

# OCCURRENCE OF THE COLON-AEROGENES GROUP OF ORGANISMS IN RAW AND IN PASTEURIZED MILK, AND ITS SIGNIFICANCE

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## INTRODUCTION

The writer has for some time been interested in the problem of the "Occurrence of the colon-aerogenes group of organisms in raw and in pasteurized milk and its significance." Many samples of milk have therefore been examined to determine, if possible, the relation of the above group of organisms to the initial contamination of milk, its age, its temperature, and to the efficiency of pasteurization.

A number of samples have been secured of raw milk produced at the college barn; raw milk retailed in Guelph; raw milk received at the pasteurizing plant of the dairy department of the college; raw milk received at the commercial pasteurizing plant in town (later referred to as Model Dairy) and, finally, of pasteurized milk from the vats of the above two plants. This work covered a full year and has been carried out in four instalments, representing in all the four seasons of the year.

## METHODS USED

### *1. Sampling*

In general, the routine methods of analysis were used as described in the "Standard Methods of Bacteriological Analysis of Milk" (2). After thorough agitation of milk 1 ounce samples were secured, using sterile pipettes and bottles. Samples of raw milk sold in town were iced immediately during warm summer weather, taken to the laboratory and plated within two hours after sampling. During the cold weather of spring, fall and winter, no attempt was made to ice the samples since the milk

was already sufficiently cold in most instances and since deliveries of milk were continued for two or three hours after the samples had been taken, so that the bacterial counts obtained really indicated the condition in which milk had been delivered to the consumers. Samples of raw milk received by the pasteurizing plants were taken from the vats after good mixing and were kept in running cold water until collected when the pasteurized milk had been cooled down to 48° to 50°F. and sampled. Night and morning samples at the college barn were taken immediately after milking and kept in running cold water in a tank with the rest of the milk.

## 2. Media

In order to obtain a general quantitative idea of the group counts it was deemed advisable to plate out every sample of milk in this series of experiments on three different media. They were made up as follows:

### *Litmus lactose agar*

	grams
"Difco" peptone per liter of medium.....	5
"Difco" beef.....	25
Agar agar.....	15
Lactose.....	10

The reaction was adjusted to +0.5, 2 cc. of litmus solution (20 grams per 1000 cc. water) were added per 100 cc. of culture medium, which was put up in flasks of 150 cc. each and finally autoclaved for one-half hour at 15 pounds pressure.

### *Litmus lactose gelatin*

	grams
"Difco" peptone per liter of medium.....	5
"Difco" beef.....	25
Gelatin.....	140
Lactose.....	10

The reaction was adjusted to +0.5, 2 cc. of litmus solution (20 grams per 1000 cc. water) were added per 100 cc. of culture medium, which was put in flasks of 150 cc. each, and finally sterilized intermittently on three consecutive days.

*Aesculin bile salt agar* (4)

	<i>grams</i>
Agar agar.....	12 to 15
Sodium taurocholate.....	2.5
Witte's peptone were boiled down in 1000 cc. distilled water....	10

The solution was neutralized with normal solution of sodium hydrate, cooled below 60°C.; the whites of two eggs or an equivalent quantity of albumen added, the mixture boiled in streaming steam and then filtered through paper. The acidity was then tested and the medium neutralized, if necessary.

One gram of aesculin and 1 gram of iron citrate were then added to the hot mixture. The reaction was again tested and if necessary made to +0.6.

This medium was used to obtain the number of organisms belonging to the *B. coli* and *B. lactis aerogenes* groups.

*3. Plating*

Several samples of milk were secured from the different sources and plated out in a preliminary way to determine the dilutions that would give satisfactory counts. The aim was to obtain agar and gelatin plates having between 30 and 500 colonies per plate, and aesculin plates having between several and 50 colonies per plate. The dilutions had to be varied according to the type of milk and season of the year, so that after a little practice there was no difficulty in getting satisfactory results. The dilutions made for agar and gelatin plates ranged from 1:50 and 1:100 to 1:10,000 and 1:20,000, while those for aesculin plates ranged from 1:10 and 1:100 to 1:5,000 and 1:10,000.

