximately five hours after they were collected. The table to be used for the disection was washed down thoroughly with a solution of mercuric chloride at a concentration of 1:1,000. The uterine horn was then placed on the table with the curved portion parallel to the table top. A longitudinal section in the middle of the curvature of the uterine horn was seared with a hot spatula. Outward pressure was applied at each outer edge of the seared area with a pair of Allis tissue forceps and a second worker immediately incised the seared area to the central canal with a flamed razor blade held with a hemostat. The exposed mucosa was immediately biopsied with a flamed biopsy sissors, and the tissue section, approximately one centimeter square and 0.2 centimeter deep, was placed into a sterile mortar along with four ml of peptone-buffered water and a small quantity of sand and macerated. When the maceration was complete, the supernatant fluid was pipetted off the residue with a sterile pipette and transferred into a sterile screw cap test tube which served as the source of inoculum for the various media employed.

Media Employed, Inoculation Techniques and Incubation Methods

Appropriate media were inoculated, as soon as practicable, with the uterine wash sample taken at Kansas City, along with the samples taken from the mucosa. Each sample was streaked upon one blood agar plate containing one per cent supplement B, one blood agar plate, one Sabouraud dextrose agar plate, one tryptose agar plate containing crystal violet at a concentration of 1:700,000, and the uterine mucosa sample, only, was streaked upon one blood agar plate. A tube of

thiol medium plus supplement B and a tube of thioglycollate broth were inoculated with a 0.1 ml of the uterine mucosa sample. The blood agar plate plus the two tubes of thiol medium and the Sabouraud agar plate, were incubated at 37° C. for 5 days. One blood agar plate inoculated from each of the paired samples and the two tubes of thiol medium were incubated in a candle jar (a sealed pressure cooker) with approximately 10 per cent CO_2 at 37° C. for five days. The tryptose agar plus crystal violet was likewise incubated in a candle jar, but for 21 days at 37° C. The remaining blood agar plate was placed in a Brewer's jar under anaerobic conditions five days at 37° C. The thioglycollate broth was incubated 12 days at 37° C.

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

Methods of Isolating Organisms

Every two days during and after the appropriate incubation times, 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 and catalase characteristics. The staphylococci were separated from the micrococci on the basis of the anaerobic fermentation of glucose by the former (Bergey's Manual of Determinative Bacteriology, 7th ed. 1953). All streptococci were typed with Lancefield A, B, C, and D antisera; the A, B, and D antisera were obtained from Difco Laboratories and the C antiserum was kindly supplied by Professor V. D. Foltz, Department of Bacteriology. 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 10 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 25. All reactions of the organisms isolated are shown in detail in appendix II, page 32.

EXPERIMENTAL RESULTS

Tables 1, 2, 3, and 4 show organisms isolated from each cow plus the number of days post-partum.

Table 1. Organisms isolated from each uterus at 0-15 days post-partum.

Days post-partum	Sample No.	Organisms Isolated
3	34	Clostridium sporogenes
4	8	Strep. agalactiae, Lancefield B
		E. coli
5	36	Strep. faecalis var. liquefaciens, Lancefield D
5	1	None
7	10	Micrococcus sp.
		C. bovis
8	9	P. boreopolis
12	5	S. epidermidis
		E. coli
12	98	S. epidermidis
12	52	Strep. sanguis
13	49	Pseudomonas sp.
13	75	S. epidermidis
		anaerobe
14	6	None
14	94	A. faecalis
15	48	C. pyogenes
15	65	C. renale
15	71	None
15	101	S. epidermidis
15	106	S. epidermidis

Table 2. Organisms isolated from each uterus at 16-30 days post-partum.

Days post-partum	Sample No.	Organisms Isolated
17	58	Pseudomonas sp.
18	56	None
18	95	Strep. faecalis var. zymogenes, Lancefield D

Table 2. (Concl.)

Days post-partum	Sample No.	Organisms Isolated
19	35	Strep. faecalis, Lancefield D
19	60	S. epidermidis
		Vibrio fetus
20	7	None
20	33	P. arvilla
20	51	Arthrobacter simplex
		A. aerogenes
20	59	Microb. flavum
20	102	S. epidermidis
		Micrococcus sp.
20	104	C. pyogenes
21	87	Pseudomonas sp.
22	57	S. aureus
		Pseudomonas sp.
		Pseudomonas sp.
22	96	None
23	18	Microb. flavum
23	32	M. caseolyticus
23	66	Micrococcus sp.
24	83	C. bovis
25	17	None
25	22	Corynebacterium sp.
25	30	C. bovis
25	37	None
25	47	Pseudomonas sp.
25	53	Pseudomonas sp.
25	54	None
26	85	Strep. zooepidemicus, Lancefield C
27	24	None
27	40	S. aureus
27	68	S. epidermidis
28	14	S. epidermidis
		C. bovis
		S. flava
28	15	S. epidermidis
28	29	C. pyogenes
28	38	M. urea
28	76	None
28	93	None
30	13	Corynebacterium sp.
		Act. bovis
30	12	Microb. flavum
30	41	None
30	64	Strep. durans, Lancefield D
30	73	Pseudomonas sp.
30	90	C. bovis

Table 3. Organisms isolated from each uterus at 31-45 days post-partum.

