The kind and number of bacteria found in colostrum obtained by milking cows before and after parturition
by Thomas F Lofthouse

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Bacteriology
Montana State University


Abstract:
Numerous investigators have shown that bovine colostrum is of great value to the well-being of the new born calf, Previously, studies on colostrum have dealt mainly with the vitamin A and carotene content of this secretion.

This investigation pertains to the number and kind Of bacteria present, in 312. colostrum samples obtained from 15 cows and 12 heifers. These animals were divided into two groups, one being pre- and postpartum milked and the other, being milked only after freshening,. Physical examinations, of the udder were made at the time of milking.

Dilutions of colostrum were plated on blood agar and incubated at 37° C for 18 hours. The results showed that there was considerable daily variation in the bacterial counts from both groups. High bacterial counts from the premilked group were generally accompanied by udder congestion and bloody secretions. The postpartum milked group showed lower counts and no bloody secretions. Two hundred and seventy-nine cultures were isolated and the physiological activities of 89 were studied. Fifty-one strains were placed in the genus Micrococcus and 38 in the genus Streptococcus. Five of the micrococci and one of the streptococci cultures could not be classified to species according to the descriptions in Bergey'S Manual.

Most of the organisms classified have been found previously in normal milk by other investigators.

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ABSTRACT

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This investigation pertains to the number and kind of bacteria present in 312 colostrum samples obtained from 15 cows and 12 heifers. These animals were divided into two groups, one being pre- and postpartum milked and the other, being milked only after freshening. Physical examinations of the udder were made at the time of milking.

Dilutions of colostrum were plated on blood agar and incubated at 37° C for 48 hours. The results showed that there was considerable daily variation in the bacterial counts from both groups. High bacterial counts from the premilked group were generally accompanied by udder congestion and bloody secretions. The postpartum milked group showed lower counts and no bloody secretions.

Two hundred and seventy-nine cultures were isolated and the physiological activities of 89 were studied. Fifty-one strains were placed in the genus Micrococcus and 38 in the genus Streptococcus. Five of the micrococci and one of the streptococci cultures could not be classified to species according to the descriptions in Bergey's Manual.

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# THE KIND AND NUMBER OF BACTERIA FOUND IN COLOSTRUM OBTAINED BY MILKING COWS BEFORE AND AFTER PARTURITION

## INTRODUCTION

The first secretion of the bovine mammary glands before or immediately following parturition of the young is defined as colostrum. This material differs markedly from the later flow of normal milk by having a higher specific gravity, greater percentages of albumin, globulin, and ash, and a lower lactose content. It also differs from normal milk in that it has a viscous consistency and can be coagulated by boiling. In appearance, colostrum varies from a white to a light straw yellow color.

Since colostrum is the first nourishment received by the new born calf and the physiological welfare of the young calf depends entirely upon colostrum, considerable attention has been paid to it in recent years. Animal physiologists have known for some time that colostrum is necessary in the proper nourishment of the calf and that it supposedly contains some antibodies to help protect the young offspring from disease during the first few days of life (Savage and McCay, 1942).

It has been known that colostrum can be obtained from the mammary glands pre- as well as postpartum. With this in mind, research workers have begun to try to find out more about milk production, udder congestion, and the general well being of dairy cows if prepartum milking was practiced. Bacteria have long been known to be present in aseptically drawn normal milk. This led other investigators to believe that bacteria could and do exist in colostrum. In an attempt to find the kind and number of bacteria

and their general effect on the udder condition of the cow, this work was undertaken.

Among the earlier workers to study the physiological effects colostrum has on the calf are Hart and Guilbert (1933) and Guilbert and Hart (1934) who found that livers removed from slaughtered new born calves contained from one twenty-fifth to one one-hundredth of the vitamin A potency of adult cow livers. This condition was found irregardless of the diet of the dam during gestation. Semb et al (1943) likewise showed that the vitamin A content of livers from new born calves was low or practically negligible. Keyes et al (1943), Lundquist and Phillips (1943), Hansen et al (1946), and Allen (1948) agreed that the supplement of vitamin A to the diet of calves improved their physical well being and increased their growth rate. Hansen et al (1946) also noted a decreased death rate of calves when vitamin A was supplemented in their diets consisting of a skim milk basic ration. Calves were found to make more rapid gains, exhibit superior physical appearance, and maintain higher blood plasma levels of vitamin A and carotene when fed colostrum only 3 days (Kaeser and Sutton, 1948). These results agree with those of Allen (1948) who also discovered that calves fed colostrum, which had been frozen for periods up to one year, made better progress when colostrum fed for 10 days than calves which were allowed colostrum only during the period when it was produced by their dams.

In their histological studies of the organs removed from calves on a vitamin A deficient diet, Reed, Huffman, and Addington (1928) found a condition similar to that noted in cotton seed meal injury which is

characterized by a degeneration of epithelium. Optic nerves, kidneys, and liver were most frequently affected by this avitaminosis while the rumen, intestines, adrenals, thyroid, pineal, and thymus were only slightly atrophied if at all. Thorp, Keener, Bechdel, and Guerrant (1942) found a similar pathological condition in dairy calves fed three different suboptimal levels of carotene.

It is evident for the foregoing literature that vitamin A, either artificially added as a dietary supplement or naturally occurring in colostrum, is necessary for the proper growth of calves. In this respect, colostrum assumes a role of great importance.

## HISTORICAL REVIEW

From a study of 6 cows, Copeland and Olson (1926) found that leucocyte counts of colostrum averaged 657,000 per ml. The bacterial counts obtained from individual quarters of the udder varied from 10 to 650,000 per ml and the bacterial counts on the colostrum from the rear quarters were found to be slightly higher than in the front quarters. In additional studies of colostrum from cows of various age groups, these workers observed that cattle past maturity had higher leucocyte and bacterial counts than found in colostrum from younger cattle. An average of 331 bacteria and 420,300 leucocytes per ml appeared in 12 cows under 4 years of age whereas 11 cows over 7 years old gave an average of 8,282 bacteria and 1,559,000 leucocytes per ml of colostrum. Considerable variation in the bacterial numbers was noted from day to day. The highest bacterial content of colostrum was observed one week after parturition.

Ragsdale et al (1929a) found that when pregnant heifers were milked at regular intervals prior to calving, that there was a high death rate among the calves. Bacteria isolated from the viscera of these dead calves indicated a Bacillus coli (Escherichia coli) infection. These authors did not draw any conclusions because they regarded their information as being too limited. An udder secretion resembling colostrum was obtained as early as the fourth month of pregnancy by Ragsdale et al (1929b). This secretion was viscous, yellowish, honey-like, and rapidly became horny upon exposure to air. Analyses showed that when cows were milked daily for a period of 10 days or more prior to parturition, that the chemical composition of the secretion was practically like that of normal milk rather than like colostrum. These reports appear to be the first work on prepartum milking.

