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The Microflora of Milk Drawn Aseptically from the U.S.A.C. Dairy Herd

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THE MICROFLORA OF MILK DRAWN ASEPTICALLY FROM THE U. S. A. C. DAIRY HERD

A

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INTRODUCTION

Early studies on the bacterial content of milk were made mainly to satisfy the interest of people who wished to determine the various materials that contained bacteris. Soon the value of bacterial counts, as an indication of the general conditions of production, of handling, and of the keeping qualities of milk, became evident and bacterial counts were used to obtain information concerning these problems. Numbers of bacteria in milk have been used also in the studies of the desirable and undesirable changes in milk.

In the last few years our citizenry has been made more conscious of the presence and importance of bacteria in milk. As the number of milk dealers have increased and our population in the cities has become more conjested, more stringent regulation of our milk supplies has been practiced. Of major importance in this regulatory program is the bacterial count of milk. Large dairy manufacturing plants, which have also recognized the importance of high bacterial counts in influencing the quality of their products, have encouraged production of low count milk even to the extent of giving bonuses to such producers and rejecting milk that did not come within their standards.

It is a well recognized fact that the producer may control the bacterial content of milk which results from external contamination, but what about the contamination coming from the interior of the udder? Not as much attention has been paid to the latter problem as to the former. From the standpoint of ordinary market milk from healthy cows, the intermal contamination does not assume much importance but to the producer of low count milk, and especially certified milk with its common standard of 10,000 bacteria per cc., this problem of interior contamination from the udder may become of major importance.

Some cows with apparently normal udders have been known to consistently give milk of high bacterial content. Some of these examples may be noted by studying cases quoted in the historical section of this thesis (14). In some instances the failure of certified milk to come up to the bacteriological requirements has been due to a few cows giving exceptionally high count milk. The exclusion of this milk has immediately brought the number of organisms within the required limits (11).*

In this investigation a study has been made of the normal number of bacteria in milk aseptically drawn from apparently normal cows of the Utah State Agricultural College dairy herd. In other words, in this

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herd what is the count of bacteria that is unavoidable, even with the greatest care in milking? Does the number of bacteria in the udders vary from month to month? Does the number between cows vary and is there a significant variation between quarters? Are there certain cows that consistently produce milk of low count? From this information it should be possible to predict the quality of milk, high or low count, the cow will produce. What types of bacteria occur in the udder? Is this flora constant or does it vary?

REVIEW OF LITERATURE

In 1877, Lister proposed the theory that milk within the udder was germ free. This theory, believed until about 1890, was based upon a single observation that two sealed samples kept for six weeks were of normal taste and reaction. In 1891, Schultz disproved this theory and in 1897 Ward (18) showed that bacteria were uniformly present in the udder. Bergey (4) reports that 32% of the 272 samples of milk drawn into sterile tubes contained no bacteria in 1cc.; 48% less than 500 bacteria per cc., and only 10.3% more than 5000 per cc. Hastings and Hoffman (14) made a study of two cows that regularly gave unusually large numbers of bacteria. Sixty one samples from one cow covering parts of two lactation periods, were taken with a minimum of 1700, and a maximum of 305,000, and an average of 30,700 bacteria per cc. From another cow, thirty one samples were taken and a minimum of 2,500, a maximum of 154,000 and an average of 38,800 bacteria per cc. were obtained.

An early important contribution to this subject of the microflora of healthy udders was carried on by Harding and Wilson (12). They isolated and identified a number of organisms but the organisms isolated are somewhat indefinite due to a later classification by Hucker(15).

Alice Evans (10) made an extensive study which included a qualitative as well as quantitative determination. She found micrococci in 58% of the samples taken. A rod which she named Bacterium lipolyticum was isolated and identified. Buchanan (6) states that "Very rarely do the number of bacteria amount to more than 100 per cc. Animals with more are usually suffering from some udder infection." Alice F. Breed (7), using Hucker's classification, identified 171 of 176 cultures of micrococci isolated from carefully drawn strippings milk. The following organisms were identified: M. aurantiacus, M. freudenreichii, M. albus, M. candidus, M. epidermidis, M. citreus, M. varians, M. flavus, M. congloneratus, and M. luteus.

As a result of their investigation, Copeland and Olson(8) state that bacterial and cell count compare quite closely. They found that lactation had no appreciable effect on the bacterial content of the udder.

