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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THOMAS F. LOFTHOUSE

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Submitted to the Graduate Faculty

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partial fulfillment of the requirements

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Master of Science in Bacteriology

at

Montana State College

Approved:

Head, Major Department

Chairman, Examining Committee

Dean, Graduate Division

Bozeman, Montana
June, 1950

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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.

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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