Days post-partum	Sample No.	Organisms Isolated
32	21	None
32	26	*P. boreopolis*
32	31	*A. metalkaligenes*
33	61	*Pseudomonas* sp.
		Pseudomonas sp.
33	86	None
33	92	None
35	3	None
35	16	*A. viscolactis*
		Micrococcus sp.
35	28	*M. roseus*
		Aspergillus sp.
35	25	*M. roseus*
35	46	*Pseudomonas* sp.
35	110	*S. epidermidis*
35	17	None
35	23	None
35	19	None
35	42	None
35	82	None
38	81	None
38	84	None
40	11	*S. aureus*
		S. epidermidis
40	27	*Corynebacterium* sp.
40	39	*Pseudomonas* sp.
40	55	*Strep. pyogenes*, Lancefield A
40	72	*Strep. faecalis*, Lancefield D
40	108	*C. bovis*
40	109	*P. vulgaris*
40	2	None
40	4	None
40	43	None
40	79	None
40	88	None
40	105	None
42	62	*S. epidermidis*
42	89	None
45	99	*Strep. anginosus*
		S. epidermidis
45	103	*S. epidermidis*
45	107	*C. bovis*
45	63	None

Table 4. Organisms isolated from each uterus at 46-60 days post-partum.

Days post-partum	Sample No.	Organisms Isolated
50	44	None
50	67	None
50	97	None
50	100	None
55	70	*E. coli*
55	74	None
55	78	None
57	77	None
60	20	None
60	69	None
60	80	None
60	91	None

Table 5 shows the results obtained when the uteri are divided into the following groups, 0-15 days post-partum, 16-30 days post-partum, 31-45 days post-partum, and 46-60 days post-partum. From these data the proportion of uteri containing bacteria, expressed as per cent of total uteri collected can be calculated.

Table 5. Per cent of uteri containing bacteria as opposed to per cent lacking bacteria.

Days post-partum at 15 day intervals	Total number of cows per group	Number of uteri lacking bacteria	Number of uteri containing bacteria.	Per cent lacking bacteria	Per cent containing bacteria
0-15	19	3	16	15.8	84.2
16-30	41	9	32	22.0	78.0
31-45	38	20	18	52.6	47.4
46-60	12	11	1	91.7	8.3

The results of table 5 can be represented by plotting per cent of uteri containing bacteria vs. days post-partum. It can be seen from figure 1, after 25 days the curve is almost a linear function.

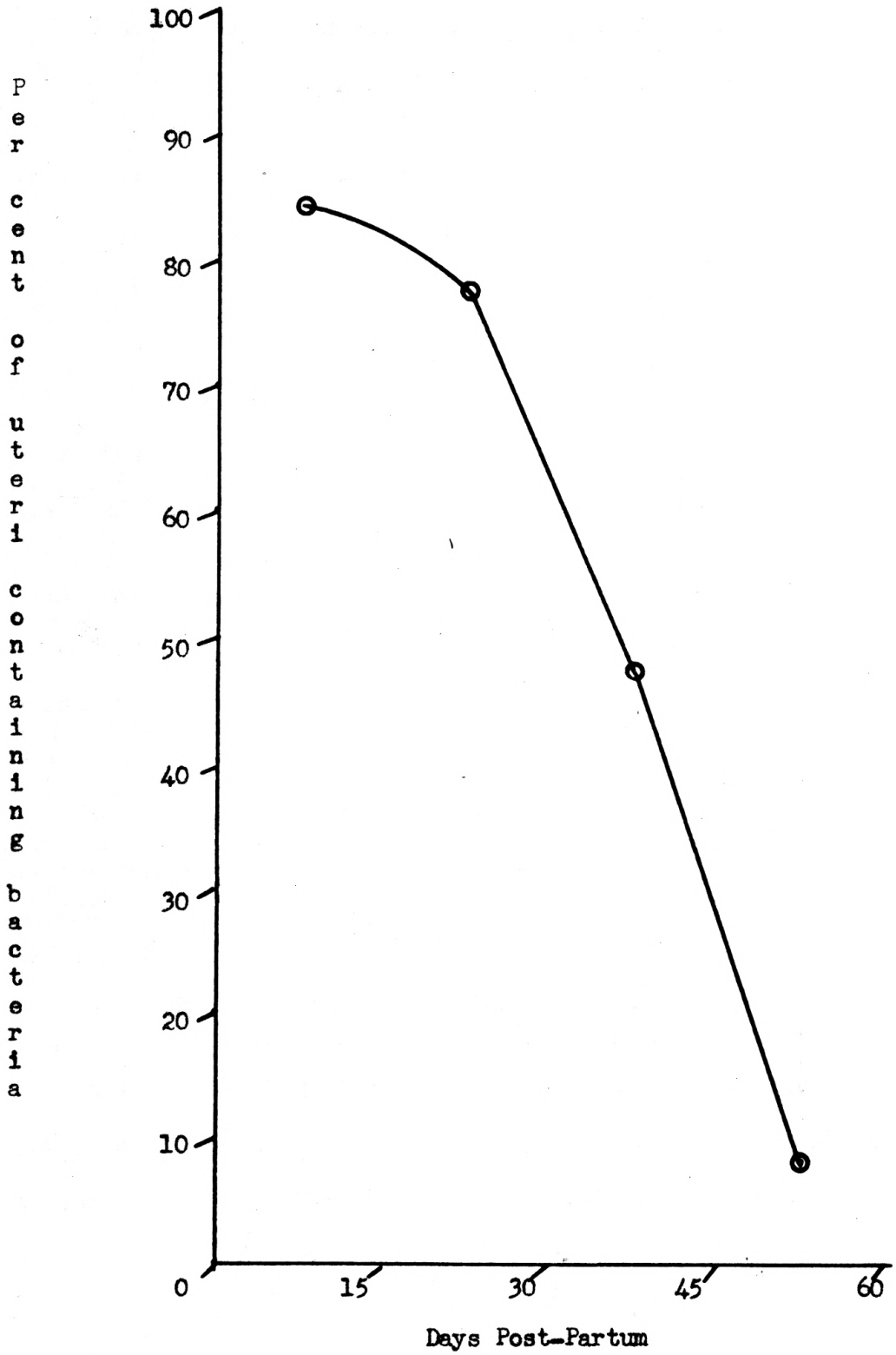

Fig. 1. Per Cent of uteri containing bacteria at various days post-partum.