A comparison of the Burri slant method and the plate method was made by W. Dorner (9). Results showed the lowest herd count obtained on Burri slants was 3,965 per cc., while the highest was 9,635 per cc., and the average count was 7,475. On standard agar plates the average counts from the individual herds varied between 530 and 4,390 per cc., the final calculated average being 2,775 per cc.

Information from the literature concerning number and kind is summarized in the table on the following page.

Table I

Author	Ave. count per cc.	No. of Cows	Percentage cocci
Schulz 1892	2330		
Russell 1894	330		
Marshall 1900	295		
Von Freudenreich 1902	295		Nearly 100%
Lux 1903	1,391	10	90 to 95%
Esten and Mason 1908			95%
Atwood and Giddings 1911	35	6	
Harding and Wilson 1913	428	78	75%
Evans 1916		161	58.8%
Burri and Hohl 1917		16	82.5%
Copeland and Olson 1926	1,546	40	
Alice F. Breed 1928	964	12	
W. Dorner 1930	7,475	132	89.3%

TECHNIQUE

The samples were collected from 10 cows in the Utah State Agricultural College dairy herd during the months of January, February, March, and April, 1937. Each cow was tested monthly. Cows selected for this experiment had never shown symptoms of infectious mastitis and recent tests showed them to be free from contagious abortion and bovine tuberculosis. Only cows giving negative tests to the brom thymol blue, Hotis, and direct microscopic tests were selected for this experiment.

Cows were carefully cleaned by washing the udder and surrounding parts with soap solution and then washing the quarters with a mercuric chloride solution (1-1000). The udder was dried with a clean dry towel.

Hands were also washed with soap and water and rinsed with the mercuric chloride solution. They were dried with a clean dry towel.

Samples were collected from the afternoon strippings milk.* Milking was done by hand into sterile test tubes, one tube being used for each quarter. The sample contained several streams of milk. Tubes were held open

*Harding and Wilson (12) found that, "Bacteria are most abundant in the first few streams or foremilk; are distinctly less abundant during the main portion of the milking, and again become more abundant in the strippings. Due to this relationship, a fairly close approximation of the germ content of the entire flow of milk can be obtained from the strippings milk."

just long enough to collect the samples and were held in a position that would expose them to a minimum amount of contamination.

The samples were taken to the laboratory and plated within two hours upon a modified form of A. P. H. A. standard agar. The modification consisted of the addition of 3 grams of glucose per liter (13), (19). The reaction was adjusted to pH 6.8-6.9 so as to approximate the normal reaction of milk. Dilutions were made so as to approximate 20 to 300 organisms on the plates. After preliminary tests, it was possible to determine the dilutions to use. In subsequent samples, if the numbers of bacteria fell outside these ranges, an adjustment of the dilution was made the succeeding month. Dilutions were made as follows: 1-10 by using 5cc. milk and 45cc. of water; 1-5 by using 5cc. of milk and 20 cc. of water; and 1-2 by using 10 cc. of milk and 10 cc. of water. Six replicates and one check were made of the sample from each quarter of the udder. Every bacterial colony occurring on the plates was counted with the aid of a hand lens, and an average of the six plates was taken. The plates were incubated for 5 days at 30° C. and an additional 2 days at 37° C.*

Counts were also made of the hemolytic organisms,

*Several investigators found that 37° C. was not the optimum temperature for many of the organisms of milk. (3), (16), (19).

using the same dilutions as used for the plate counts. Three plates and one check were made on each quarter of the udder. The medium used was Bacto Blood Agar Base Dehydrated, to which was added 5% sterile defibrinated blood. This medium was used immediately after it was made and sterilized. The blood used was collected with a sterile cannula, from animals killed at the local slaughter house. After slitting the skin the cannula was inserted into the jugular vein and the blood collected into a sterile container which contained glass beads to facilitate defibrination. Rapid shaking of the blood for several minutes defibrinated it. This blood was first plated and incubated to insure its sterility before using.

NUMBERS OF BACTERIA PRESENT

Probably the most apparent thing to be noted from the standard agar plate counts is their variability when the counts from one cow are compared with those of another. This is not surprising, considering the wide variation that occurs between individual cows and quarters. Variation in the numbers occurring in the same quarter of the udder of the same cow at different months is of interest. The variation in the types of bacteria from month to month between cows as well as their quarters is not quite so evident and recourse to Analysis of Variance as a tool to determine the variance between the different factors was made in order to determine its significance.

