THE BACTERIAL FLORA OF RAW BULK TANK MILK OF FOURTEEN FARMS IN THE WINNIPEG AREA

by

David H. Ashton, B.S.A.

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A Major Thesis submitted to the

Faculty of Graduate Studies and Research

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in candidacy for the degree of

Master of Science

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ABSTRACT

The types of bacteria found in the raw bulk tank milk of fourteen farms in the Winnipeg area were determined over a ten month period, from 7-9 samples per farm, by plating in duplicate on Standard Plate Count Agar, incubating for 48 hrs. at 32° C, and picking 25 colonies at random from each countable plate for detailed study. Pure cultures were separated on the basis of the Gram stain, catalase test, coagulase test, gas production in lactose, and spore stain into eight categories. Of the 5550 pure cultures studied, 51.4% were micrococci, 20.0% Gram -ve rods, 19.2% streptococci, 2.7% asporogenous Gram +ve rods, 2.2% coliform organisms, 2.0% staphylococci, 0.2% bacilli, and less than 0.1% lactobacilli.

The fourteen farms used in the study were classified as "good", "fair", or "poor" on the basis of the cleanliness as determined by visual examination of equipment surfaces with which the milk had come in contact. As conditions on the farm deteriorated, the percentage of streptococci gradually increased, while the percentage of Gram -ve rods gradually decreased. However, the milk samples of several farms

possessed a characteristic flora irrespective of the colony count or the cleanliness of the equipment with which the milk had been handled.

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THE BACTERIAL FLORA OF RAW BULK TANK MILK OF FOURTEEN FARMS IN THE WINNIPEG AREA

INTRODUCTION

For many years, raw milk was stored on the farm and shipped daily to the local dairy plant in eight or ten-gallon cans. These cans were refrigerated by immersion in cold water. Samples of the milk were collected at the dairy plant by health authorities and subjected to laboratory tests designed to indicate the sanitary conditions of production. The most widely accepted laboratory tests measured the reducing ability of microorganisms present in the milk sample (1). It was assumed that dyes placed in milk: (i) produced by healthy cows, (ii) handled with clean utensils, and (iii) cooled quickly to a temperature which minimized bacterial growth, would not be reduced as quickly as in milk treated with less care on the farm. The results of the laboratory test determined, to some extent, the price paid for the milk, since milk in which the dye was reduced slowly under the conditions of the test was given a high grade and hence a premium price.

Bulk tank coolers, because of superior efficiency in cooling and handling the milk, have recently replaced cans as a means of storing raw milk on the farm. Laboratory tests developed for raw milk in the can storage era are no longer satisfactory (13) for the following reasons;

(i) Bacterial growth in the bulk tank is virtually eliminated because of the low temperatures of milk storage, and unsatisfactory conditions of production may exist on the farm without being detected by the laboratory test.

(ii) The milk is now collected from the farm every other day. During this time it is held at 34-40° F (1.1-4.4° C) which inactivates the bacteria present.

To overcome these two factors, Johns (13) suggested "Preliminary Incubation" (PI) of the raw milk sample, for 18 hrs. at 55° F (12.8° C). The incubation period would allow the bacteria time to regain their normal metabolic activities. Any increase in bacterial population during PI would be due to multiplication of saprophytic microorganisms derived from dirty equipment, since udder flora is composed mainly of micrococci with little ability to multiply at 55° F (8).

As a result of favorable reaction to PI in several countries (2, 10, 19) Dr. C. K. Johns, Canada Department of

Agriculture, in 1961 initiated co-operative experiments to assess existing laboratory tests, especially PI, in an attempt to find a bacteriological test for raw milk which would indicate sanitary conditions of production even when milk was stored cold in a bulk tank. The experiments were carried out at the University of Alberta, the University of Manitoba, and the University of Guelph. In the course of the experiments it soon became evident that there was not as great an increase in the Standard Plate Count (SPC) of bacteria after PI at Edmonton as there was at the other two centres (15). It was postulated that some organism or group of organisms which was multiplying during PI at Guelph and Winnipeg was not doing so in the milk at Edmonton. In order to test this hypothesis, a knowledge of the groups of organisms comprising the flora of raw milk at the three centres was required.