Table 6 shows each organism isolated and its frequency of isolation. These data show which organisms predominanted.

Table 6. Per cent of total isolations each organism was obtained.

Organism	: Number of times : isolated	: Per cent of total : organisms isolated
S. epidermidis	15	18.3
Pseudomonas sp.	12	14.6
Streptococcus	12	14.6
C. bovis	7	8.4
S. aureus	3	3.6
Micrococcus sp.	3	3.6
E. coli	3	3.6
C. pyogenes	3	3.6
Corynebacterium sp.	3	3.6
Mic. flavum	3	3.6
M. roseus	2	2.4
P. boreopolis	2	2.4
M. caseolyticus	1	1.2
M. urea	1	1.2
A. aerogenes	1	1.2
P. arvilla	1	1.2
C. renale	1	1.2
P. vulgaris	1	1.2
Act. bovis	1	1.2
A. viscolactis	1	1.2
A. metalkaligenes	1	1.2
Arth. simplex	1	1.2
Cl. sporogenes	1	1.2
V. fetus	1	1.2
anaerobe	1	1.2
Aspergillus	1	1.2
Total number of organisms isolated	82	

It was found that of the total number of organisms isolated, the family Micrococcacae was isolated 30.5 per cent of the time. Also, out of a total of 110 uteri cultured, 67 or 60.9 per cent showed a microflora.

DISCUSSION

From the results obtained, it appears that the normal bovine uterus becomes essentially free from bacteria at about 60 days post-partum. The first

group of workers would seem to agree with this statement, i.e. the group composed of Denzler (1904), Carpenter (1919-1921), Beller (1927), Klimmer et.al. (1929), Haupt (1930), Fitch and Bishop (1932), Clark and Stevenson (1949), Kampelmacher (1952), and Wulf and Dracy (1952), who state that the normal bovine uterus contains few or no bacteria in the healthy state. On the other hand, Hagan (1916), Day (1917), Wagner (1928), Conklin et.al. (1931), Dawson (1950), Eastly et.al. (1951), Gunter et.al. (1955), and Martinez (1955) who reported the normal bovine uterus to contain a moderate to a fairly large number of organisms, would be in disagreement.

Some of the conflict would seem to be solved if one considers the included graph. It can be readily seen, that a great deal would depend upon the length of the post-partum period prior to sampling. If the samples were taken very soon after parturition, the presence of bacteria should be noted in about 85 per cent of the samples, but, if the samples were taken at a late post-partum date organisms should be noted in only a small percentage of the samples.

An explanation would also be in order to account for the high percentage of organisms found by some workers in the gravid uterus. Conklin et.al. lists 88.7 per cent of the uteri they examined to contain a bacterial flora. Out of this 88.7 per cent, they recovered the genus Bacillus 26.2 per cent of the time. This fact would leave some doubt about their 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 or never reported. Also, some of the workers employed a speculum and a pipette or swab to obtain their samples, and it would not be unfair to assume that the end of the speculum would become contaminated as it passes through the vagina and pass this contamination

into the uterus. From the work of Weitz (1947), it can be seen that the vaginal flora differs greatly from that of the uterus; therefore, if the uterus had been contaminated with bacteria from the vagina, the uterine microflora would be changed. The flush technique utilized by Wulf and Dracy (1952) could be severly criticized even though they did run a comparison of sterile with non-sterile flush solution and found no great difference in numbers and types. It would seem that even with the sterile solution, there would be a great chance of contamination throughout the operation.

These criticisms would point out that there is a great need for caution during the process of sample collection. This worker has faith in the methods employed during this survey, especially the technique of opening the uterus, because after the streaked plates had been incubated, very few contaminants were found. It was also felt that the media employed would isolate a very broad spectrum of bacteria. The 37° C. incubation temperature 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. Two to three days incubation were added as a safety factor. 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. Time elapsing between collection was not felt to give a drastic reduction in numbers, because peptone-buffered water is a good transport medium, (Straka and Stokes 1957). The thiol medium and thioglycollate broth inoculated at the slaughterhouse would also serve as added protection.

It would seem, from the data collected during this survey, that the normal bovine uterus becomes, to a large extent, essentially free from bacteria

at 60 days post-partum. A statement very similar to this one was made by Beller (1927). It would seem logical that the normal uterus could become very easily contaminated as parturition, which would account for the presence of bacteria at early days post-partum.