The media in flasks were steamed for one half hour, cooled down and poured into the plates (within one half hour after inoculation) till the melted material covered the plate to the nearly uniform depth of  $\frac{1}{8}$  inch. Mixing and uniform distribution were facilitated by a moderate circular movement of the plates. During the warm weather gelatin plates were quickly hardened by being placed on the level top of a box-like tank which had cold water circulating through it. After the material had solidified, the plates (other than gelatin) were inverted and incubated.

#### 4. Incubation and counting

The agar plates were incubated at 37.5°C. for two days and then at room temperature for one day. The aesculin plates were incubated at 37.5°C. for two days, while the gelatin plates at 20°C. for five days. At the end of these respective incubating periods, plates were counted, use being made of a magnifying lens 2½ diameters and a counting plate. The reported figures represent the total count and the various group counts per cubic centimeter of milk.

#### 5. Differential group counts

The differential group counts were obtained in order to get an idea of the proportions in which they were present at different seasons of the year and in different types of milk.

*Total count.* High counts in market milk indicated, in a general way, carelessness, while low counts indicated care, either in production or in handling, or in both. But bacteriological analyses of individual samples did not furnish enough data for comment. Therefore, in the summary tables that follow, the average of bacterial counts of a specified number of samples of the same type of milk were given, while the analyses of the individual samples were reported in the appendix.

*Acid count.* This was taken as an indication of initial contamination or subsequent growth, or both. This group included all colonies turning blue litmus red. Most of these were of the harmless lactic acid type, but "standard methods are not such as to give a proper count of lactic acid bacteria" (2). It must be borne in mind, therefore, that the figures reported under this heading are rather an underestimate of the actual conditions.

*Liquefied count.* This group included all colonies liquefying gelatin, whether rapidly or slowly. These were considered undesirable in milk on account of their putrefactive nature.

*Colon-aerogenes count.* Under the head of *B. coli* or colon group, we include all colonies which gave a brown or black coloration on aesculin bile salt agar. We know that this includes many varieties of colon

which have been described by various writers under specific names (e.g., *B. communior*). It also includes the *B. lactis aerogenes* group which is found in large numbers in the faeces of cattle. We regard any member of these groups as indicating "manurial impurities" in milk. A large number of this group present in a sample shows either carelessness and uncleanness in milking, subsequent keeping at a high temperature, or as most frequently happens, a combination of both (4).

In obtaining this group count no attention was paid to lactose fermenting yeasts and to some molds that give the same blackening on aesculin agar. Their colony growth was different from that of the members of colon-aerogenes group.

#### *Other types*

Other types not included above, were unimportant and not considered here. They consisted of chromogenic bacteria of little significance in milk and of inert or neutral bacteria producing no acid and having no noticeable effect on milk. According to Conn and Esten (5) these neutral bacteria make up a large percentage of the total bacteria in fresh milk, and a considerable percentage of the total bacteria in old milk, kept at a low temperature.

#### DISCUSSION

##### *1. Raw milk at the college barn*

The college barn is a modern building, well lighted and well ventilated. It is equipped with concrete floors and gutters and is easily kept clean. No particular time or labor-consuming precautions are taken outside of keeping the cows decently clean, milking in a cleanly manner, straining the milk through several thicknesses of cheesecloth which is later washed and boiled, using well-cleaned and steamed utensils and practising immediate cooling of milk by setting the cans in a tank of running cold water.

The average analyses of morning milk, which was three hours old at the time of plating, for the different seasons of the year, are shown in summary table 1.