Examination of Table II indicates that there are certain cows that consistantly produce milk of low count. During the period of this experiment cows Nos. 2, 5, 7, and 9 were typical examples of such producers, while Nos. 1, 3, 8, and 10 rather consistently produced milk of high count. In Nos. 1 and 3, this higher count may be attributed to an abnormally high count in the right front quarter, and in the right rear quarter in the case of No. 8. One quarter of the udder may give a high count and the other may be quite low. Cows were noted that give a high count in one or two quarters and in succeeding months gradually decreased in number while others gained from month to month.

The average number of bacteria for all cows occurring per cc. in this investigation was 239. This number of bacteria may appear rather low when compared with the average results of other workers. A probable explanation for this is that special care in this experiment was exercised in obtaining animals free from udder infection. It is interesting to note that in this experiment the right front quarter gave the highest average count with the right rear quarter next in number. Fewest numbers were obtained from the left rear quarter.*

*Other workers have failed to substantiate these

An examination of Table II readily discloses a high variance in the number of bacteria occurring between cows. An example of this may be found in the comparison of the total average of 511 for cow No. 1 with 49 for cow No. 5 or 87 for cow No. 2. The high significance of this variance may be seen in Table III. This leads to the conclusion that there is a great deal of difference between the number of bacteria in milk produced by different cows.

Factors such as physiological differences make the entrance of bacteria into the udder more easy. Environmental condition would not be identical nor would the same general condition of good health exist among the cows.

By examining Table III a significant variance compared with the error variance is found for the months, i.e., the number of bacteria produced by all the cows is different for each month. It will be noted that the variance between months is not nearly so significant, compared with the error variance, as the variance between cows.

A surprisingly high variance is noted between quarters. From this it is evident that the numbers occurr-

results although Atwood and Giddings (1) found the bacterial content of the milk from the front quarters to be higher per cc. than that from the hind quarters.

Harding and Wilson (12), however, reported about three times as many bacteria per cc. in hind quarters as in that from the front quarters.

Table II

Showing Average Number of Colonies per cc. for Cows and Quarters. (Each figure average of 4 months)

Cows	Left Front	Left Rear	Right Front	Right Rear	Average
1	327	260	1171	285	511
2	25	14	279	31	87
3	467	260	941	210	470
4	149	174	304	116	123
5	76	51	36	33	49
6	134	59	236	37	117
7	126	107	38	138	102
8	156	199	237	800	348
9	114	158	118	80	117
10	521	292	292	513	404
Ave.	210	157	365	224	239

	5%	1%
Significant Difference between Quarters	19.34	25.66

Table III

The Analysis of Variance of the Number of Colonies per cc. for Cows, Months, and Quarters.

Source of Variance	DF	Sum of Sq.	Variance	F	5%	1%
Total	159	15,556,379.4				
Cows	9	4,439,261.65	492,251.3	260.94	2.06	2.74
Months	3	92,720	30,906.67	16.36	2.72	4.04
Quarters	3	947,657.3	315,885.5	167.26	2.72	4.04
Interactions						
Cow and Month	27	5,022,455.75	186,016.88	98.49	1.65	2.03
Cow and Quarter	27	4,444,741.95	164,620.07	87.16	1.65	2.03
Month and Quarter	9	465,568.1	51,729.8	27.39	2.06	2.74
Cow, Month and Quarter	81	152,974.65	1,888.57			

Table IV

Showing Average Number of Colonies per cc. for ~~Cows~~ quarters and Months. (Each figure average 10 cows)

Months	LF	LR	RF	RR	Average
1	283	191	327	289	272
2	256	143	250	233	221
3	113	155	402	178	212
4	187	140	482	196	252
Average	210	157	365	224	239

Table V

Showing Average Number of Colonies per cc. for Cows and Months. (Each figure average of 4 quarters)

Cows	1st. Month	2nd. Month	3rd. Month	4th. Month	Average
1	508	180	485	871	511
2	92	22	0	234	87
3	285	478	575	541	470
4	121	281	140	165	123
5	79	13	88	17	49
6	225	98	28	116	117
7	233	47	67	51	102
8	471	478	258	186	348
9	200	116	66	86	117
10	507	485	379	246	404
Ave.	272	221	212	252	239

Table VI

Showing Number of Colonies per cc. Developing from Different Quarters at Different Dates.