Keyes et al (1944) reported the finding of streptococci of all 3 hemolytic types in the bovine udder secretion whether the animal was premilked or not. These organisms, constituting the majority of organisms found, were present in highest concentrations in the first milkings. Leucocyte counts showed a good correlation with the bacterial counts. The streptococci were generally found to decrease in numbers until 4 days after freshening when they could not be detected in most animals. These results were based upon uncentrifuged slide preparations. Microscopic observations were made of the sediment from centrifuged colostrum and displayed the presence of streptococci at all times indicating that they constitute part of the normal flora of colostrum. Injury to the udder or illness of the animal brought about a higher concentration of streptococci in the udder

secretion. According to Keyes (1950), normal milk was produced earlier in the premilked cow than in the non-premilked animal and that after calving, the bacterial counts of colostrum from the premilked cow dropped rapidly. Udder congestion was also relieved by premilking the animal.

Since the literature consulted revealed no detailed study of micro-organisms from bovine colostrum, bacteriological investigations were started in the Fall of 1948.

## PURPOSE OF STUDY

The underlying reasons for undertaking such a problem were to determine the numbers and kinds of bacteria in bovine colostrum and to ascertain whether or not these organisms differed from those previously isolated by other investigators from normal milk.

Analyses for phosphorus and calcium in colostrum as well as carotene, vitamin A, phosphorus, and calcium in the bovine blood were made by the members of the Montana State College chemistry department; the vitamin A and carotene content of colostrum was determined and the condition of the udder and secretion at the time of milking was recorded by the dairy department.

Any correlation of these bacteriological, chemical, and physiological findings is beyond the scope of this paper and will be discussed in other publications.

## MATERIALS AND METHODS

The colostrum studied was from 20 cows and 7 heifers which were divided into two groups, one of which was premilked approximately 7 days

before parturition, and the other, a control group, which was only milked following calving. Bacteriological studies on both groups were started on the first day of milking and continued through the fourth day following the end of gestation, when the secretion was considered to be normal milk.

Drawing the sample:

During the early phases of the study, individual samples of about 4 ml were taken from each quarter of the udder at 5:00 AM and 5:00 PM. Since there was no appreciable variation among the numbers of bacteria found in the different quarters and no great differences between the bacteria present in the morning and evening colostrum, composite samples from the 4 quarters were then employed from the 5:00 PM milking only.

In order to avoid external contamination during sampling, the udder teats, and teat orifices were washed with a tepid diversol solution containing between 100-200 ppm. chlorine. The first few streams of milk were discarded in order to flush out the organisms in the teat canal. This procedure was followed irregardless of the amount present in the udder. In some instances, no colostrum remained after these strippings were removed. The sample was collected in a clean, sterile, screw-cap vial, taken to the laboratory, and plated within one hour.

Plating media employed:

During preliminary work with 2 plating media, it was found that bacto-blood agar base containing 8 percent defibrinated virgin heifer blood supported approximately 10 times as much growth as tryptone-glucose extract agar. These findings are in agreement with those of Roots (1943) who

noted that ox or sheep blood supported more growth than any other medium employed and was especially desirable when only a few bacteria were present in the sample. Therefore, blood agar was used throughout the remainder of the quantitative studies as the plating medium.

Diluting the colostrum:

In order to break clumps of bacteria in the colostrum, the vials were shaken laterally against the hand 25 times. The extreme viscosity of some samples, however, may have prevented thorough mixing even by this procedure. One ml of colostrum was transferred to a sterile 99 ml water blank and shaken 25 times as rapidly as possible in an arc of approximately 10 inches.

When milking was begun on a cow or heifer, dilutions of colostrum ranging from 1:100 to 1:100,000 were made, plated in duplicate, and incubated for 48 hours at 37° C after which time the colonies developing were counted using a Quebec Colony Counter. Representative colonies from petri plates indicating counts over 10,000 to 15,000 per ml were generally picked to tryptose-phosphate broth containing 0.2 percent agar or to cystine-trypticase agar and incubated for 24 hours at 37° C.

Types of organisms found:

Smears were made from the 24 hour growth, stained with Hucker's modification of the gram stain, and the bacterial morphology and gram reaction recorded. The organisms obtained in such a manner from the colostrum were gram positive cocci, the majority of which formed grape-like clusters although some formed chains of variable length. Eighty-nine strains varying in size, shape, and grouping, and resembling members of the genera

Micrococcus and Streptococcus were selected for further study employing a number of physiological tests listed in Bergey's Manual (Breed et al, 1948).

Media employed for the micrococci:

The ability of micrococci to utilize ammonium dihydrogen phosphate and urea as sources of nitrogen was regarded as valuable criteria by Hucker (1924). He recommended the following medium:

| | |
|---|---|
| Agar | 15.0 g |
| $NH_4H_2PO_4$ | 1.0 g |
| KCl | .2 g |
| $MgSO_4$ | .2 g |
| Glucose | 10.0 g |
| Water | 1,000.0 ml |
| Brom cresol purple | |
| pH 7.0 | |

For the preparation of urea agar, 1 g urea was substituted for the $NH_4H_2PO_4$. In addition to these media, nitrate broth, litmus milk, nutrient gelatin, nutrient agar, and mannitol broth were used.

Media employed for the streptococci:

Fermentation of trehalose, sorbitol, mannitol, glycerol, and lactose using cystine-trypticase agar as the basal medium were studied as well as growth at 10° C, 37° C, and 45° C using litmus milk. Tolerance tests were performed using cystine-trypticase agar adjusted to the pH of 9.6 and the same medium prepared with 6.5 NaCl. In addition, 0.1 percent methylene blue in skim milk was used.

Slavin (1948) developed a medium containing sodium hippurate and aesculin for the detection of Streptococcus agalactiae which is one of the few streptococci listed in Bergey's Manual to split sodium hippurate to

glycine and benzoic acid. His formula follows:

| | |
|---|---|
| Difco yeast extract | 0.5 % |
| Difco tryptose-phosphate | 0.5 % |
| Di-potassium phosphate | 0.2 % |
| Glucose | 0.05 % |
| Arginine mono-hydrochloride | 0.3 % |
| Aesculin | 0.1 % |
| Sodium hippurate | 0.1 % |
| pH 7.4 | |

The test reagent for the detection of the splitting of sodium hippurate and aesculin is 12 percent ferric chloride in 2.5 percent HCl. Eight tenths of a milliliter of the reagent was added to one ml of the inoculated and 24 hour incubated broth and examined for a heavy precipitate which would indicate the hydrolysis of sodium hippurate. A dark green coloration would indicate utilization of aesculin.