Other reasons for studying the flora of raw milk may be cited. Several different incubation temperatures are used in laboratory tests which evaluate the bacteriological quality of raw milk. For example, the methylene blue reduction test at 35-37° C, the resazurin reduction test at 35-37° C, the SPC at 32 or 35° C, storage quality tests at

25° C, and the psychrophilic bacteria count at 5-7° C (1). PI, as suggested by Johns, introduces yet another temperature. Since the bacterial content of raw milk is made up of many different types of microorganisms, varying in optimal growth temperatures, it appears that the various tests are weighted in favor of different types of microorganisms. Only with a thorough knowledge of the flora of raw milk can this weighting be evaluated.

The flora of raw bulk tank milk should also be investigated and reported as a matter of academic interest. It would be of value to answer, with some accuracy, questions concerning the flora of raw bulk tank milk in the Winnipeg area.

The purpose of this investigation was, therefore, to determine the bacterial flora of raw bulk tank milk of fourteen farms in the Winnipeg area.

REVIEW OF LITERATURE

More interest has been shown in the various component groups of microorganisms found in raw milk than in a complete survey of bacterial types present. The coliform group, the streptococci, the lactobacilli, and the pathogenic bacteria of raw milk have received considerable attention. There is general agreement that when milk leaves the udder of a healthy cow the flora consists mainly of micrococci together with smaller numbers of staphylococci, and corynebacteria (7, 8, 12, 24). Other types of bacteria found in raw milk are the result of contamination from the exterior of the udder, equipment coming in contact with the milk, and the air. McKenzie and Bowie (17) showed that different production conditions will result in differences in the bacterial flora of raw milk. Their results are recorded in Table 1.

In a study of the effect of added hypochlorite on the numbers and kinds of bacteria in raw milk, McKenzie and Booker (16) determined the initial bacterial flora. They found 68.8% micrococci, 19.0% streptococci, 9.4% Gram +ve rods, and 3.0% Gram -ve rods in a total of 137 cultures

TABLE 1

The Bacterial Flora of Raw Milk Produced on Farms Classed as Poor or Good on Visual Examination

	Total Colonies	Micro-cocci	Strepto-cocci	Rods Gram +ve	Rods Gram -ve Acid	Rods Gram -ve Alkaline
Poor Farms	78	26(33%)	9(12%)	9(12%)	3(4%)	31(39%)
Good Farms	61	14(23%)	33(54%)	4(7%)	4(7%)	6(9%)

TABLE 2

Distribution in Percentage of Bacterial Types in Fresh Milk

Group*	Micrococci	Streptococci	Gram +ve rods	Gram -ve rods
1	90.0	2.1	4.5	2.1
2	97.2	0.0	0.0	1.1
3	72.8	0.0	2.0	22.2
4	75.3	11.1	1.2	9.9
5	69.1	5.8	22.3	0.7
6	37.7	49.8	5.4	7.0
7	78.5	7.6	3.2	7.5
8	31.8	43.9	1.2	21.9

*Each group is the average of 32 samples.