	First Month					Second Month				
Cows	LF	LR	RF	RR	Ave.	LF	LR	RF	RR	Ave.
1	415	380	1050	185	508	180	80	370	90	180
2	0	0	370	0	92	30	25	21	12	22
3	500	170	320	150	285	700	375	695	140	478
4	122	120	50	192	121	215	105	740	65	281
5	90	75	40	110	79	30	20	0	0	13
6	230	70	550	50	225	225	35	130	0	98
7	380	330	60	160	233	40	50	40	100	47
8	220	135	130	1400	471	125	320	295	1170	478
9	135	350	135	180	200	110	140	125	90	116
10	735	275	560	460	507	905	282	87	665	485
Ave.	283	191	327	289	272	256	143	250	233	221

	5%	1%
Significant Difference between Months	19.34	25.66
Significant Difference between Cows	30.59	40.55

Table VI (Continued)

	Third Month					Fourth Month					
Cows	LF	LR	RF	RR	Ave.	LF	LR	RF	RR	Ave.	Tot. Ave.
1	122	100	1680	36	485	590	480	1585	830	871	511
2	0	0	0	0	0	70	32	725	110	234	87
3	220	270	1550	260	575	450	225	1200	290	541	470
4	120	340	105	135	140	140	130	320	70	165	123
5	150	90	90	20	88	36	20	12	0	17	49
6	20	20	36	35	28	62	110	230	62	116	117
7	40	30	32	168	67	44	16	20	126	51	102
8	120	180	275	455	258	160	160	250	175	186	348
9	65	50	150	0	66	145	90	60	50	86	117
10	270	470	100	675	379	175	140	420	250	246	404
Ave.	113	155	402	178	212	187	140	482	196	252	239

	5%	1%
Significant Difference between Months	19.34	25.66
Significant Difference between Cows	30.59	40.55

LF equals left front, LR equals left rear, RF equals right front, and RR equals right rear quarter, respectively.

ing in the different quarters are quite different. The total count on the right front quarter is high and the left rear quarter low as compared with the other quarters. (Table IV)

All the interactions proved to be highly significant. Of these, Table III reveals the interaction between cows and months to be the most significant, and that the number of bacteria produced by the different cows is not the same for the different months. An excellent example of this interaction is seen in Table V between cows Nos. 6 and 7. During the first and third months cow No. 7 gave the higher average count, but in the second and fourth months cow No. 6 had the higher average count.

The highly significant interaction between cows and quarters shows that the number of bacteria produced by each cow was different for the various quarters. The right front quarter gave the highest average count, but in cows Nos. 5 and 7, this quarter gave the lowest count. Other excellent examples may be noted in Table II.

The interaction between month and quarter, although not as significant as the other interactions, is never-the-less highly significant.

TYPES OF BACTERIA OCCURRING IN THE UDDER

The percentages of cocci and bacilli were determined in conjunction with the total count. The percentage of

Table VII

The Analysis of Variance of the Percentage of Cocci per cc. for Cows, Months, and Quarters.

Source of Variance	DF	Sum of Sq	Variance	F	5%	1%
Total	159	129,780				
Between Cows	9	21,366.89	2,374.1	4.13	2.06	2.74
Between Months	3	4,019.72	1,339.91	2.33	2.72	4.04
Between Quarters	3	4,637.92	1,545.97	2.69	2.72	4.04
Interactions						
Between Cow and Month	27	32,702.16	1,211.19	2.11	1.65	2.03
Between Cow and Quarter	27	15,016.42	556.16	.97	1.65	2.03
Between Month and Quarter	9	5,533.13	614.79	1.07	2.06	2.74
Between Cow, Month, and Quarter	81	46,503.76	574.12			

Table VIII

Showing Average Percentage of Cocci per cc. for Cows and ~~Months~~ quarters. (Each figure average of 4 ~~quarters~~ months)

Between Cows and Quarters

Cows	Left Front	Left Rear	Right Front	Right Rear	Average
1	49	78	75	72	68.5
2	43	20	38	33	34
3	86	64	71	59	70
4	73	89	83	73	79
5	91	72	59	45	67
6	65	71	77	46	65
7	52	54	60	56	56
8	58	67	73	70	67
9	73	52	84	47	64
10	85	82	69	49	72
Ave.	68	65	69	55	64.2

Table IX

Showing Average Percentage of Cocci per cc. for Cows and Months. (Each figure average of 4 quarters)

Between Cows and Months (Microflora)

Cows	1st. Month	2nd. Month	3rd. Month	4th. Month	Average
1	68	66	71	68	68
2	15	53	0	65	34
3	41	82	77	79	70
4	80	84	79	72	79
5	66	50	89	62	67
6	69	44	62	84	65
7	65	24	55	78	56
8	61	78	69	61	67
9	66	82	32	76	64
10	68	63	76	79	72
Ave.	60	63	61	72	64.2