Finally, it could be said that many of the organisms reported could be pathogenic or potential pathogens, but it must be remembered that most micro-organisms are pathogens of opportunity. It will be granted that organisms such as Vibrio fetus, certain corynebacteria and certain streptococci could be considered as being pathogenic, but, most of the organisms listed could be reasonably classified as saprophytes, i.e. Micrococcus, Pseudomonas, enterics, Microbacterium, non pathogenic Clostridium and coagulase negative Staphylo-coccus, which constitute 55 per cent of the total organisms isolated. It would seem, therefore, even though no histories were available, that this group of animals should be a fairly representative sample of cows with normal uteri, due to the facts the cows had recently been pregnant and most of the organisms isolated could not be considered pathogens.

SUMMARY

A group of 110 post-partum bovine uteri was obtained directly from the slaughterhouse and examined for the presence of bacteria.

It was found that there was a distinct correlation between length of the post-partum period and the presence of microorganisms. From 0-15 days post-partum approximately 85 per cent of the uteri examined contained microorganisms and this regressed until at 60 days post-partum only about 10 per cent of the uteri contained organisms.

Bacteria isolated from the uterus of the post-partum cow were: Proteus,

Staphylococcus, Micrococcus, Streptococcus, Esherichia, Aerobacter, Pseudomonas, Corynebacterium, Microbacterium, Actinomyces, Alcaligenes, Arthrobacter, Clostridium, a non spore forming anaerobe, and Vibrio, and also the mold, Aspergillus.

The results obtained during this survey would indicate that the normal bovine uterus becomes essentially free from bacteria at 60 days post-partum.

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APPENDIX I

Common Biochemical Tests and Stains Employed

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 per cent 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 per cent potassium nitrate broth. A 0.5 ml quantity of sulfanilic acid reagent and a 0.5 ml quantity of dimethyl alpha naphthylamine reagent was added to five ml of the broth culture and was then observed for a red color. If no color appeared, a small quantity to zinc dust was added to determine if a red color would develop.

Starch hydrolysis test. Starch agar was allowed to incubate until good colony formation was observed. The plate was then flooded with Gram's iodine and read for starch hydrolysis.

Coagulase test. Bovine plasma was contributed by Professor V. D. Foltz, Department of Bacteriology. Small agglutination tubes were used to hold one ml quantities of the plasma and this plasma was then inoculated with a heavy suspension of Staphylococcus. The tubes were incubated in a water bath set at 37° C. and observed for coagulation at one-half hour, one hour, two hour, three hour and 24 hour intervals. Positive controls were run with the test.

Gelatin hydrolysis test. Frazier's gelatin agar (modified 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 per cent nutrient gelatin medium contained in test tubes were utilized to check for different forms of liquefaction.

Indole test. Tryptone broth tubes were inoculated and incubated until heavy growth appeared. Ehrlich's reagent #1 was then layered on top of the culture and observed for the presence of a purple ring at the interface.

Hydrogen sulfide test. Kligler's iron agar was inoculated by stabbing and streaking. Lactose and glucose fermentation was observed along with hydrogen sulfide production.

M. R. - V. P. test. M. R. - V. P. Medium was inoculated and incubated until slight turbidity was observed. To one ml of the culture, 0.6 ml of five per cent alcoholic alpha naphthol solution was added and mixed. Then 0.4 ml of 40 per cent aqueous potassium hydroxide was added and mixed. A red color formed at the top of the culture within 30 minutes was considered as being a positive test.

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

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.

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.

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 causes an alkaline pH.

Test for the aerobic or anaerobic fermentation of glucose and mannitol. Tubes of glucose and mannitol BCP agar were utilized. The tubes were melted and cooled at 45° C. They were then immediately inoculated and plunged into an ice bath. A yellowing progressing from the bottom up or throughout the entire tube was read as the anaerobic utilization of glucose. Yellowing progressing from the top downward was read as being aerobic utilization of glucose.

Action on milk test. Litmus milk was utilized for most milk tests. It was inoculated and then incubated for 14 days, being observed the first, second, fourth, seventh, tenth, and fourteenth day. The litmus milk was observed for acid curd, rennet curd, peptonization, reduction of litmus, gas production, and pigment production.

One tenth per cent methylene blue milk was used to determine the reduction of methylene blue. A whitening of the tube of milk was a positive test.

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 two hour intervals.

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, which would be considered to be a positive test.

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

Optimum temperature determination and oxygen relationship determination.

Shake cultures of 0.1 per cent glucose yeast extract agar were utilized for this test. The tubes of agar were steamed to the boiling point and then cooled to 45° C. in a waterbath. Four tubes were stabbed from each culture and immediately plunged into an ice water bath. From each set of four, one tube was incubated at 45° C., one at 37° C., one at 30° C., and one at 20° C. The tube containing the largest amount of growth would be read as being incubated at the optimum temperature. Tubes containing cultural growth from the top to bottom would be termed facultative, tubes containing cultural growth at the bottom only, would be termed anaerobic, tubes containing cultural growth only on the top were said to be aerobic, and tubes containing cultural growth only in the middle of the tube were termed as being microaerophillic.

Sodium hippurate hydrolysis test. The medium was inoculated and incubated for four days. Hippurate hydrolysis was shown if a precipitate appeared within 15 minutes after the addition of a 0.2 ml of ferric chloride reagent to 0.8 ml of culture.

Hemolysis of red blood cells. Cultures to be determined were streaked on blood agar plates prepared with blood agar base plus the addition of five per cent sterile ovine blood. Alpha or beta hemolysis, if present, could be 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 at 24 to 48 hours incubation time.