All test media were inoculated in duplicate from 24 hour cultures and incubated at 37° C for 14 days except the sodium hippurate and nitrate broths. The reactions in these two media were determined after 24 hours growth had occurred. Carbohydrate utilization was determined for the cultures maintained in tryptose-phosphate broth by employing Durham tubes containing nutrient broth with 0.5 percent carbohydrate. The organisms carried on cystine-trypticase agar, a product of the Baltimore Biological Laboratories, were tested on this same type of medium plus carbohydrate. Production of acid in the Durham tubes was determined by adding brom cresol purple.

Table I

Number of organisms found in colostrum and udder condition of control cows examined twice daily

| Days post-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| **Cow 67** | | | |
| 1 | AM | 7,500 | Normal |
| 1 | PM | 6,500 | Slightly caked |
| 2 | AM | < 3,000 | " " - normal colostrum |
| 2 | PM | < 3,000 | " " " " |
| 3 | AM | 5,000 | Normal |
| 3 | PM | < 3,000 | " |
| 4 | AM | < 3,000 | " |
| 4 | PM | < 3,000 | " |
| **Cow 11** | | | |
| 1 | AM | < 3,000 | Udder slightly caked - prepartum milk fever |
| 1 | PM | 3,000 | Udder slightly caked |
| 2 | AM | 20,000 | Cow ill |
| 2 | PM | 13,500 | Milk fever recurred |
| 3 | AM | 48,500 | No caking - normal colostrum |
| 3 | PM | 69,500 | Normal |
| 4 | AM | 4,000 | " |
| 4 | PM | 5,000 | " |
| **Cow 52** | | | |
| 1 | AM | < 3,000 | Normal |
| 1 | PM | 11,500 | " |
| 2 | AM | < 3,000 | Slight swelling - no congestion |
| 2 | PM | < 3,000 | Normal |
| 3 | AM | 4,500 | " |
| 3 | PM | < 3,000 | " |
| 4 | AM | Blood agar contaminated | |
| 4 | PM | " " | " |

Table II

Number of organisms found in colostrum and udder condition of control cows examined twice daily

| Days post-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| **Cow 106** | | | |
| 1 | AM | < 3,000 | Hard spots in all quarters |
| 1 | PM | 3,000 | LR caked badly - others moderate |
| 2 | AM | 5,000 | " " " " " |
| 2 | PM | Blood agar contaminated | |
| **Cow 108** | | | |
| 1 | AM | 197,000 | LF blind - others swollen |
| 1 | PM | 351,000 | " " " " |
| 2 | AM | < 3,000 | " " " " |
| 2 | PM | 8,500 | " " " " |
| 3 | AM | < 3,000 | " " " " |
| 3 | PM | < 3,000 | " " " " |
| 4 | AM | < 3,000 | " " " " |
| 4 | PM | < 3,000 | " " " " |
| **Cow 62** | | | |
| 1 | AM | < 3,000 | Normal |
| 1 | PM | < 3,000 | RF some congestion |
| 2 | AM | 4,000 | " " " |
| 2 | PM | 3,500 | " " " |
| 3 | AM | 5,000 | " " " |
| 3 | PM | 3,500 | RF congestion reducing - slight congestion LF |
| 4 | AM | 7,500 | Congestion reducing |
| 4 | PM | 5,000 | " " |

LR - Left rear quarter
LF - Left front quarter
RF - Right front quarter

Table III

Number of organisms found in colostrum and udder condition of control cows examined twice daily

| Days post-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| Cow 38 | | | |
| 1 | AM | > 300,000 | Normal |
| 1 | PM | > 300,000 | " |
| 2 | AM | 3,000 | Milk fever - normal colostrum |
| 2 | PM | > 300,000 | " " " " |
| 3 | AM | 8,500 | " " " " |
| 3 | PM | < 3,000 | " " " " |
| 4 | AM | 12,500 | " " " " |
| 4 | PM | | " " " " |

Table IV

Number of organisms found in colostrum and udder condition of control cows examined in the evening

| Days post-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| **Cow 55** | | |
| 1 | > 300,000 | Normal |
| 2 | 445,000 | " |
| 3 | 64,000 | " |
| 4 | < 3,000 | " |
| **Cow 138** | | |
| 1 | 3,500 | Some congestion - normal colostrum |
| 2 | 3,000 | RR and LR very congested - LF and RF slightly congested |
| 3 | 3,500 | LF and LR very congested - RF and RR free |
| 4 | 4,500 | LF and LR very congested - RF and RR free |
| **Cow 98** | | |
| 1 | < 3,000 | All quarters congested |
| 2 | < 3,000 | " " " |
| 3 | < 3,000 | LF and LR congested - RF and RR practically free |
| 4 | < 3,000 | LR congested - other quarters free |
| **Cow 140** | | |
| 1 | < 3,000 | Some congestion |
| 2 | < 3,000 | " " |
| 3 | < 3,000 | " " |
| 4 | < 3,000 | " " |

RR - Right rear quarter
LR - Left rear quarter
LF - Left front quarter
RF - Right front quarter

Table V

Number of organisms found in colostrum and udder condition of control cows examined in the evening

| Days post-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| Cow 93 | | |
| 1 | < 3,000 | Swelling but no hardness |
| 2 | < 3,000 | " " " " |
| 3 | < 3,000 | " " " " |
| 4 | 5,500 | Normal |

Table VI

Summary of number of organisms found in colostrum
of control cows following parturition

| Cow No. | Days postpartum 1 AM | 1 PM | 2 AM | 2 PM | 3 AM | 3 PM | 4 AM | 4 PM |
|---|---|---|---|---|---|---|---|---|
| 67 | 7,500 | 6,500 | < 3,000 | < 3,000 | 5,000 | < 3,000 | < 3,000 | < 3,000 |
| 11 | 3,000 | < 3,000 | 20,000 | 13,500 | 48,500 | 69,500 | 4,000 | 5,000 |
| 52 | < 3,000 | 11,500 | < 3,000 | < 3,000 | 4,500 | < 3,000 | * | * |
| 106 | < 3,000 | 3,000 | 5,000 | + | + | + | + | + |
| 108 | 197,000 | 351,000 | < 3,000 | 8,500 | < 3,000 | < 3,000 | < 3,000 | < 3,000 |
| 62 | < 3,000 | < 3,000 | 4,000 | 3,500 | 5,000 | 3,500 | 7,500 | 5,000 |
| 38 | > 300,000 | > 300,000 | 3,000 | > 300,000 | 8,500 | < 3,000 | 12,500 | + |
| 55 | | > 300,000 | | 445,000 | | 64,000 | | < 3,000 |
| 138 | | 3,500 | | 3,000 | | 3,500 | | 4,500 |
| 98 | | < 3,000 | | < 3,000 | | < 3,000 | | < 3,000 |
| 140 | | < 3,000 | | < 3,000 | | < 3,000 | | < 3,000 |
| 93 | | < 3,000 | | < 3,000 | | < 3,000 | | 5,500 |