Table X

Showing Average Percentage of Cocci per cc. for Months and Quarters. (Each figure average of 10 cows)

	Left Front	Left Rear	Right Front	Right Rear	Ave.
1st. Month	67	50	64	58	60
2nd. Month	72	74	59	46	63
3rd. Month	59	64	70	52	61
4th. Month	72	71	83	64	72
Average	68	65	69	55	64.2

Probability	5%	1%
Significant Difference between Quarters	10.66	14.14
Significant Difference between Months	10.66	14.14

Table XI

Showing Percentages of Cocci per cc. Developing in Different Cows, and Quarters at Different Dates.

	First Month					Second Month				
Cows	LF	LR	RF	RR	Ave.	LF	LR	RF	RR	Ave.
1	45	75	70	80	68	56	80	67	60	66
2	0	0	62	10	15	80	40	10	83	53
3	84	21	44	13	41	85	84	70	89	82
4	62	93	70	94	80	87	100	85	65	84
5	74	13	75	100	66	100	100	0	0	50
6	80	100	45	50	69	47	40	90	0	44
7	90	43	100	25	65	8	44	25	20	24
8	86	48	35	74	61	64	83	94	71	78
9	80	43	81	60	66	100	68	100	60	82
10	70	66	55	80	68	96	96	45	13	63
Ave.	67	50	64	58	60	72	74	59	46	63

Table XI (Continued)

Showing Percentages of Cocci per cc. Developing in Different Cows, and Quarters at Different Dates.

	Third Month					Fourth Month					
Cows	LF	LR	RF	RR	Ave.	LF	LR	RF	RR	Ave.	Tot. Ave.
1	33	81	91	80	71	60	75	72	66	68	68
2	0	0	0	0	0	90	41	80	50	65	34
3	86	60	88	73	77	88	89	80	60	79	70
4	80	83	92	62	79	61	70	84	71	72	79
5	100	100	77	80	89	91	75	83	0	62	67
6	60	60	83	46	62	74	84	90	89	84	65
7	25	67	34	93	55	86	63	80	85	78	56
8	67	61	80	67	69	16	75	84	67	61	67
9	34	30	63	0	32	76	67	90	70	76	64
10	100	93	88	22	76	74	73	87	80	79	72
Ave.	59	64	70	52	61	72	71	83	64	72	64.2

	5%	1%
Significant Difference between Cows	16.86	22.34
Significant Difference between Months	10.66	14.14

LF equals left front, LR equals left rear, RF equals right front, and RR equals right rear quarter, respectively.

cocci for all comparisons was 64.2% and the rods 35.8%.

Analysis of Variance tables have been worked out in a similar manner to those on the total numbers of bacteria in milk. As the percentage of bacilli varied inversely with the percentage of cocci, only the percentages of cocci have been used in the tables.

The highest variance in the percentage of organisms occurred between cows. (Table VIII)

The variance between months is just approaching significance, hence the percentage of cocci may be considered as being more or less constant from month to month, or at least there is not sufficent data here to prove otherwise. (Table X)

The difference in the variance for percentage of cocci between quarters approaches significance when based upon the probability of .05. (Table VII)

Table X discloses that the right rear quarter is responsible for much of the variance due to quarters.

The only interaction of significance is that between cows and months. This indicates that the types of bacteria produced by the different cows is different for the different months. (Table VII)

THE RELATIONSHIP OF THE HEMOLYTIC COUNT TO THE PLATE COUNT

Results obtained by the hemolytic plate count are found in the Appendix. This test was run to determine its relationship to the plate count.

In this experiment a high plate count was not always accompanied by a high hemolytic count. Sometimes a rather high hemolytic count was obtained and the plate count remained low.

An insignificant correlation coefficient was obtained when the hemolytic count was compared with the plate count.

Whether or not the hemolytic organisms appearing on the plates were Streptococcus mastitis or Streptococcus epidemicus were not determined. It appears from the work of Ayers and Mudge (2) that Streptococcus mastitis may be found in normal udders, although in smaller numbers than in cases of mastitis. Non-pathogenic hemolytic organisms have also been reported by Minett and Stableforth (17). Hammer states, "There is no close correlation between infected udders and high counts on the milk at the time it is drawn and in some instances milk coming from such udders has an unusually low bacterial count.