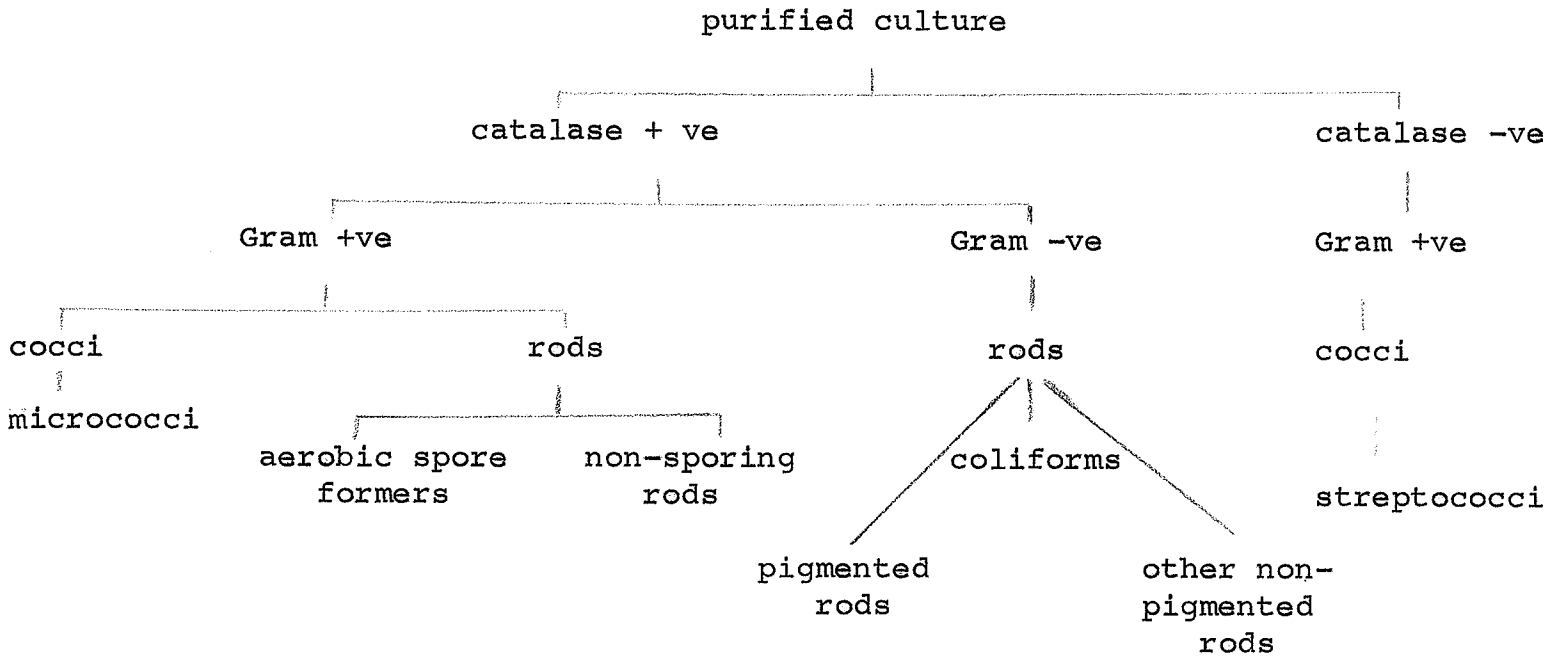
isolated.

During the 13 and 14th years of a mastitis control program, Schalm and Lasmanis (21) identified the bacteria found in the raw milk of a single 250 cow herd. The milk was found to contain 70.8% micrococci, 11.2% coliforms, 8.5% streptococci, 1.0% proteus, 0.2% corynebacteria, 0.1% pseudomonas and 8.2% of all others.

Carreira et al (5) also determined the types of bacteria in raw milk. These are recorded in Table 2.

Recently a comprehensive study of the bacterial flora of raw milk, handled in cans and shipped daily to the dairy plant, was made by Thomas et al (23). These workers isolated and identified 2065 bacterial cultures from 87 farm milk supplies. Samples of raw milk were plated on yeastrel-milk agar and incubated at 30° C for 72 hrs. Approximately 24 colonies were picked at random from each countable plate (30-300 colonies), using a Harrison disc, and inoculated into yeast-dextrose-Lemco broth, which was incubated at 30° C for 24-72 hrs. The resulting pure cultures were identified according to the following scheme:

Fig. 1. Scheme for the Differentiation of the Main Types of Bacteria Isolated from Raw Milk, Thomas et al (1962).



The results obtained by Thomas *et al* appear in Table 3.

TABLE 3

Percentages and Numbers of Bacterial Types Found in Raw Milk

Bacterial Type	% of Total Cultures Isolated	No. of Cultures Isolated
Micrococci	38.4	793
Streptococci	15.2	314
Gram +ve asporogenous rods	11.0	227
Coli-aerogenes	6.5	134
Pigmented Gram -ve rods	9.7	200
Non-pigmented Gram -ve rods	11.2	231
Aerobic spore-forming rods	5.4	112
Unclassified	2.6	54
Total	100.0	2065

TABLE 4

Incidence of Different Types of Bacteria Isolated from Raw Bulk Tank Milk According to Range of Colony Count of Milk Samples

% (in brackets) and numbers of cultures isolated from milk samples with colony counts within the ranges:

Type of Organism	<5000	6000 – $2x10^4$	$>2x10^4$ – $2x10^5$	$>2x10^5$	Total
Micrococci	(67.8)	(54.2)	(33.8)	(28.0)	(46.2)
	217.0	671.0	358.0	560.0	1806.0
Streptococci	(16.3)	(22.5)	(37.0)	(13.0)	(26.5)
	52.0	279.0	392.0	26.0	749.0
Asporogenous Gram +ve rods	(5.9)	(6.3)	(7.0)	(11.5)	(6.9)
	19.0	78.0	74.0	23.0	194.0
Gram -ve rods	(4.1)	(7.9)	(14.2)	(35.5)	(11.8)
	13.0	98.0	151.0	71.0	333.0
Bacilli	(3.1)	(3.5)	(2.7)	(8.5)	(3.5)
	10.0	43.0	29.0	17.0	99.0
Coliform	(0.3)	(0.4)	(0.6)	(0.5)	(0.5)
	1.0	5.0	6.0	1.0	13.0
Unclassified	(2.5)	(5.2)	(4.7)	(3.0)	(4.6)
	8.0	66.0	50.0	6.0	130.0
Total	320	1240	1060	704	3324

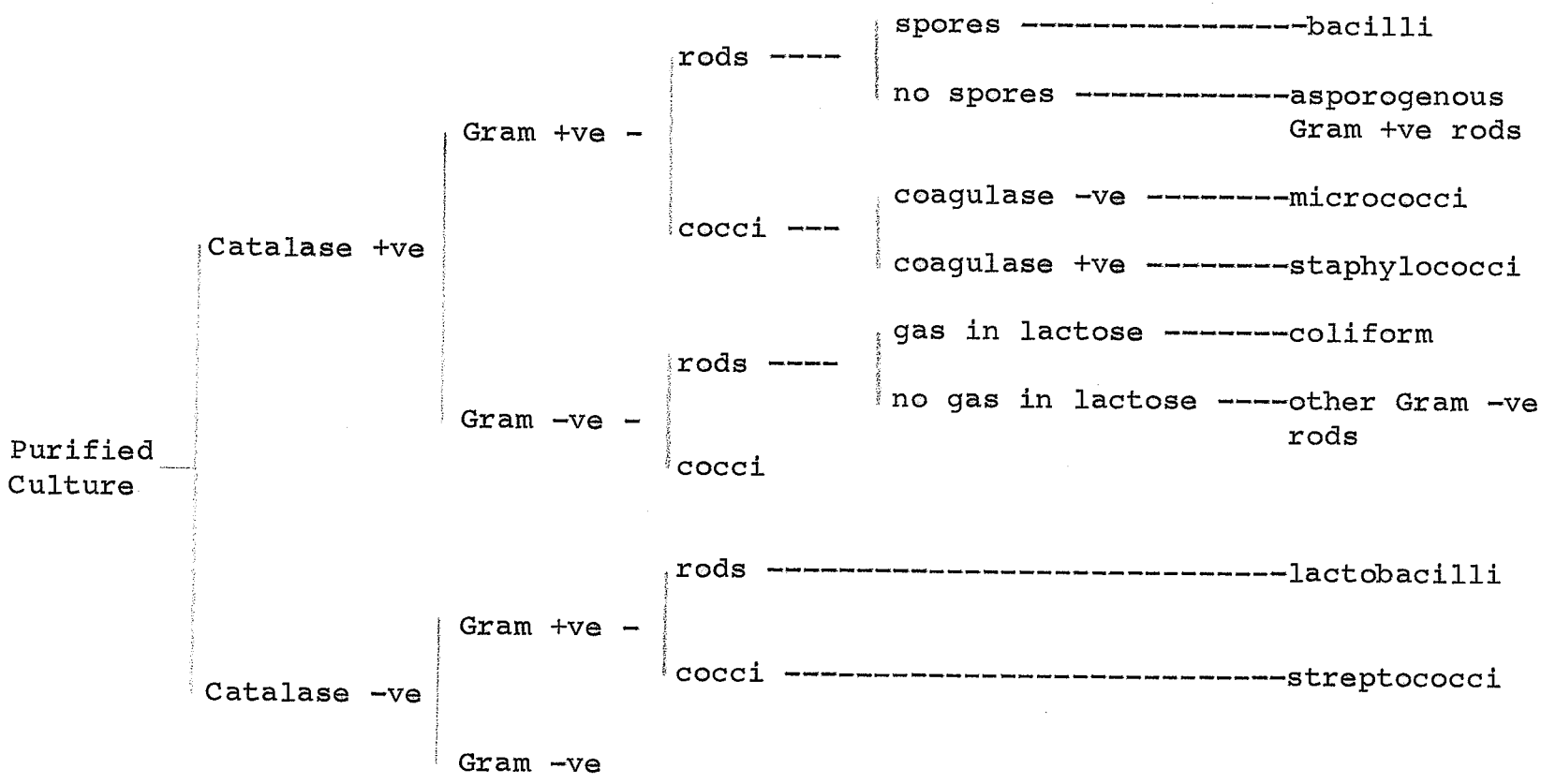
They reported a predominance of micrococci in milk of low count, a co-dominance of streptococci, corynebacteria, and micrococci in milk with colony counts between $2x10^4$ and $2x10^5$, and the dominance of non-pigmented Gram -ve rods and streptococci in the high count milks. Several milk samples, irrespective of colony count level, had a characteristic individual flora dominated by either corynebacteria, aerobic spore formers, coli-aerogenes organisms, fluorescent pseudomonads, or flavobacteria.