* Blood agar contaminated
+ No sample
Blank spaces - no sample taken in morning

Table VII

Number of organisms found in colostrum and udder condition of premilked cow 53 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 7 | AM | 8,500 | Normal |
| 7 | PM | 8,000 | " |
| 6 | AM | 24,500 | " |
| 6 | PM | 19,000 | " |
| 5 | AM | > 300,000 | " |
| 5 | PM | > 300,000 | Bloody colostrum from all quarters |
| 4 | AM | No sample | " " " " " |
| 4 | PM | > 300,000 | " " " " " |
| 3 | AM | > 300,000 | " " " " " |
| 3 | PM | > 3,000,000 | Normal |
| 2 | AM | 90,000 | " |
| 2 | PM | 73,000 | " |
| 1 | AM | > 3,000,000 | " |
| 1 | PM | > 3,000,000 | Some caking in all quarters |
| Days post-partum | | | |
| 1 | PM | 16,500 | Some caking in all quarters |
| 2 | AM | 13,000 | " " " " " |
| 2 | PM | 26,000 | Less caking in front quarters |
| 3 | AM | 120,000 | " " " " " |
| 3 | PM | 96,000 | " " " " " |
| 4 | AM | 7,500 | " " " " " |
| 4 | PM | 5,000 | Normal |

Table VIII

Number of organisms found in colostrum and udder condition of premilked cow 104 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 5 | AM | 8,500 | Congestion extending into teats |
| 5 | PM | No sample | " " " " |
| 4 | AM | < 3,000 | " " " " |
| 4 | PM | 8,000 | " " " " |
| 3 | AM | < 3,000 | " " " " |
| 3 | PM | 4,000 | " " " " |
| 2 | AM | 7,000 | " " " " |
| 2 | PM | 5,500 | " " " " |
| 1 | AM | 15,500 | " " " " |
| 1 | PM | 4,000 | Very congested |
| Days post-partum | | | |
| 1 | AM | 28,000 | " " |
| 1 | PM | 5,000 | " " |
| 2 | AM | 70,500 | " " |
| 2 | PM | 20,500 | " " |
| 3 | AM | 19,000 | " " but decreasing |
| 3 | PM | 21,000 | " " " " |
| 4 | AM | 45,500 | Congestion absent from teats |
| 4 | PM | 4,000 | " " " " |

Table IX

Number of organisms found in colostrum and udder condition of premilked cow 56 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 6 | PM | 3,000 | Normal |
| 5 | AM | < 3,000 | " |
| 5 | PM | 4,000 | " |
| 4 | AM | < 3,000 | " |
| 4 | PM | < 3,000 | " |
| 3 | AM | < 3,000 | " |
| 3 | PM | < 3,000 | " |
| 2 | AM | < 3,000 | " |
| 2 | PM | < 3,000 | " |
| 1 | AM | 7,500 | Some swelling - colostrum normal |
| 1 | PM | 5,500 | " " " " |
| **Days post-partum** | | | |
| 1 | AM | < 3,000 | Normal |
| 1 | PM | 8,500 | " |
| 2 | AM | < 3,000 | Udder swelling but not hard |
| 2 | PM | 3,000 | " " " " " |
| 3 | AM | No sample | Some congestion |
| 3 | PM | < 3,000 | " " |
| 4 | AM | < 3,000 | All quarters slightly caked |
| 4 | PM | No sample | " " " " |

Table X

Number of organisms found in colostrum and udder condition of premilked cow 41 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 1 | AM | > 3,000,000 | Severe caking |
| 1 | PM | > 3,000,000 | " " |
| Days post-partum | | | |
| 1 | AM | > 3,000,000 | Caking reduced |
| 1 | PM | > 3,000,000 | Udder hard and full |
| 2 | AM | | " " " " |
| 2 | PM | 201,500 | " " " " |
| 3 | AM | 39,000 | Swelling reduced |
| 3 | PM | > 300,000 | " " |

Table XI

Number of organisms found in colostrum and udder condition of premilked cow 12 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 3 | AM | 300,000 | Badly caked |
| 3 | PM | 26,500 | " " |
| 2 | AM | > 300,000 | " " - colostrum brown |
| 2 | PM | > 300,000 | " " - " bloody |
| 1 | AM | 70,000 | Severely congested - colostrum bloody |
| 1 | PM | 97,000 | Severely congested - colostrum bloody |
| Days post-partum | | | |
| 1 | AM | 582,500 | Badly caked - colostrum bloody |
| 1 | PM | 681,000 | " " - " " |
| 2 | AM | 576,000 | " " - " " |
| 2 | PM | 75,000 | Congestion decreasing - less bloody |
| 3 | AM | 29,000 | Congestion leaving front quarters |
| 3 | PM | 49,000 | Swelling reduced in front quarters |
| 4 | AM | 120,000 | " " " " " |
| 4 | PM | 480,000 | Rear quarters still swollen - colostrum not bloody |

Table XII

Number of organisms found in colostrum and udder condition of premilked cow 54 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 3 | AM | < 3,000 | Udder swollen but not hard - normal colostrum |
| 3 | PM | < 3,000 | Badly congested - normal colostrum |
| 2 | AM | < 3,000 | " " - " " |
| 2 | PM | 3,500 | " " - " " |
| 1 | AM | > 300,000 | RR most congested - " " |
| 1 | PM | < 3,000 | Congestion reduced - " " |
| **Days post-partum** | | | |
| 1 | AM | < 3,000 | Some congestion |
| 1 | PM | 7,000 | LR most congested - others reduced |
| 2 | AM | No sample | " " " - " " |
| 2 | PM | < 3,000 | Congestion reduced |
| 3 | AM | < 3,000 | LR most congested - " " |
| 3 | PM | < 3,000 | LR badly congested - others normal |
| 4 | AM | < 3,000 | " " " - " " |
| 4 | PM | < 3,000 | " " " - " " |