SUMMARY TABLE 1

(Derived from tables 1 A, B, C)

*Raw milk, college dairy barn; morning's milk*

SEASON OF THE YEAR	AVERAGE NUMBER OF SAMPLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELATIN	LIQUEFIERS	AESCULIN COUNT	GRADE, MONTREAL, 1914, STANDARD
July, 1917.....	14	83,000	20,375	96,958	13,363	5,100	707	A—t. B—l; a
Distribution, per cent.....		100	24.4	100	14	5.3	0.85	
October, 1917.....	22	35,500	2,585	28,500	2,800	4,350	74	A—t; a. B—l
Distribution, per cent.....		100	7	100	10	15	0.2	
February, 1918.....	21	9,600	2,100	11,800	1,400	1,400	49	A—t; l; a
Distribution, per cent.....		100	22	100	12	12	0.5	
Average per sample per year.....	57	36,000	7,300	41,300	5,500	2,800	250	A—t; a. B—l
Distribution, per cent.....		100	20	100	13	7	0.7	

During the summer months there is an indication of bacterial growth having taken place in all of the groups mentioned. This is explained by the fact that ice was not used in the cooling tank, and that the cooling water was not any too cold.

During the other seasons of the year there seems to have been no important change in the relative proportion of the different groups of bacteria, so that the figures given may be taken as a measure of initial contamination at the barn.

Of the average total bacterial content per morning sample at the barn for the entire year it may be said that it was very satisfactory, being below 50,000 per cubic centimeter.

It is of interest to note here that the colon-aerogenes count on aesculin agar during the fall and winter was below 100 per cubic centimeter, presumably indicating the initial contamination under conditions somewhat better than those found on the average farm.

Summary table 2 shows the results obtained with raw night milk produced at the college barn under the same conditions as already indicated, but which was eighteen hours old when plated, and which was kept during this time in running cold water.

The counts of night samples are very much similar to those obtained from the morning samples. They indicate during the summer that bacterial growth has taken place in all of the groups mentioned (but chiefly in the inert group not shown here) to a greater extent than in the morning milk on account of the insufficiently cold cooling water and on account of the longer exposure (eighteen hours as compared to three hours).

The counts of night samples for fall and winter show that there is practically no bacterial increase in any of the groups in milk in eighteen hours, if kept in running cold water.

It may be added that the colon-aerogenes count, too, remains unchanged under this condition of temperature during the fall and winter months.

The averages per night sample for the year are higher than per morning sample, but this is wholly owing to the undue influence of the night samples for the summer.

SUMMARY TABLE 2  
(Derived from tables 1 A, B, C)  
*Raw milk, college dairy barn; night's milk*

SEASON OF THE YEAR	AVERAGE NUMBER OF SAMPLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELATIN	LIQUEFIERS	AESCULIN COUNT	GRADE, MONTREAL, 1914, STANDARD
July, 1917.....	14	276,333	63,250	611,777	40,857	10,115	1,850	B—t; l; a
Distribution, per cent.....		100	22.9	100	6.7	1.6	0.67	
October, 1917.....	22	39,450	1,138	23,300	840	590	60	A—t. B—l; a
Distribution, per cent.....		100	3	100	3.5	2.5	0.15	
February, 1918.....	19	7,000	1,500	14,300	1,500	1,600	110	A—t; l; a
Distribution, per cent.....		100	21	100	10	11	1.5	
Averages per sample per year.....	55	81,800	17,200	151,500	11,900	4,600	595	A—t. B—l; a
Distribution, per cent.....		100	21	100	8	3	0.73	



## *2. Raw milk at consumer's door*

Getting a license was the only local regulation affecting the distribution of milk in Guelph. Raw milk at consumer's door was sold either by numerous small milk producers from the immediate vicinity or by many small dealers buying their milk from nearby farms, so that very little of it was more than eighteen hours old and a good deal of it was only six hours old when delivered. Most of the milk was dipped but some of it was bottled the previous night. Hardly any attempt was made to cool the milk properly at any time, particularly in the summer when no ice whatsoever was used and the only protection that the milk received was that of being placed on a covered milk delivery wagon. During the other seasons of the year reliance was placed on the cold weather to keep the milk cool.

Summary table 3 shows the average analyses of the indicated number of samples. The highest total counts were obtained, as would be expected, during the summer, due perhaps to some extent to the pressure of other farm work and the consequent neglect of the proper care of the milk, but chiefly to the failure to cool the milk and to keep it cold.