Sugar fermentation test. Brom thymol blue fermentation broth base was prepared and 0.5 per cent of desired sugar was added to the base and tubed with a Durham tube in each tube of culture media. It was then autoclaved for 15 minutes at 121° C. The inoculated tubes were observed after the 1st, 2nd,

4th, 7th, 10th, 14th and 21st days 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 and was used on all Gram negative organisms. Eighteen to twenty hour culture slants were washed with two to three 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 meticulously cleaned slide were air dried and stained by Leifson's method.

Heat test for spores. A week old culture of the organism was suspended in three ml of sterile water. This tube of suspension was immersed in a water bath adjusted to 85° C. for 10 minutes. At the end of this time, the tube was cooled and poured onto a plate of suitable medium and observed for growth for one week.

Cellulose utilization test. Cellulose salts medium containing filter paper strips was inoculated, incubated at the optimum temperature and observed for 21 days. The test was considered positive when damage to the filter paper could be observed.

Coagulated albumin broth. The broth was prepared and inoculated. Damage to the albumin was read as a positive test.

Milk tube. Tubes of skim milk were inoculated and observed for action of the organism on the milk.

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

Spore stain. One ml of the culture was placed in a test tube and carbol

fuchsin was added until a deep red color was noted. This mixture was steamed for 10 minutes and a large drop placed on a clean slide. Nigrosin was added, the mixture was mixed and streaked out, allowed to air dry and observed.

Lancefield typing. The antigen was prepared from 20 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 used in the identification of the Clostridium were steamed and then rapidly cooled immediately before inoculation. The media used for the identification of Vibrio fetus were inoculated and incubated under 10 per cent CO_2.

All media employed for biochemical tests, techniques utilized and methods are listed in Determinative Bacteriology, (Lord 1959) or The Manual of Microbiological Methods, 1957.

APPENDIX II

CHARACTERISTICS OF BACTERIA ISOLATED

Micrococcaceae

	Organism and uterus number	Oxygen relationship	Optimum temperature	Morphology	Litmus milk	Gelatin hydrolysis	NO_3 reduction test	Indole production	H_2S production	Glucose $(NH_4)_3 PO_4$	Glucose urea	Mannitol agar	Glucose agar	2% NaCl agar	7% NaCl agar	12% NaCl agar	Potato slant	Urea broth	Catalase test	Xylose	Lactose	Sucrose	Maltose	Inulin	Glycerol	Inositol	Coagulase test
5	Staphylococcus epidermidis	facultative aerobic	37° C.	Gram + cocci	acid coagulation	+	+	-	-	-	+	-	aerobic anaerobically	+	+	-	white growth	+	+	-	+	+	+	-	+	-	-
10	Micrococcus sp.	aerobic	30° C.	Gram + cocci	alkaline	+	-	-	-	-	slight	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-
11	S. aureus	facultative aerobic	37° C.	Gram + cocci	acid	+	+	-	-	+	-	fermented anaerobically	fermented anaerobically	+	+	+	white growth	-	+	-	+	+	+	-	+	-	-
11	S. epidermidis	facultative aerobe	37° C.	Gram + cocci	acid	+	+	-	-	-	+ slight	-	fermented anaerobically	+	+	+	white growth	-	+	-	+	+	+	+	+	-	-
14	S. epidermidis	facultative aerobe	37° C.	Gram + cocci	acid slight reduction	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white growth	-	+	-	+	+	-	-	+	-	-
14	Sarcina flava	aerobic	30° C.	Gram + cocci	alkaline	+	-	-	-	-	-	-	utilized aerobically	-	-	-	yellow growth	-	+	-	-	-	-	-	-	-	-
15	S. epidermidis	facultative aerobic	37° C.	Gram + cocci	acid	+	-	+	-	slight	slight +	-	fermented anaerobically	+	+	-	white growth	-	+	-	+	+	+	-	-	-	-
16	Micrococcus sp.	aerobe	37° C.	Gram + cocci	no change	+	-	-	-	-	-	-	-	+	-	-	colorless growth	-	+	-	-	-	-	-	-	-	-
25	M. roseus	aerobic	30° C.	Gram + cocci	slightly alkaline	+	+	-	-	+	-	fermented aerobically	-	+	-	-	red growth	-	+	+	-	-	-	-	+	-	-
28	M. roseus	aerobic	30° C.	Gram + cocci	no change	+	+	+	-	+	-	-	-	+	+	-	red growth	-	+	+	-	-	-	-	+	+	-
32	M. caseolyticus	aerobic	25° C.	Gram + cocci	acid reduction	+	-	-	-	+	-	fermented aerobically	utilized aerobically	-	-	-	slight white	-	+	-	+	+	+	-	+	-	-
38	M. urea	aerobic	25° C.	Gram + cocci	alkaline	-	-	-	-	+	+	-	utilized aerobically	+	+	-	grayish white	+	+	-	-	-	-	-	-	-	-
40	S. aureus	facultative	37° C.	Gram + cocci	acid	+	+	-	-	-	-	fermented anaerobically	fermented anaerobically	+	+	+	grayish white	-	+	-	+	+	+	-	+	-	-
57	S. aureus	facultative	37° C.	Gram + cocci	acid reduction	+	+	-	-	-	-	fermented anaerobically	fermented anaerobically	+	+	+	white	-	+	-	+	+	+	-	+	-	-
60	S. epidermidis	facultative	37° C.	Gram + cocci	no change	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	-	+	-	+	+	+	-	+	-	-
62	S. epidermidis	facultative	37° C.	Gram + cocci	acid	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	-	+	-	+	+	+	-	+	-	-
66	Micrococcus sp.	aerobic	37° C.	Gram + cocci	alkaline	+	-	-	-	-	+	-	utilized aerobically	+	+	+	white	-	+	+	-	-	-	-	+	-	-

Micrococcaceae (Concl.)