RR - Right rear quarter
LR - Left rear quarter

Table XIII

Number of organisms found in colostrum and udder condition of premilked cow 29 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 7 | AM | < 3,000 | Normal |
| 7 | PM | < 3,000 | " |
| 6 | AM | < 3,000 | " |
| 6 | PM | < 3,000 | " |
| 5 | AM | < 3,000 | " |
| 5 | PM | < 3,000 | " |
| 4 | AM | < 3,000 | " |
| 4 | PM | < 3,000 | " |
| 3 | AM | < 3,000 | " |
| 3 | PM | < 3,000 | " |
| 2 | AM | < 3,000 | Rear quarters slightly swollen |
| 2 | PM | < 3,000 | Swelling decreased in rear quarters |
| 1 | AM | 4,000 | " " " " " |
| 1 | PM | 4,500 | Some swelling - udder filling rapidly - normal colostrum |
| **Days post-partum** | | | |
| 1 | AM | 7,000 | Some swelling - no hardness |
| 1 | PM | 6,000 | Swelling reduced |

Table XIV

Number of organisms found in colostrum and udder condition of premilked cow 35 examined twice daily

| Days pre-partum | Time of day | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|---|
| 6 | AM | 402,000 | Soft, pliable - colostrum bloody |
| 6 | PM | 642,000 | " " - " " |
| 5 | AM | 525,000 | " " - " " |
| 5 | PM | > 300,000 | " " - " " |
| 4 | AM | 169,000 | " " - " " |
| 4 | PM | 117,000 | " " - " " |
| 3 | AM | 1,550,000 | Swelling - " " |
| 3 | PM | 1,340,000 | " - " " RR |
| 2 | AM | 89,000 | Swelling in LF and RF - RR and LR hard |
| 2 | PM | 92,000 | Swelling in LF and RF - LR hard and bloody |
| 1 | AM | 340,000 | Swelling in LF and RF - rear quarters bloody |
| 1 | PM | 355,000 | Swelling in LF and RF - rear quarters bloody |
| Days post-partum | | | |
| 1 | AM | 12,500 | Swelling in LF and RF - rear quarters bloody |
| 1 | PM | 48,000 | Swelling in LF and RF - rear quarters bloody |
| 2 | AM | 36,000 | Rear quarters hard |
| 2 | PM | 60,500 | All quarters hard |
| 3 | AM | 120,000 | " " " - normal colostrum |
| 3 | PM | 70,000 | " " caked - " " |
| 4 | AM | 140,000 | " " " - " " |
| 4 | PM | 54,500 | " " " - " " |

RR - Right rear quarter
LF - Left front quarter
RF - Right front quarter
LR - Left rear quarter

Table XV

Number of organisms found in colostrum and udder condition of premilked cow 131 examined in the evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 16 | 26,500 | Bloody |
| 15 | 300,000 | Not bloody |
| 14 | 41,500 | LR colostrum bloody |
| 13 | 26,500 | RR colostrum bloody |
| 12 | 55,500 | " " " |
| 11 | 125,500 | " " " |
| 10 | 85,500 | " " " |
| 9 | 46,000 | No blood - becoming like normal milk |
| 8 | 69,500 | No blood - becoming like normal milk |
| 7 | 31,500 | No blood - becoming like normal milk |
| 6 | 31,000 | Increased secretion |
| 5 | 4,500 | " " |
| 4 | 3,500 | Bloody colostrum |
| 3 | 13,000 | LR secretion bloody |
| 2 | 26,000 | Udder congested |
| 1 | < 3,000 | Udder filling rapidly and congested |
| **Days post-partum** | | |
| 1 | 27,500 | LR congested - normal colostrum |
| 2 | 8,500 | All quarters congested |
| 3 | 5,000 | " " " |
| 4 | 5,000 | LF, RF, and RR normal - LR congested |

LR - Left rear quarter
RR - Right rear quarter
LF - Left front quarter
RF - Right front quarter

Table XVI

Number of organisms found in colostrum and udder condition of premilked cow 90 examined in the evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 6 | 131,500 | Normal - bloody colostrum |
| 5 | 50,500 | " - " " |
| 4 | 113,500 | " - " " |
| 3 | 29,000 | Swelling - " " - coming to milk |
| 2 | 44,500 | Swelling - no congestion - coming to milk |
| 1 | 28,000 | Swelling - no congestion - slightly bloody |
| **Days post-partum** | | |
| 1 | 51,000 | LR congested - bloody colostrum |
| 2 | 7,000 | RR most congested - bloody colostrum - increased secretion |
| 3 | 7,500 | RR most congested - bloody colostrum |
| 4 | 8,000 | RR most congested - bloody colostrum |

LR - Left rear quarter
RR - Right rear quarter

Table XVII

Number of organisms found in colostrum and udder condition of premilked cow 14 examined in evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 5 | 35,000 | RF congested - bloody colostrum |
| 4 | 85,000 | Swelling - " " |
| 3 | 23,000 | " - " " |
| 2 | 90,500 | " - " " |
| 1 | 54,000 | Udder inflamed - " " |
| Days post-partum | | |
| 1 | 71,000 | " " - hard and caked - bloody colostrum |
| 2* | 60,500 | Udder inflamed - hard and caked - bloody colostrum |
| 3 | 64,000 | Udder inflamed - hard and caked - bloody colostrum |
| 4 | 672,000 | Udder inflamed - hard and caked - bloody colostrum |

* placenta removed by veterinarian
RF - Right front quarter

Table XVIII

Number of organisms found in colostrum and udder condition of premilked cow 23 examined in the evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 17 | 3,000 | Normal |
| 16 | 3,000 | " |
| 15 | No sample | " |
| 14 | 4,000 | " |
| 13 | 3,000 | " |
| 12 | 3,000 | " |
| 11 | 590,000 | " |
| 10 | 4,000 | " |
| 9 | 3,000 | " |
| 8 | 3,000,000 | " |
| 7 | 1,525,000 | RF has lumpy secretion |
| 6 | 29,500 | " " " " |
| 5 | 23,000 | " " " " |
| 4 | 19,000 | " " " " |
| 3 | 11,000 | RF improving |
| 2 | 30,000 | Increased secretion |
| 1 | 67,000 | Normal |
| Days post-partum | | |
| 1 | 59,000 | " |
| 2 | 67,000 | " |
| 3 | 21,500 | " |
| 4 | 7,500 | " |

RF - Right front quarter

Table XIX

Number of organisms found in colostrum and udder condition of premilked cow 134 examined in the evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 7 | < 3,000 | RF very congested |
| 6 | < 3,000 | " " " |
| 5 | < 3,000 | All quarters congested |
| 4 | < 3,000 | " " " |
| 3 | < 3,000 | " " " |
| 2 | < 3,000 | Congestion decreasing |
| 1 | < 3,000 | " " |
| Days post-partum | | |
| 1 | < 3,000 | " " |
| 2 | < 3,000 | " " |
| 3 | < 3,000 | All quarters normal except LR |
| 4 | < 3,000 | " " " " " |