The lowest total counts were obtained in the fall of the year and may be taken as an indication of the initial contamination at the farm. These figures are in strong contrast with the summer figures and show that even better results may be accomplished by cooling immediately after milking and on purpose.

All differential group counts show that no probable growth of bacteria in the given time took place in the milk in the fall, that some growth took place in the spring, probably before the milk reached the temperature of the surrounding air, and that very vigorous growth took place in milk during the summer.

The total and differential count for the winter (break in the prevailing severe weather came during the time that these samples were taken) shows either increased initial contamination at the farm, a slow growth extending over a longer period (eighteen hours) or both.

As in the preceding table, the average per sample for the year is unduly raised by the high summer count.

SUMMARY TABLE 3  
(Derived from tables 2 A, B, C, D)  
*Raw milk at consumer's door*

SEASON OF THE YEAR	AVERAGE NUM- BER OF SAMPLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELATIN	LIQUEFIERS	AESCULIN COUNT	AVERAGE TEMPER- ATURE OF SAM- PLES REPORTED	GRADE, MONTREAL, 1914, STANDARD
April, 1917.....	50	172,312	64,702	126,000	51,409	17,895	1,914	43°F.	B—t; l; a
Distribution, per cent.....		100	36	100	40	14	1.1		
July, 1917.....	46	1,803,000	536,238	2,028,484	735,703	90,976	23,254	63°F.	C—t; l. D—a
Distribution, per cent.....		100	29.4	100	36.2	4.5	1.3		
October, 1917.....	31	184,000	16,900	127,000	30,300	4,000	588	40°F.	B—t; l; a
Distribution, per cent.....		100	9	100	24	3	0.3		
February, 1918.....	29	490,000	234,000	710,000	343,000	17,000	2,100		B—t; l; a
Distribution, per cent.....		100	48	100	48	2	0.5		
Average per sample per year	156	647,000	175,000	765,000	295,000	43,000	7,600		B—t. C—l; a
Distribution, per cent.....		100	27	100	38	5.6	1.2		

### *3. Raw and pasteurized milk from the dairy department of college*

The milk at the dairy department was pasteurized every other day, so that in many instances all or some of the milk that reached the pasteurizing vat was forty-eight hours old. The machine in which the operation was carried out was modern in construction and equipped with a heating and cooling coil. The supply of steam for heating the water was abundant. Cooling was effected by the use of water. Pasteurization proper was done by different men at different times, but each one of them had some dairy school training and understood the process.

Summary table 4 gives the average results of bacteriological analyses of raw milk intended for pasteurization. In the spring samples the age of milk is indicated by the total count and the liquefier-count and the insufficiently low temperature is indicated by the acid count. In the summer samples both temperature and age are indicated by the total count and by the different group counts. The high colon-aerogenes count is particularly suggestive of high temperatures.

The winter, fall and spring averages resemble one another except that they grade into one another in the ascending scale in the order mentioned. The explanation for this lies no doubt in the temperature, the other factors, such as initial contamination and age, remaining fairly constant on the whole.

The cause of high average liquefier content for winter milk seems to be that this group is favored by the temperature of the season.

Where milk is to be held any length of time, either in transit or in storage, temperature appears to be more important than the initial contamination.

Summary table 5 shows the results obtained in pasteurized milk. Regardless of the varying quality of the raw milk received from day to day, and of the different persons doing the work, the milk pasteurized at the dairy department had made a uniformly good showing throughout the year, proving the importance of good equipment and understanding of the process on the part of the operator. There is no reason, however, why the raw milk

SUMMARY TABLE 4

(Derived from 3 A, C, E, G)

*Efficiency of pasteurization, dairy department, Ontario Agricultural College; raw milk intended for pasteurization*