Organism and uterus number	Oxygen relationship	Optimum temperature	Morphology	Litmus milk	Gelatin hydrolysis	NO_3 reduction test	Indole production	H_2S production	Glucose $(NH_4)_3 Po_4$	Glucose urea	Mannitol agar	Glucose agar	2% NaCl agar	7% NaCl agar	12% NaCl agar	Potato slant	Urea broth	Catalase test	Xylose	Lactose	Sucrose	Maltose	Inulin	Glycerol	Inositol	Coagulase test
68 S. epidermidis	facultative	37° C.	Gram + cocci	acid reduction	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	yellow	-	+	-	+	+	+	-	+	-	-
75 S. epidermidis	facultative	37° C.	Gram + cocci	acid	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	yellow	-	+	-	+	+	+	-	+	-	-
98 S. epidermidis	facultative	37° C.	Gram + cocci	no change	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	-	+	-	+	+	+	-	+	-	-
99 S. epidermidis	facultative	37° C.	Gram + cocci	reduction peptonization	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	-	+	-	-	-	+	-	+	-	-
101 S. epidermidis	facultative	37° C.	Gram + cocci	no change	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	-	+	-	+	+	+	-	+	-	-
102 Micrococcus sp.	aerobic	37° C.	Gram + cocci	peptonization reduction	+	+	-	-	+	+	-	-	+	+	+	white	-	+	-	-	-	-	-	-	-	-
102 S. epidermidis	facultative	37° C.	Gram + cocci	acid reduction	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	-	+	-	-	+	+	-	+	-	-
103 S. epidermidis	facultative	37° C.	Gram + cocci	no change	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	yellow	-	+	-	+	+	+	-	+	-	-
106 S. aureus	facultative	37° C.	Gram + cocci	acid	+	+	-	-	-	-	fermented anaerobically	fermented anaerobically	+	+	+	white	-	+	-	+	+	+	-	+	-	-
110 S. epidermidis	facultative	37° C.	Gram + cocci	acid reduction	+	+	-	-	-	-	-	fermented anaerobically	+	+	+	white	+	+	-	+	+	+	-	+	-	-

Streptococcus

	Organism and uterus number	Morphology	Catalase test	2% NaCl broth	6.5% NaCl broth	0.1% methylene-blue milk	pH 9.6 nutrient broth	5% sucrose broth	Litmus milk	Motility test agar	NO_3 reduction test	1% hippurate broth	Glycerol	Inulin	Lactose	Maltose	Raffinose	Sorbitol	Sucrose	Trehalose	Xylose	Gelatin	Starch agar	H_2S production	Oxygen relationship	Lancefield type	Hemolysis	Optimum temperature
8	Streptococcus agalactiae	Gram + cocci	-	+	-	-	-	growth	acid	-	-	+	+	-	+	+	-	-	+	+	-	-	-	-	facultative	B	beta	37° C.
35	Strep. faecalis	Gram + cocci	-	+	+	+	+	growth	acid	-	-	+	+	-	+	+	-	+	+	-	-	+	-	-	facultative	D	alpha	37° C.
36	Strep. faecalis var. liquefaciens	Gram + cocci	-	+	+	+	+	growth	acid peptonized	-	-	+	+	-	-	+	-	-	+	-	-	+	-	-	facultative	D.	alpha	37° C.
50	Strep. zooepidemicus	Gram + cocci	-	+	-	-	-	growth	acid	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	facultative	C	beta	37° C.
52	Strep. sanguis	Gram + cocci	-	+	-	-	-	viscid	acid	-	+	-	-	-	-	+	+	-	+	+	-	-	-	-	facultative	-	beta	37° C.
55	Strep. pyogenes	Gram + cocci	-	+	-	-	-	no growth	acid	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	facultative	A	beta	37° C.
64	Strep. durans	Gram + cocci	-	+	+	+	+	growth	acid	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	facultative	D	beta	37° C.
72	Strep. faecalis	Gram + cocci	-	+	+	+	+	growth	acid	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	facultative	D	alpha	37° C.
85	Strep. zooepidemicus	Gram + cocci	-	-	-	-	-	growth	acid	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	facultative	C	beta	37° C.
95	Strep. faecalis var. zymogenes	Gram + cocci	-	+	+	+	+	growth	acid reduction	-	-	-	/	-	+	+	+	-	+	+	-	+	-	-	facultative	D	beta	37° C.
99	Strep. anginosus	Gram + cocci	-	+	-	-	-	growth	acid	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	facultative high CO_2 tension required	-	beta	37° C.