RF - Right front quarter
LR - Left rear quarter

Table XX

Number of organisms found in colostrum and udder condition of premilked cow 60 examined in the evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 6 | * | Normal |
| 5 | 8,000 | " |
| 4 | 12,000 | Udder filling rapidly |
| 3 | 15,000 | " " " |
| 2 | 15,500 | " " " |
| 1 | 18,500 | RF and LR congested |
| **Days post-partum** | | |
| 1 | 4,500 | Udder swollen - LF has hard spot |
| 2 | < 3,000 | Congestion in RF and LR |
| 3 | 6,500 | Congestion gone |
| 4 | 4,000 | RF slightly congested |

*Blood agar contaminated
RF - Right front quarter
LR - Left rear quarter
LF - Left front quarter

Table XXI

Number of organisms found in colostrum and udder condition of premilked cow 91 examined in the evening

| Days pre-partum | No. of organisms per ml | Condition of udder and secretion |
|---|---|---|
| 6 | 21,000 | Normal |
| 5 | 14,000 | " |
| 4 | 7,500 | " - coming to milk |
| 3 | 3,500 | " - " " " |
| 2 | 8,000 | Increased secretion |
| 1 | < 3,000 | " " |
| **Days post-partum** | | |
| 1 | < 3,000 | Udder filling rapidly |
| 2 | 3,500 | Increased normal secretion |
| 3 | 8,500 | Normal |
| 4 | < 3,000 | " |

Table XXII

Number of organisms x $10^3$ from colostrum of premilked cows

| Cow No. | Time | Days prepartum 7 | 6 | 5 | 4 | 3 | 2 | Days postpartum 1 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | AM | 8.5 | 24.5 | > 300.0 | | > 300.0 | 90.0 | > 3000.0 | | 13.0 | 120.0 | 7.5 |
| | PM | 8.0 | 19.0 | > 300.0 | > 300.0 | > 3000.0 | 73.5 | > 3000.0 | 16.5 | 26.0 | 96.0 | 5.0 |
| 104 | AM | * | | 8.5 | < 3.0 | < 3.0 | 7.0 | 15.5 | 28.0 | 70.5 | 19.0 | 45.5 |
| | PM | * | | | 8.0 | 4.0 | 5.5 | 4.0 | 5.0 | 20.5 | 21.0 | 4.0 |
| 56 | AM | * | | < 3.0 | < 3.0 | < 3.0 | < 3.0 | 7.5 | < 3.0 | < 3.0 | | < 3.0 |
| | PM | * | 3.0 | 4.0 | < 3.0 | < 3.0 | < 3.0 | 5.5 | 8.5 | 3.0 | < 3.0 | |
| 41 | AM | * | | | | | | > 3000.0 | > 3000.0 | | 39.0 | |
| | PM | * | | | | | | > 3000.0 | > 3000.0 | 201.5 | > 300.0 | |
| 12 | AM | * | | | | 300.0 | > 300.0 | 70.0 | 582.5 | 576.0 | 29.0 | 120.0 |
| | PM | * | | | | 26.5 | > 300.0 | 97.0 | 681.0 | 75.0 | 49.0 | 480.0 |
| 54 | AM | * | | | | < 3.0 | < 3.0 | > 300.0 | < 3.0 | | < 3.0 | < 3.0 |
| | PM | * | | | | < 3.0 | 3.5 | < 3.0 | 7.0 | < 3.0 | < 3.0 | < 3.0 |
| 29 | AM | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | 4.0 | 7.0 | | | |
| | PM | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | 4.5 | 6.0 | | | |
| 35 | AM | * | 402.0 | 525.0 | 169.0 | 1550.0 | 89.0 | 340.0 | 12.5 | | | |
| | PM | * | 642.0 | > 300.0 | 117.0 | 1340.0 | 92.0 | 255.0 | 48.0 | 60.5 | 70.0 | 54.5 |
| 131 | PM | 31.5 | 31.0 | 4.5 | 3.5 | 13.0 | 26.5 | < 3.0 | 27.5 | 8.5 | 5.0 | 5.0 |
| 90 | PM | * | 131.5 | 50.5 | 113.5 | 29.0 | 44.5 | 28.0 | 51.0 | 7.0 | 7.5 | 8.0 |
| 14 | PM | * | | 35.0 | 85.0 | 23.0 | 90.5 | 54.0 | 71.0 | 60.5 | 64.0 | 672.0 |
| 23 | PM | 1525.0 | 29.5 | 23.0 | 19.0 | 11.0 | 30.0 | 67.0 | 59.0 | 67.0 | 21.5 | 7.5 |
| 134 | PM | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 | < 3.0 |
| 60 | PM | * | + | 8.0 | 12.0 | 15.5 | 15.5 | 18.5 | 4.5 | < 3.0 | 6.5 | 4.0 |
| 91 | PM | * | 21.0 | 14.0 | 7.5 | 3.5 | 8.0 | < 3.0 | < 3.0 | 3.5 | 3.5 | < 3.0 |

* Cow freshened prior to scheduled date

\+ Blood agar contaminated

Table XXIII

Physiological reactions of the micrococci isolated from colostrum

| Culture number | Ammonium di-hydrogen phosphate | Urea | Pig-ment | Nitrate reduction | Litmus milk | Gelatin lique-faction | Mann-itol | Classified as |
|---|---|---|---|---|---|---|---|---|
| 35-2 | - | - | LO | + | A | - | + | M. aurantiacus |
| 35-6 | - | - | LO | + | A | - | + | " " |
| 35-7 | - | - | LO | + | A | - | + | " " |
| 35-11 | - | - | W | - | A | + | - | M. candidus |
| 35-23 | - | - | W | - | A | + | - | " " |
| 35-25 | - | - | W | - | A | + | - | " " |
| 35-30 | - | - | W | - | A | + | - | " " |
| 35-33 | - | - | W | - | A | + | - | " " |
| 35-37 | - | - | W | - | A | + | - | " " |
| 35-39 | + | + | W | - | B | + | + | M. ureae |
| 38-1 | + | - | LY | + | ARCW | + | + | * |
| 38-3 | + | + | LY | + | ARCW | + | + | * |
| 41-1 | - | - | LY | + | A | - | + | M. aurantiacus |
| 41-7 | - | - | LY | + | A | + | + | " " |
| 53-1 | - | - | Y | + | ARC | + | + | M. citreus |
| 53-5 | - | - | Y | + | A | + | + | " " |
| 53-8 | - | - | Y | + | AC | + | + | " " |
| 53-9 | - | - | Y | + | A | + | + | " " |
| 53-10 | - | - | Y | + | ARC | + | + | " " |
| 53-20 | + | - | Y | - | A | - | + | M. luteus |

C = cream color, LY = light yellow, Y = yellow, LO = light orange, W = white
A = acid, R = reduction, C = curd, W = whey, P = proteolysis
* Description does not fit any recognized species in Bergey's Manual

Table XXIII contd.