SEASON OF THE YEAR	AVER- AGE NUM- BER OF SAM- PLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELA- TIN	LIQUEFIERS	AESCULIN COUNT	GRADE, MONTREAL, 1914 STANDARD
May, 1917.....	10	5,358,000	774,555	6,736,666	878,000	564,000	6,386	D—t; 1. C—a
Distribution, per cent.....		100	12.6	100	12.9	8.4	0.12	
July and August, 1917.....	10	22,333,333	9,062,500	20,285,714	11,000,000	444,444	299,500	D—t; 1; a
Distribution, per cent.....		100	40	100	54	2.2	1.3	
October and November, 1917	12	556,700	62,800	649,200	146,000	32,000	5,070	B—t; a. C—1
Distribution, per cent.....		100	11	100	22	5	0.9	
March, 1918.....	5	258,000	38,000	196,000	42,500	61,000	1,100	B—t; a. C—1
Distribution, per cent.....		100	15	100	21	26	0.4	
Average per sample per year	37	7,300,000	2,960,000	8,150,000	4,560,000	340,000	100,000	D—t; 1; a
Distribution, per cent.....		100	40	100	56	4.2	1.4	

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SUMMARY TABLE 5

(Derived from tables 3 B, D, F, H)

*Efficiency of pasteurization, dairy department, Ontario Agricultural College; pasteurized milk*

SEASON OF THE YEAR	AVER- AGE NUM- BER OF SAM- PLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELA- TIN	LIQUE- FIERS	AESCU- LIN COUNT	HOW PASTEURIZED	GRADE, MONTREAL, 1914 STANDARD
May, 1917.....	10	7,266	1,666	2,000	1,120		26	145°F., 20-30 mins.	A
Distribution, per cent.....		100	23	100	56		0.35		
July and August, 1917.....	10	11,914	4,150	11,600	3,600	888	77	145°F., 20 mins.	B—t; 1
Distribution, per cent.....		100	34.8	100	31	7.7	0.7		
October and November, 1917....	16	9,100	570	9,900	2,800	315	36	140°F., 30 mins.	A—t; 1
Distribution, per cent.....		100	6	100	28	3	0.4		
March, 1918.....	5	2,410	900	4,650	600	460	27	145°F.	A—t; 1
Distribution, per cent.....		100	37	100	13	10	1		
Average per sample per year....	41	8,300	1,600	8,200	2,500	550	42		A—t. B—1
Distribution, per cent.....		100	19	100	30	6.7	0.5		

*Note:* The Montreal Standard's requirements for aesculin count in pasteurized milk have not been considered here.

intended for pasteurization should first be allowed to deteriorate in quality.

Pasteurization by the holding process destroyed practically all of the colon-aerogenes group in milk.

Pasteurization by the holding process allowed a good many lactic acid bacteria to remain in the milk and thus to control the subsequent fermentation.

#### *4. Raw and pasteurized milk from the Model Dairy, Guelph*

The Model Dairy is the only plant in town that sells pasteurized bottled milk. The plant is equipped with the old type of pasteurizing machine. The operator is an intelligent man who has other duties to attend to and does not always secure uniform results on this account. Some of the farmers deliver their raw milk at the plant, but not daily; other farmers deliver their milk to the man collecting it for the dairy. The milk is pasteurized every morning, is bottled, put away in storage and is delivered the following morning.

Summary table 6 shows the results obtained in the experiments at the Model Dairy. The raw milk throughout the year shows the effects of age and temperature, obliterating entirely the initial contamination.

The milk was at its worst in the summer, with little to choose between the spring and winter milk, and a somewhat improved quality in the fall. Particularly striking is the high seasonal level reached by the colon-aerogenes group in the summer.