Pseudomonas

	Organism and uterus number	Morphology	Flagella stain	Motility test agar	H_2S production	15% gelatin medium	Glucose	Sucrose	Lactose	Inositol	Nutrient broth	5% NaCl broth	Cellulose salts medium	Litmus milk	NO_3 reduction test	Indol production	Frazier's gelatin	Starch agar	Acetyl methyl carbinol	Methyl red	Urea broth	Catalase test	Citrate agar	Pigment	Optimum temperature	Oxygen relationship
9	Pseudomonas boreopolis	Gram rods	polar	+	-	+	+	(+)	-	-	turbid	-	-	peptonization	+	-	+	-	-	-	-	+	-	yellow brown	37° C.	facultative
26	P. boreopolis	Gram rods	polar	+	-	+	+	-	-	-	turbid	-	-	peptonization reduction	+	-	+	-	-	-	-	+	+	white	37° C.	facultative
33	P. arvilla	Gram rods	polar	+	-	-	+	-	-	-	turbid	-	-	peptonization reduction	-	-	-	-	-	-	-	+	-	white brown	37° C.	facultative
39	Pseudomonas sp.	Gram rods	polar	+	-	+	+	-	+	+	turbid	-	-	acid peptonization reduction	-	-	+	-	-	-	-	+	-	white	37° C.	facultative
46	Pseudomonas sp.	Gram rods	polar	+	+	+	+	-	+	-	turbid	-	-	peptonization reduction	-	-	+	-	-	-	+	+	-	white brown	30° C.	facultative
47	Pseudomonas sp.	Gram rods	polar	+	+	+	+	-	+	-	turbid	-	-	peptonization reduction	-	-	+	-	-	-	-	+	-	white	30° C.	facultative
49	Pseudomonas sp.	Gram rods	polar	+	+	+	+	-	+	+	turbid	-	-	rennet curd reduction	-	-	+	-	-	-	-	+	-	white	30° C.	facultative
53	Pseudomonas sp.	Gram rods	polar	+	+	+	+	-	+	-	turbid	-	-	rennet curd reduction	-	-	+	-	-	-	+	+	-	white	30° C.	facultative
57	Pseudomonas sp.	Gram rods	polar	+	+	+	(+)	(+)	-	-	turbid	-	-	acid peptonization reduction	+	+	+	-	-	+	+	+	-	white	37° C.	facultative
57	Pseudomonas sp.	Gram rods	polar	+	-	+	+	+	+	-	turbid	-	-	peptonization reduction	-	-	+	-	-	-	-	+	-	white	30° C.	facultative
58	Pseudomonas sp.	Gram rods	polar	+	+	+	+	-	+	-	turbid	-	-	rennet curd peptonization reduction	-	-	+	-	-	-	+	+	-	white brown	30° C.	facultative
61	Pseudomonas sp.	Gram rods	polar	+	+	-	-	-	+	-	turbid	-	-	peptonization reduction	-	-	-	-	-	-	-	+	-	white	30° C.	facultative
61	Pseudomonas sp.	Gram rods	polar	+	+	-	-	-	+	-	turbid	-	-	rennet curd reduction	-	-	+	-	-	-	+	+	+	white	30° C.	facultative
73	Pseudomonas sp.	Gram rods	polar	+	+	+	+	-	+	-	turbid	-	-	rennet curd peptonization	-	-	+	-	-	-	-	+	-	white	30° C.	facultative
87	Pseudomonas sp.	Gram rods	polar	+	+	+	(+)	(+)	-	-	turbid	-	-	no change	+	+	+	-	-	+	+	+	-	white	37° C.	facultative

Enterobacteriaceae

Organism and uterus number	Morphology	Flagella stain	Frazier's gelatin	Litmus milk	Citrate agar	Acetyl methyl carbinol	Methyl red	Nutrient broth	Indole production	NO_3 reduction test	Urea broth	Mannitol	Maltose	Glucose	Galactose	Sucrose	Lactose	15% gelatin medium	H_2S production	Motility test agar	Catalase test	Optimum temperature	Oxygen requirement	Pigment
5 Escherichia coli	Gram - rod	peritrichous	-	acid	-	-	+	turbid	+	+	-	(+)	+	(+)	+	(+)	(+)	-	-	+	+	37° C.	facultative	white
8 E. coli	Gram - rod	peritrichous	-	acid	-	-	+	turbid	+	+	-	(+)	(+)	(+)	(+)	(+)	(+)	-	-	+	+	37° C.	facultative	white
16 Alcaligenes viscolactis	Gram - rod	none	-	alkaline	-	-	-	turbid	-	+	-	-	-	-	-	-	-	-	-	-	+	37° C.	aerobic	white
31 A. metalkaligenes	Gram - rod	none	-	alkaline	-	-	-	turbid	-	+	-	-	-	-	-	-	-	-	-	-	+	25° C.	facultative	white
51 Aerobacter aerogenes	Gram - rod	none	-	acid	+	+	-	turbid	-	+	+	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-	+	37° C.	facultative	white
70 E. coli	Gram - rod	peritrichous	-	acid reduction	-	-	+	turbid	-	+	-	(+)	(+)	(+)	(+)	(+)	(+)	-	-	+	+	37° C.	facultative	white
94 A. faecalis	Gram - rod	peritrichous	-	alkaline	-	-	-	turbid	-	+	-	-	-	-	-	-	-	-	-	+	+	37° C.	facultative	white
109 Proteus vulgaris	Gram - rod	peritrichous	+	alkaline peptonization	-	-	-	turbid	+	+	+	-	+	+	-	+	-	+	+	+	+	37° C.	facultative	white