Physiological reactions of the micrococci isolated from colostrum

| Culture number | Ammonium di-hydrogen phosphate | Urea | Pig-ment | Nitrate reduction | Litmus milk | Gelatin lique-faction | Mann-itol | Classified as |
|---|---|---|---|---|---|---|---|---|
| 55-1 | - | - | Y | + | ARC | - | + | M. aurantiacus |
| 55-3 | - | + | C | + | A | - | + | " |
| 55-4 | - | + | C | + | A | - | + | " |
| 55-5 | + | - | C | + | A | + | + | M. conglomeratus |
| 55-6 | - | + | C | + | A | - | + | M. aurantiacus |
| 55-7 | - | - | C | + | A | - | + | " |
| 55-10 | - | - | C | + | A | - | + | " |
| 90-1 | - | - | C | + | ARC | + | + | M. pyogenes var. aureus |
| 90-2 | - | - | C | + | ARC | - | + | M. aurantiacus |
| 90-5 | - | + | C | + | ARCW | - | + | " |
| 90-7 | + | - | Y | + | ARCP | + | - | M. caseolyticus |
| 90-9 | + | - | Y | + | ARCP | + | - | " |
| 90-12 | + | + | C | + | A | - | + | M. varians |
| 90-14 | - | + | C | + | ARC | - | + | * |
| 90-15 | - | - | C | + | ARC | - | + | * |
| 90-16 | - | + | C | + | ARC | - | + | * |
| 91-1 | + | - | YO | + | ARCP | + | - | M. caseolyticus |
| 91-2 | - | - | W | + | ARC | - | - | M. epidermidis |
| 91-3 | - | - | Y | + | A | + | - | M. aurantiacus |
| 91-4 | + | - | YO | + | ARCW | + | - | " |
| 91-5 | - | - | C | + | A | + | - | " |

C = cream color, LY = light yellow, Y = yellow, LO = light orange, W = white
A = acid, R = reduction, C = curd, W = whey, P = proteolysis
* Description does not fit any recognized species in Bergey's Manual

Table XXIII contd.

Physiological reactions of the micrococci isolated from colostrum

| Culture number | Ammonium dihydrogen phosphate | Urea | Pigment | Nitrate reduction | Litmus milk | Gelatin liquefaction | Mannitol | Classified as |
|---|---|---|---|---|---|---|---|---|
| 108-1 | - | - | W | + | A | - | - | M. epidermidis |
| 108-2 | - | - | W | + | A | - | - | " " |
| 108-3 | - | - | W | + | A | - | - | " " |
| 131-5 | + | - | Y | + | ARCP | + | - | M. caseolyticus |
| 131-10 | - | - | Y | + | A | - | - | M. aurantiacus |
| 131-11 | - | - | Y | + | A | - | - | " " |
| 131-12 | - | - | Y | + | ARC | - | - | " " |
| 131-19 | + | - | Y | + | ARCP | + | - | M. caseolyticus |
| 131-22 | + | + | Y&W | + | ARCP | + | - | " " |
| 131-29 | - | - | Y | + | ARC | - | - | M. aurantiacus |

C = cream color, LY = light yellow, Y = yellow, LO = light orange, W = white
A = acid, R = reduction, C = curd, W = whey, P = proteolysis
* Description does not fit any recognized species in Bergey's Manual

Table XXIV

Physiological reactions of the streptococci isolated from colostrum

| Culture number | Treh-alose | Glyc-erol | Sorb-itol | Mann-itol | Lit-mus milk | Sodium hippurate | Lac-tose | Methyl-ene blue (.1%) | 10°C | 45°C | pH 9.6 | 6.5% NaCl | Classified as |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 60-3 | + | - | - | - | AC | - | + | - | - | - | - | - | S. pyogenes |
| 60-4 | + | - | - | - | AC | - | + | - | - | - | - | - | S. pyogenes |
| 60-10 | + | - | - | - | AC | - | + | - | - | - | - | - | S. pyogenes |
| 60-11 | - | - | - | - | AC | - | + | - | - | - | - | - | S. bovis |
| 60-18 | + | - | - | - | AC | - | + | - | - | - | - | - | S. pyogenes |
| 60-26 | - | - | - | - | AC | - | + | - | - | - | - | - | S. bovis |
| 23-13 | + | - | - | - | AR | - | + | - | - | - | - | - | S. pyogenes |
| 23-19 | + | - | - | - | ARC | - | + | - | - | - | - | - | S. pyogenes |
| 23-22 | - | - | - | - | ARC | - | + | - | - | - | - | - | S. bovis |
| 23-26 | + | - | + | - | AR | - | + | + | - | - | - | - | * |
| 23-27 | + | - | - | - | ARC | - | + | - | - | - | - | - | S. pyogenes |
| 14-1 through 14-27 | + | - | - | - | ARC | + | + | - | - | - | - | - | S. agalactiae |

A = acid, R = reduction, C = coagulated
* Description does not fit any recognized species in Bergey's Manual

## RESULTS

The numbers of organisms counted by plating colostrum from 12 control cows and the condition of their udders and secretions are shown in tables I through V. Table VI gives a summary of the quantitative data obtained in this phase of the work. It will be noted that the number of bacteria from 8 cows ranged from less than 3,000 to 11,500 per ml of colostrum. However, only 2 of these 8 cows appeared to have normal udders and secretions when examined at the time of milking. In the case of the other 4 control cows, the counts ranged from less than 3,000 to 445,000 organisms per ml and only one udder (cow 55) was considered normal. This would tend to indicate very little correlation between the number of organisms and the physical condition of the udder and secretion.

In 11 of the 15 premilked cows studied (tables VII through XXI), it was possible to correlate high bacterial counts (over 10,000 to 15,000 per ml) with abnormal udder conditions including caking or congestion, and sometimes bloody secretion. One cow (number 91), had one count over 15,000 but the udder remained normal. Two of the 15 animals had normal udders and secretions as well as low bacterial counts ranging from less than 3,000 to 8,500 per ml. In cow 134, less than 3,000 bacteria were observed during the test period although there was marked congestion throughout the first 5 days of milking which gradually decreased thereafter.

The number of bacteria from premilked animals before and after parturition is shown in table XXII. In a number of instances, it will be noted that the cow freshened before the scheduled date, thus limiting the

number of samples studied.

The number of organisms from cows 53, 12, and 23 generally decreased during the days following calving. In some cases, (cows 131 and 90), this decrease was observed before parturition of the calf whereas with cows 35 and 14 the counts increased after calving. Cows 56, 41, 54, 134, 60, and 91 show a rather constant count and the data from cows 29 and 104 are inconclusive.