Summary table 7 shows the results obtained with pasteurized milk at the same plant. The samples were obtained directly from the pasteurizing vat immediately after the milk had been cooled. Higher counts would undoubtedly be obtained from the same milk at the consumer's door. When the work was started, they were pasteurizing supposedly at 140°F. for thirty minutes. The reduction in bacterial numbers was unsatisfactory—a high total number of bacteria as well as those of the colon-aerogenes group survived the treatment. During the summer and fall, therefore, they were advised to pasteurize at 145°F. for twenty

SUMMARY TABLE 6

(Derived from tables 4 A, C, E, G)

*Efficiency of pasteurization, Model Dairy, Guelph; raw milk intended for pasteurization*

SEASON OF THE YEAR	AVERAGE NUMBER OF SAM- PLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELATIN	LIQUEFIERS	AESCULIN COUNT	GRADE, MONTREAL, 1914 STANDARD
April and May, 1917.....	19	6,888,421	2,360,555	7,477,333	2,330,714	288,438	50,828	D—t; a; l
Distribution, per cent.....		100	34	100	31	3.9	0.74	
July and August, 1917.....	11	11,975,000	6,887,500	17,037,500	7,431,250	530,000	260,000	D—t; l; a
Distribution, per cent.....		100	57	100	44	3	2.2	
October and November, 1917.....	10	1,931,000	548,750	1,125,700	414,300	126,600	5,000	C—t; a. D—l
Distribution, per cent.....		100	28	100	36	11	0.25	
March, 1918.....	6	4,066,000	2,232,000	6,850,000	2,800,000	400,000	22,500	C—t. D—l; a
Distribution, per cent.....		100	55	100	42	5.8	0.55	
Average per sample per year.....	46	6,290,000	2,900,000	8,345,000	3,220,000	340,000	92,000	D—t; l; a
Distribution, per cent.....		100	46	100	38	4	1.5	

SUMMARY TABLE 7  
(Derived from tables 4 B, D, F, H)

*Efficiency of pasteurization, Model Dairy, Guelph; pasteurized milk*

SEASON OF THE YEAR	AVERAGE NUMBER OF SAMPLES	TOTAL ON AGAR	ACID ON AGAR	TOTAL ON GELATIN	ACID ON GELATIN	LIQUEFI- ERS	AESCU- LIN COUNT	HOW PASTEURIZED	GRADE, MONTREAL, 1914, STANDARD
April and May, 1917.....	19	214,842	52,789	127,526	47,947	7,526	572	Mostly 140°F., 20-30 mins.	D—t. C—1
Distribution, per cent....		100	24	100	39	6	0.45		
July and August, 1917....	10	37,286	11,050	59,840	25,877	4,450	12	145°F., 20 mins.	B—t; 1
Distribution, per cent....		100	29	100	43	7	0.03		
October and November, 1917.....	17	63,900	14,600	79,000	25,000	1,990	46	See table, 4°F.	C—t. B—1
Distribution, per cent....		100	23	100	31	2.5	0.07		
March, 1918.....	7	126,000	82,000	142,000	61,000	1,600	165	140°F., 20-30 mins.	D—t. B—1
Distribution, per cent....		100	66	100	43	1	0.1		
Average per sample per year.....	53	110,500	39,000	102,000	39,000	5,000	324		D—t. B—1
Distribution, per cent....		100	31	100	39	5	0.26		



minutes, and as a result a marked improvement was made, both in the total count and in the colon-aerogenes count.

In the winter, however, the management changed hands and with the intention of saving fuel went back to pasteurizing at 140°F. for twenty to thirty minutes. As a result, the count of bacteria increased again.

These experiments show that the critical temperature for the destruction of the colon-aerogenes group of bacteria is at about 145°F.; that juggling of temperature at pasteurizing plants should be made impossible by the health boards defining the process of proper pasteurization, and that a check should be kept on the efficiency of pasteurization by periodical bacteriological analysis for the sake of protecting the consumers from a false sense of security.

#### SUGGESTIONS TO THE MEDICAL HEALTH OFFICER OF GUELPH<sup>1</sup>

In view of the results obtained and reported in this study, the following suggestions for improving the milk supply of Guelph seem to be justified.

*1. Establishment of a bacterial standard for grading all raw milk sold in the city*

*Grade A.*<sup>2</sup> Total count up to 100,000 per cubic centimeter; colon-aerogenes count up to 500 per cubic centimeter.