"Occasionally an animal is encountered in which one or more quarters shift back and forth from a normal to an abnormal condition throughout the entire lactation period."

KINDS OF ORGANISMS PRESENT

From the plates which were inoculated with aseptically drawn milk, 68 cultures of micrococci were isolated in pure culture. In isolating these care was taken to get as many different types as possible present as indicated by cultural characteristics under the low power of the microscope. The exact identity of 38 typical colonies was determined according to Bergey's Manual of Determinative Bacteriology (5), and Studies on the Coccaceae (15). The following organisms arranged in the frequency of their occurrence were identified: Micrococcus casei, 8 cultures; Micrococcus cinnebareus, 5 cultures; Micrococcus roseus, 5 cultures; Micrococcus flavus, 3 cultures; Micrococcus aurantiacus, 3 cultures; Micrococcus aureus, 2 cultures; Micrococcus candidus, 2 cultures; Micrococcus albus, 1 culture; Micrococcus citreus, 1 culture; Micrococcus luteus, 1 culture; and Micrococcus varians, 1 culture.

The organisms present in milk drawn aseptically belong to rather definite types and are not of a wide variety of species. To multiply in the udder, an organism must have the ability to grow in contact with living tissue, and this is a character lacking in many common types.

CONCLUSIONS

1. Milk drawn aseptically from each quarter of the udder of ten cows, over a four months period, gave an average bacterial count of 239.

2. There was a high significant difference in the number of bacteria produced by the different quarters, cows, and months.

3. The interactions between cows and months, between cows and quarters, and quarters and months, were all highly significant.

4. Results in this experiment indicated that some cows were consistently low producers, while others were consistently high.

5. Of the total number of organisms appearing on the plates, 64.2% were cocci and 35.8% were rods.

6. The greater significant difference in the percentage of cocci per cc. occurred between cows.

7. The percentage of cocci produced by the different cows may be considered as being more or less constant from month to month.

8. The difference in the variance for the percentage of cocci between quarters approaches significance when based upon the probability of .05. The right rear quarter accounted for most of the variance.

9. A significant interaction between cow and quarter indicates that the percentage of cocci per cc. produced by

the different cows is different for the different quarters.

10. The percentage of cocci per cc. occurring in the udder is much more constant than the number of colonies per cc.

11. There appears to be no correlation between the number of colonies per cc. and the hemolytic colonies.

12. Organisms isolated were: Micrococcus casei, Micrococcus cinnebareus, Micrococcus roseus, Micrococcus flavus, Micrococcus aurantiacus, Micrococcus aureus, Micrococcus candidus, Micrococcus albus, Micrococcus citreus, Micrococcus luteus, and Micrococcus varians.

APPENDIX

The complete data obtained are given in detail in the following tables:

Table 12

Bacteriological Analysis of Milk from Cow No. 1

Quarter of Udder	Month of Lact.	Dilu-tion	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 9						
LF	5	1-5	380	342	38	100
LR		1-5	330	150	180	75
RF		1-5	60	30	30	0
RR		1-5	160	40	120	50
Total			930	562	368	225
Feb. 4						
LF	6	1-5	40	3	37	0
LR		1-5	50	22	28	0
RF		1-5	40	10	30	0
RR		1-5	100	20	80	0
Total			230	55	175	0
March 5						
LF	7	1-2	40	10	30	0
LR		1-2	30	15	15	0
RF		1-2	32	11	21	0
RR		1-2	168	157	11	12
Total			270	193	77	12
April 3						
LF	8	1-2	44	38	6	0
LR		1-2	16	10	6	0
RF		1-2	20	16	4	0
RR		1-2	126	107	19	0
Total			206	171	35	0

LF equals left front, LR equals left rear, RF equals right front, and RR equals right rear of quarter, respectively.

Age of cow, 3 years.

Table 13

Bacteriological Analysis of Milk from Cow No. 2

Quarter of Udder	Month of Lact.	Dilu-tion	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 9						
LF	3	1-5	0	0	0	0
LR		1-5	0	0	0	0
RF		1-5	370	229	141	0
RR		1-5	0	0	0	0
Total			370	229	141	0
Feb. 4						
LF	4	1-2	30	24	6	0
LR		1-2	25	10	15	0
RF		1-2	21	2	19	0
RR		1-2	12	10	2	0
Total			88	46	42	0
March 3						
LF	5	1-2	0	0	0	0
LR		1-2	0	0	0	0
RF		1-2	0	0	0	0
RR		1-2	0	0	0	0
Total			0	0	0	0
April 3						
LF	6	1-2	70	63	7	0
LR		1-2	32	13	19	0
RF		1-2	725	580	145	6
RR		1-2	110	55	55	0
Total			937	711	226	6

LF equals left front, LR equals left rear, RF equals right front, and RR equals right rear of quarter, respectively.