In tables XXIII and XXIV the physiological reactions of the 89 cultures selected for taxonomic study are given. The majority of bacteria classified are reported in Bergey's Manual to have been isolated from normal milk or dairy products. A summary of the number of cultures isolated, the names of the bacteria, and their habitats follow:

| Number of cultures | Name of bacteria | Habitat |
|---|---|---|
| 27 | S. agalactiae | bovine udders |
| 21 | M. aurantiacus | milk, cheese, dust |
| 7 | S. pyogenes | bovine udders |
| 6 | M. candidus | milk, dairy products |
| 5 | M. caseolyticus | " " " |
| 5 | M. citreus | skin, mucous membranes of vertebrates |
| 4 | M. epidermidis | Skin, mucous membranes |
| 3 | M. luteus | skim milk, dairy products and dust |
| 1 | M. varians | body secretions, dairy products and utensils, dust and water |
| 1 | M. pyogenes var. aureus | skin and mucous membranes |
| 1 | M. ureae | stale urine and soil containing urine |

| Number of cultures | Name of bacteria | Habitat |
|---|---|---|
| 1 | M. conglomeratus | infections, milk, dairy products and utensils, water |
| 6 | unclassified | |
| 89 | | |

It is evident from a survey of the above summary that all of the bacteria named could have possible origin either in the colostrum, mucous membranes which line the teat canal, or from the straw bedding on which urine had been excreted.

In some instances, organisms bearing the same name (table XXIII) differ slightly. This may be explainable on the basis that the authors of Bergey's Manual have "lumped" many of the micrococci together. Also some allowance has been made for strain variations. Hence, 14 days may not have been long enough to incubate the Micrococcus epidermidis cultures 108-1, 2, and 3 (table XXIII) since these cultures liquefy gelatin slowly according to Bergey's Manual.

The predominating colonies isolated from colostrum of cow 14 were beta hemolytic streptococci. Cultures of these colonies 14-1 through 27 (table XXIV) reacted similarly in all tests performed and are listed inclusively. Inflammation, congestion, and a bloody secretion marked the condition of the udder and secretion of this animal. This was the only cow in which inflammation was observed in the udder and from which Streptococcus agalactiae was isolated.

## DISCUSSION

At present there appears to be no accurate explanation for the association of udder congestion or caking and bloody secretions with high bacterial counts made on the colostrum. Various degrees of congestions appeared quite frequently in the udders of the cows studied which would indicate that generally, this condition is not too serious. One recommended treatment for udder congestion is to prevent the animal from being on full feed. When the animal is not on a full ration, her milk production falls below normal and for this reason, congestion is of great concern to the dairyman.

There now appears 3 possible reasons for the daily variation in the bacterial counts and for the post parturition decrease in the number of bacteria found. Certain investigators have found a bacteriostatic substance in the whey fraction of normal milk which they have called lactenin. Among the early workers to study lactenin were Jones and Little in 1927 (Foley and Lee, 1948), who showed that Streptococcus agalactiae becomes adapted to lactenin and grows at an uninterrupted logarithmic rate when transferred to fresh milk which has a strong inhibitory activity against unadapted cultures of this organism. Jones and Simms (1930) studied the inhibitory action of lactenin against in vitro cultures of the streptococcus causing scarlet fever. These investigators demonstrated that alcohol and ammonium sulfate precipitations of lactenin inactivated the lactenic property. Dialyzed whey showed no appreciable lactenic activity over that of the untreated whey control. In addition to reducing the hemolytic

zones of Streptococcus scarlatiniae (Streptococcus pyogenes), lactenin was found to be stable for 1½ hours at the pH of 4 and 10. In demonstrating the modus operandi of lactenin against the various cultures of bacteria, Foley and Lee (1948) noted that lactenin is not effective against Micrococcus pyogenes var. aureus, moderately so against S. agalactiae, and relatively inactive against Streptococcus faecalis. If lactenin is also present in colostrum, there may exist an explanation for the post parturition decrease of the bacteria present in this secretion and for the daily variation noted in the bacterial counts. However, literature was not found regarding daily variation of lactenic activity before or after freshening.

Since colostrum has a higher globulin content than normal milk and since Boyd (1947) states that antibodies are known to be of a modified globulin composition, one can then understand that colostrum is richer in antibodies than normal milk. If the organisms in the colostrum are antigenic, an antigen-antibody reaction could occur in the colostrum and cause a clumping (agglutinin) or lysing (lysin) of the bacteria provided that serum complement is present in the lytic reaction.

From the data recorded on the physiological condition of the udder, it was noted that the milker had observed a marked increase in secretion starting approximately 24 hours before calving and this increase continued during the following 5 days. The bacterial decrease then, could be brought about by the diluting action of the increase in secretion provided that the numbers of bacteria remained relatively stable. Certainly other conditions must prevail when one attempts to explain the intermittent bacterial

increases noted after calving in cows 106, 38, 53, 104, 41, 12, 35, and 14. Other conditions must enter in when one attempts to explain why the bacterial counts decreased prepartum where dilution was of no concern.

## SUMMARY

A comprehensive bacteriological study has been made of 312 bovine colostrum samples. The number of bacteria in these samples was determined using blood agar and physical examinations of the udder were made at the time of milking.

The literature cited indicates the importance of colostrum to the nutrition and well being of the new born calf.

Colostrum was studied from 15 cows milked pre- and postpartum and from 12 cows milked postpartum only. Results show that there was considerable daily variation in the bacterial counts which ranged from less than 3,000 to over 3,000,000 per ml of colostrum in the premilked cows and from less than 3,000 to 445,000 in the control (postpartum milked) cows.

Congestion in the udder generally occurred simultaneously with high bacterial counts in the premilked cows. Congestion occurred so frequently that the author was led to believe that this condition was not too serious from the cow's standpoint, but to the producer of milk, congestion would be of great importance.

Good correlation was not observed between the counts and abnormal udders in the case of the control cattle.

Bloody secretions were generally closely associated with the high bacterial counts in the premilked cows. In the control group, bloody

colostrum was not observed.

The bacteria found in the colostrum of 3 premilked animals showed a marked decrease after parturition; 2 others showed this decrease before freshening. In 2 cows, the bacteria in the colostrum increased after calving while in 6 other animals, the counts remained rather constant. The bacteriological data from the other 2 cows were inconclusive.

Eighty-nine representative cultures were selected from 279 isolates and their physiological reactions studied. Fifty-one were placed in the genus Micrococcus and 38 in the genus Streptococcus and classified to species. Five of the micrococci and 1 of the streptococci were unclassified as to species.

Most of the organisms classified have been found previously in normal milk by other investigators.

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