*Grade B.* Total count up to 500,000 per cubic centimeter; colon-aerogenes count up to 5000 per cubic centimeter.

*Grade C.* Total count over 500,000 per cubic centimeter; colon-aerogenes count up to 5,000 per cubic centimeter.

The above standard practically corresponds with the Montreal 1914 standard.

At least five samples are to be taken from each dealer during four given months of the year representing the different quarters, such as January, April, July and October, and the grades are to be obtained from the average of these samples.

<sup>1</sup> These suggestions are also applicable to other small towns of similar size.

<sup>2</sup> The bacterial counts are to be considered at the time of delivery to the consumer.

## 2. *Temperature requirement*

Insistence on having all milk cooled to 50°F. or lower, particularly during the warm weather.

## 3. *Publication in the local press of results of grading for any given quarter of the year*

No consumer wants to buy second grade milk, if the price of the first grade is within reach. Grading milk will help the consumer to buy intelligently from a careful dealer. Grading will put a premium on clean milk and would remunerate the producer for the extra effort of producing sanitary milk. Grading will make the careless producer lose his trade or turn over a new leaf.

## 4. *Inspection of dairy farms and dealers' premises*

To be carried out by a competent inspector, with a concentration of helpful effort on places and men producing and handling the poorest milk.

## 5. *Control of pasteurizing plants*

This would include equipment, process, operators, and results obtained. A bacterial standard for pasteurized bottled milk at the time of delivery to the consumer should be taken as a measure of sanitation and efficiency.

The following grades are suggested:

	RAW MILK FOR PASTEURIZATION		PASTEURIZED MILK	
	Total count per cubic centimeter	Colon-aerogenes count per cubic centimeter	Total count per cubic centimeter	Colon-aerogenes count per cubic centimeter
Grade A.....	Up to 200,000	Up to 500	Up to 10,000	Up to 50
Grade B.....	Up to 1,000,000	Up to 5,000	Up to 50,000	Up to 100
Grade C.....	Over 1,000,000	Over 5,000	Over 50,000	Over 100

In the above standard the total count for raw milk corresponds to the New York Board of Health Standard; the colon-aerogenes count for raw milk practically corresponds to the Montreal 1914 Standard, while the standard for pasteurized milk is original.

### *6. Pasteurization of all milk by the municipality*

This is for the sake of safety and economy, as an alternative to the preceding measures.

This is an ideal plan for a city of the size of Guelph (population of about 20,000). No certified milk is sold in the city at present, but raw milk needed in special cases on doctor's orders could be made available.

The city could operate a model municipal pasteurizing and bottling plant in a new modern building and under expert supervision. It could perhaps be done in connection with the dairy department at the college. This plan would work for the general good of the community from the point of view of safe milk and saving due to the economics of the larger centralized business and consolidation of gathering and distributing routes, together with elimination of wasteful competition, bottle-losses, bad debts, etc.

### **SUMMARY**

1. Ordinary decency in the barn, clean cows and sterile utensils made it possible to produce milk with an initial total count lower than 50,000 per cubic centimeter (summary tables 1 and 2).

2. Immediate cooling of milk to 50°F. or lower checked the increase of the total count of bacteria in general and of the colon-aerogenes group in particular (summary tables 1 and 2).

3. When the temperature of milk was not sufficiently low (50°F. or above) there was evidence of increase at first in all of the groups studied, i.e., the lactic acid bacteria, the liquefiers and the members of the colon-aerogenes group (summary tables 1, July; 2, July; 3; 4; 6).

4. In raw milk initially contaminated at the barn, members of the colon-aerogenes group of bacteria were present only to a small extent averaging less than 100 per cubic centimeter where care was used and 588 per cubic centimeter where varying indifferent methods were used (summary tables 1 and 2, October and February; 3, October).

5. In raw milk having a temperature of 60°F. or higher, a rapid increase in the colon-aerogenes count took place, depending on

the temperature and time allowed for growth, and ranging from 2000 to 300,000 per cubic centimeter (summary tables 3, 4, 6).