Age of cow, 3 years.

Table 14

Bacteriological Analysis of Milk from Cow No. 3

Quarter of Udder	Month of Lact.	Dilution	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 9						
LF	2	1-5	500	420	80	130
LR		1-5	170	36	134	5
RF		1-5	320	140	180	40
RR		1-5	150	20	130	10
			1140	616	524	185
Feb. 4						
LF	3	1-10	700	595	105	500
LR		1-5	375	315	60	0
RF		1-10	695	487	208	200
RR		1-5	140	125	15	20
Total			1910	1522	388	720
March 3						
LF	4	1-10	220	190	30	60
LR		1-5	270	160	110	0
RF		1-10	1550	1360	190	640
RR		1-5	260	190	70	0
Total			2300	1900	400	700
April 3						
LF	5	1-10	450	395	55	80
LR		1-5	225	200	25	0
RF		1-10	1200	960	240	200
RR			290	175	115	5
Total			2165	1730	435	285

LF equals left front, LR equals left rear, RF equals right front, and RR equals right rear quarter, respectively.

Age of cow, 3.

Table 15

Bacteriological Analysis of Milk from Cow No. 4

Quarter of Udder	Month of Lact.	Dilu-tion	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 9						
LF	1	1-5	122	76	46	0
LR		1-5	120	112	8	0
RF		1-5	50	35	15	0
RR		1-5	192	180	12	0
			484	403	81	0
Total						
Feb. 4						
LF	2	1-5	215	187	28	105
LR		1-5	105	105	0	40
RF		1-5	740	630	110	60
RR		1-5	65	42	23	5
Total			1125	964	161	210
March 3						
LF	3	1-5	120	96	24	0
LR		1-5	340	282	58	5
RF		1-5	105	97	38	0
RR		1-5	135	84	21	5
Total			700	559	141	10
April 3						
LF	4	1-5	140	85	55	0
LR		1-5	130	90	40	0
RF		1-5	320	270	50	0
RR		1-5	70	50	20	0
Total			660	495	165	0

LF equals left front, LR equals left rear, RF equals right front, RR equals right rear of quarter, respectively.

Age of cow, 4 years.

Table 16

Bacteriological Analysis of Milk from Cow No. 5

Quarter of Udder	Month of Lact.	Dilu-tion	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan.11						
LF	7	1-5	90	67	23	0
LR		1-5	75	10	65	0
RF		1-5	40	30	10	5
RR		1-5	110	110	0	0
Total			315	217	98	5
Feb. 10						
LF	8	1-2	30	30	0	0
LR		1-2	20	20	0	0
RF		1-2	0	0	0	0
RR		1-2	0	0	0	0
Total			50	50	0	0
March 6						
LF	9	1-2	150	150	0	2
LR		1-2	90	90	0	0
RF		1-2	90	70	20	0
RR		1-2	20	16	4	0
Total			350	326	24	2
April 5						
LF	10	1-2	36	33	3	0
LR		1-2	20	15	5	0
RF		1-2	12	10	2	0
RR		1-2	0	0	0	0
Total			68	58	10	0

LF equals left rear, LR equals left right, RF equals right front, RR equals right rear quarter of udder, respectively.

Age of cow, 3 years.

Table 17

Bacteriological Analysis of Milk from Cow No. 6

Quarter of Udder	Month of Lact.	Dilution	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 12						
LF	1	1-5	230	185	45	5
LR		1-5	70	70	0	0
RF		1-5	550	245	305	15
RR		1-5	50	25	25	0
Total			900	525	375	20
Feb. 6						
LF	2	1-2	225	106	119	10
LR		1-2	35	14	21	0
RF		1-2	130	117	13	10
RR		1-2	0	0	0	0
Total			390	237	153	20
March 8						
LF	3	1-2	20	12	8	0
LR		1-2	20	12	8	0
RF		1-2	36	30	6	0
RR		1-2	35	16	19	0
Total			111	70	41	0
April 6						
LF	4	1-2	62	46	16	4
LR		1-2	110	92	18	0
RF		1-2	230	205	25	24
RR		1-2	62	54	8	0
Total			464	397	67	28

LF equals left front, LR equals left rear, RF equals right front, RR equals right rear of quarters, respectively.