( ) means gas

Gram ≠ rods

	Organism and uterus number	Morphology	Metachromatic granule stain	Litmus milk	Frazier's gelatin	NO_3 reduction test	H_2S production	Acetyl methyl carbinol	Methyl red	Indole production	Starch agar	Glucose	Lactose	Sucrose	Maltose	Mannitol	Catalase test	Cellulose medium	Potato slant	Optimum temperature	Oxygen relationship	Heat shock	Motility
10	Corynebacterium bovis	Gram ≠ rod	banded	no change	-	-	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-
12	Microbacterium flavum	Gram ≠ rod	banded	alkaline slightly	≠	-	-	-	-	-	-	≠	-	≠	-	-	≠	-	-	37° C.	facultative	-	-
13	Corynebacterium sp.	Gram ≠ rod	banded	peptonization	≠	-	-	-	-	-	-	≠	-	-	-	≠	≠	-	-	37° C.	facultative	-	-
14	C. bovis	Gram ≠ rod	banded	no reaction	-	-	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-
18	M. flavum	Gram ≠ rod	banded	alkaline	-	≠	-	-	-	-	-	≠	-	≠	-	-	≠	-	-	37° C.	facultative	-	-
22	Corynebacterium sp.	Gram ≠ rod	banded	acid	≠	-	-	-	-	-	≠	≠	≠	≠	≠	≠	≠	-	-	37° C.	facultative	-	-
27	Corynebacterium sp.	Gram ≠ rod	banded	growth	-	-	-	-	-	-	-	≠	-	≠	-	-	≠	-	-	37° C.	facultative	-	-
29	C. pyogenes	Gram ≠ rod	banded	coagulation	-	-	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-
30	C. bovis	Gram ≠ rod	banded	no change	≠	-	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-
48	C. pyogenes	Gram ≠ rod	banded	acid coagulation	-	-	-	-	-	-	-	≠	≠	≠	-	-	≠	-	-	37° C.	facultative	-	-
51	Arthrobacter simplex	Gram ≠ rod	banded	peptonization	≠	≠	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	aerobic	-	-
59	M. flavum	Gram ≠ rod	banded	no reduction	-	-	-	-	-	-	-	≠	-	≠	-	-	≠	-	-	37° C.	facultative	-	-
65	C. renale	Gram ≠ rod	banded	acid reduction	-	≠	-	-	-	-	-	≠	≠	≠	-	-	≠	-	white gray	37° C.	facultative	-	-
83	C. bovis	Gram ≠ rod	banded	no reaction	-	-	-	-	-	-	-	-	-	-	≠	-	≠	-	-	37° C.	facultative	-	-
104	C. pyogenes	Gram ≠ rod	banded	acid reduction	≠	-	-	-	-	-	≠	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-
107	C. bovis	Gram ≠ rod	banded	alkaline	-	-	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-
108	C. bovis	Gram ≠ rod	banded	alkaline	-	-	-	-	-	-	-	-	-	-	-	-	≠	-	-	37° C.	facultative	-	-

Miscellaneous

Organism and uterus number	Morphology	Frazier's gelatin	Glucose	Sucrose	Mannitol	Maltose	Salicin	Litmus milk	Oxygen relationship	Optimum temperature
13 Actinomyces bovis	granules, club-shaped hyphae, Gram +	-	+	+	-	+	-	acid	micro-aerophillic	37° C.

Organism and uterus number	Morphology	Cellulose medium	15% gelatin medium	Coagulated albumin broth	Milk tube	Motility test agar	Litmus milk	NO_3 reduction test	Glucose	Fructose	Galactose	Maltose	Lactose	Sucrose	Salicin	Glycerol	Mannitol	Inulin	Hemolysis	Optimum temperature	Oxygen relationship	Spore stain
34 Clostridium sporogenes	Gram + rods, subterminal spores	-	+	liquefaction	digested	+	peptonization	+	(+)	(+)	-	(+)	-	-	-	-	-	-	beta	37° C.	anaerobic	subterminal spores

Organism and uterus number	Morphology	Litmus milk	Glucose	Lactose	Sucrose	Maltose	Frazier's gelatin	Indole production	NO_3 reduction test	Optimum temperature	Catalase test	Oxygen relationship
60 Vibrio fetus	Gram - spirillum to short curved rods	no growth	-	-	-	-	-	-	+	37° C.	+	grows only under 10% Co_2

THE BACTERIAL FLORA OF THE UTERUS OF THE POST-PARTUM COW

by

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The repeat breeder cow has been the focus of many investigations throughout the past years due to the economic importance of these animals. This large economic loss stimulated the initiation of a project at Kansas State University which was designed to investigate the possible role of microorganisms and antigen-antibody reaction in early embryo loss in cattle. Due to the fact that the study was designed to be an intensive long range investigation dealing with the causes of infertility, it was felt that before a program dealing with bacterial infection as a possible cause of embryo loss could be formulated, the bacterial flora of the normal uterus must be known.

With this in mind, a total of 110 post-partum uteri were gathered directly at the slaughterhouse of Armour and Company located at Kansas City, Kansas. It was felt that post-partum cows should give a fairly representative sample of cows with normal uteri. These uteri, which had been dated according to days post-partum, were then cultured, with a wide variety of isolation media, for the presence of bacteria.

It was found that the bacterial flora of the normal post-partum uterus changes greatly with the days post-partum. Bacteria were found to be present in approximately 85 per cent of the samples at 0-15 days post-partum and in only about 10 per cent of the samples at 46-60 days post-partum.

In view of the results obtained from this investigation, it would be reasonable to assume that the largest percentage of the bovine uteri are free from bacteria at 60 days post-partum.

The genera isolated during this study were as follows: Staphylococcus, Micrococcus, Streptococcus, Pseudomonas, Escherichia, Aerobacter, Proteus, Alcaligenes, Corynebacterium, Microbacterium, Arthrobacter, Clostridium, Vibrio, Actinomyces, Aspergillus, and an anaerobe. These isolates were obtained in significant numbers.