6. Fresh cold milk carefully produced contained from 600 to 4000 liquefiers per cubic centimeter.

Milk produced on average farms, indifferently cooled and allowed to age, contained from 30,000 to 500,000 liquefiers per cubic centimeter (summary tables 1, 2, 4, 6).

7. Lactic acid bacteria were present in all milk and increased rapidly at 60°F. or higher (summary tables 3, 4, 6).

8. Proper pasteurization by the holding method destroyed practically all colon-aerogenes organisms per cubic centimeter of milk, leaving an average of 42 per cubic centimeter and showing some samples with 0 per cubic centimeter of milk. The critical temperature for the destruction of these organisms appeared to be about 145°F. (summary tables 5; 7, July, October).

Proper pasteurization caused a great reduction of the liquefying organisms per cubic centimeter, but allowed the surviving lactic acid bacteria to predominate and control the subsequent fermentation, at the ordinary temperature (summary tables 5, 7).

9. Properly pasteurized milk resembles a good quality of raw milk from the bacteriological standpoint, except that it is safer, more uniform, and likely to have a lower content of the colon-aerogenes organisms, fewer liquefiers and is surer to sour normally at ordinary temperatures.

Consumer received greater value for the extra cost (summary tables 1, 2, 5).

Improperly pasteurized milk is not safe, not uniform; has a high total count, a high acid count and a high colon-aerogenes count. In these respects it resembles raw milk of average quality, but costs the consumer more (summary tables 3, 7).

#### CONCLUSIONS

1. In raw milk initially contaminated at the barn, members of the colon-aerogenes group of bacteria were present only to a small extent, averaging less than 100 per cubic centimeter where care was used, and 588 per cubic centimeter where varying indifferent methods were used.

2. Temperature of 50°F. or lower checked the increase of the colon-aerogenes group of bacteria in raw milk.

3. Temperature of 60°F. or higher resulted in a rapid increase of the colon-aerogenes count in raw milk.

4. Proper pasteurization by the holding method destroyed practically all colon-aerogenes organisms in milk, leaving an average of 42 per cubic centimeter of milk and showing some samples with 0 per cubic centimeter of milk. The critical temperature for the destruction of these organisms appeared to be about 145°F.

5. The colon-aerogenes count in milk obtained on the aesculin bile salt agar immediately after pasteurization becomes a very valuable aid in connection with the agar plate count in controlling the process of pasteurization.

6. A standard for the colon-aerogenes count on aesculin bile salt agar used in connection with the standard total counts should prove of great value in the milk-grading work.

The following standard is suggested:

*Proposed standard for grading raw and pasteurized milk*

GRADE	RAW. TOTAL COUNT PER CUBIC CENTIMETER ON AGAR	RAW MILK INTENDED FOR PASTEURIZING. TOTAL COUNT PER CUBIC CENTIMETER ON AGAR	PASTEURIZED MILK. TOTAL COUNT PER CUBIC CENTIMETER ON AGAR	RAW MILK. COLON-AEROGENES COUNT PER CUBIC CENTIMETER ON AESCULIN BILE SALT AGAR	PASTEURIZED MILK. COLON-AEROGENES COUNT PER CUBIC CENTIMETER ON AESCULIN BILE SALT AGAR
A	Below 100,000*	Below 200,000*	Below 10,000*	Below 500†	Below 50
B	Below 1,000,000*	Below 1,000,000*	Below 50,000*	Below 5,000†	Below 100
C	Above 1,000,000*	Above 1,000,000*	Above 50,000*	Above 5,000†	Above 100

\* Corresponds to the New York Board of Health Standard.

† Corresponds to the Montreal Standard, 1914.

#### ACKNOWLEDGMENTS

Sincere thanks are due to Prof. H. H. Dean of the Dairy Department of the Ontario Agricultural College for suggesting this work and for ready coöperation in carrying on the pasteurizing experiments; also to Prof. Dan. H. Jones, of the Department of Bacteriology, Ontario Agricultural College for timely advice and helpful criticism.

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