Age of cow, 2 years.

Table 18

Bacteriological Analysis of Milk from Cow No. 7

Quarter of Udder	Month of Lact.	Dilution	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 9						
LF	5	1-5	380	342	38	20
LR		1-5	330	150	180	10
RF		1-5	60	30	30	0
RR		1-5	160	40	120	15
Total			930	562	368	45
Feb. 4						
LF	6	1-5	40	3	37	0
LR		1-5	50	22	28	0
RF		1-5	40	10	30	0
RR		1-5	100	20	80	0
Total			230	55	175	0
March 5						
LF	7	1-2	40	10	30	0
LR		1-2	30	15	15	0
RF		1-2	32	11	21	0
RR		1-2	168	157	11	6
Total			270	193	77	6
April 3						
LF	8	1-2	44	38	6	0
LR		1-2	16	10	6	0
RF		1-2	20	16	4	0
RR		1-2	126	107	19	0
Total			206	171	35	0

LF equals left front, LR equals left rear, RF equals right front, RR equals right rear of quarters, respectively.

Age of cow, 3 years.

Table 19

Bacteriological Analysis of Milk from Cow No. 8

Quarter of Udder	Month of Lact.	Dilution	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 12						
LF	6	1-5	220	190	30	0
LR		1-5	135	65	70	0
RF		1-5	130	45	85	45
RR		1-5	1400	1040	360	45
Total			1885	1340	545	90
Feb. 6						
LF	7	1-2	125	80	45	0
LR		1-2	320	266	54	12
RF		1-2	295	276	19	0
RR		1-2	1170	836	234	30
Total			1910	1458	352	42
March 8						
LF	8	1-5	120	80	40	35
LR		1-5	180	110	70	0
RF		1-5	275	220	55	10
RR		1-5	455	305	150	0
Total			1030	715	315	45
LF	9	1-2	160	25	135	0
LR		1-2	160	120	40	0
RF		1-2	250	210	40	0
RR		1-2	175	105	70	10
Total			745	460	285	10

LF equals left front, LR equals left rear, RF equals right front, RR equals right rear of quarters, respectively.
Age of cow, 4 years.

Table 20

Bacteriological Analysis of Milk from Cow No. 9

Quarter of Udder	Month of Lact.	Dilution	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 12						
LF	2	1-5	135	108	27	5
LR		1-5	350	150	200	0
RF		1-5	135	110	25	0
RR		1-5	180	108	72	0
Total			800	476	144	5
Feb. 6						
LF	3	1-5	110	110	0	55
LR		1-5	140	95	45	0
RF		1-5	125	125	0	90
RR		1-5	90	54	15	0
Total			465	384	60	145
March 8						
LF	4	1-5	65	22	43	0
LR		1-5	50	15	35	0
RF		1-5	150	95	55	0
RR		1-5	0	0	0	0
Total			265	132	133	0
April 6						
LF	5	1-2	145	110	35	0
LR		1-2	90	60	30	0
RF		1-2	60	54	6	0
RR		1-2	50	35	16	0
Total			345	259	86	0

LF equals left front, LR equals left rear, RF equals right front, RR equals right rear of quarters, respectively.

Age of cow, 7 years.

Table 21

Bacteriological Analysis of Milk from Cow No. 10

Quarter of Udder	Month of Lact.	Dilution	No. of Bacteria	No. of Cocci	No. of Rods	Hemolytic
Jan. 11						
LF	5	1-5	735	515	220	30
LR		1-5	275	180	95	5
RF		1-5	560	310	250	60
RR		1-5	460	370	90	0
Total			2030	1375	655	95
Feb. 10						
LF	6	1-5	905	869	36	100
LR		1-5	282	271	11	65
RF		1-5	87	39	48	10
RR		1-5	665	86	579	10
Total			1939	1265	674	185
March 6						
LF	7	1-5	270	270	0	10
LR		1-5	470	437	33	20
RF		1-5	100	88	12	10
RR		1-5	675	149	526	70
Total			1515	944	571	110
April 5						
LF	8	1-5	175	131	44	0
LR		1-5	140	102	38	0
RF		1-5	420	365	55	20
RR		1-5	250	200	50	0
Total			985	798	187	20

LF equals left front, LR equals left rear, RF equals right front, RR equals right rear of quarters, respectively.

Age of cow, 6 years.

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