The only available report of the bacterial flora of raw bulk tank milk is that of Jackson (11) whose results are shown in Table 4. The methods used in collecting the milk samples and in isolating pure cultures reported in Table 4 were identical to those which will be reported in this thesis.

METHODS

Farms within a 25 mile radius of the city of Winnipeg, shipping bulk tank milk, and consistently producing raw milk with a SPC $< 5x10^4$ under conditions of machine milking in the absence of a milk pipeline were considered for the co-operative experiments initiated by Dr. Johns in 1961. The fourteen farms to be used in the study were selected at random from those eligible. Each week three farms were visited at a time when the bulk tanks contained milk from two milkings. The milk was mechanically agitated for 4 minutes and samples were taken aseptically. While at the farm, a record (see Appendix p. 66) was made of the sanitary condition, as determined by visual examination, of all equipment with which the milk had come in contact. The samples were refrigerated at 35° F until returned to the laboratory where they were stored overnight at 38° F. A SPC was made on each sample and plates containing 30-300 colonies were selected. From each of the duplicate plates, 25 colonies were picked into yeast-dextrose litmus milk giving 50 cultures per sample. The colonies to be picked were selected by means of a 5x5 line grid giving a pattern of squares with 25 intersections. The colony nearest

Fig. 2. Scheme for Differentiation of the Main Types of Bacteria Isolated from Raw Bulk Tank Milk.



each intersection was picked. After incubation at 32° C for 3 days, reactions of the litmus milk to the bacteria were recorded. Twenty-four hr. litmus milk cultures were streaked onto SPC agar plates and incubated at 32° C for 18 hrs. From the agar streaks, smears were made for Gram staining (22) and were examined microscopically; the staining reaction and morphology being recorded. Following a catalase test using 10% (v/v) hydrogen peroxide on the plate cultures, Gram -ve rods were inoculated into brilliant green bile broth (Difco) in tubes containing inverted vials and incubated at 32° C for 48 hrs. to check for gas production. Catalase +ve cocci were tested for the presence of coagulase using Bacto Plasma (Difco) in a slide test (4, 18). Gram +ve, catalase +ve rods were streaked on slants of SPC agar containing 1 ml. of 0.001% (w/v) Mn^{++} as $MnSO_4$ per liter (25) and incubated at 32° C for 15 days after which time smears were made and spore stains carried out (22).

From the results of these tests and with the aid of Bergey's Manual (3) it was possible to classify the isolates according to the scheme shown in Fig. 2.

RESULTS

The numbers and percentages of bacterial types found in raw bulk tank milk of fourteen farms in the Winnipeg area are reported in Table 5. The most prominent types of organisms were micrococci 51%, Gram -ve rods 20%, and streptococci 19%, which together made up 90% of the cultures identified. Gram +ve asporogenous rods, coliform organisms and staphylococci made smaller contributions. The incidence of lactobacilli and bacilli was negligible.

The percentage of milk samples in which the different types of bacteria were detected, and the proportion in which the different types constituted 50% or more of the bacterial flora, i.e. in which they were dominant, are given in Table 6. Micrococci, Gram -ve rods, and streptococci were found in a high proportion of the samples. Micrococci were detected in 100% of the samples examined and were dominant in the bacterial flora of 55.8%. Though Gram -ve rods were found in 89.2% of the milk samples they were frequent or dominant in only 16.2%. Streptococci were detected in 88.3% of the samples and were dominant in 10.8%. Gram +ve asporogenous

TABLE 5

Numbers and Percentages of Bacterial Types Found in Raw Bulk Tank Milk of Fourteen Farms in the Winnipeg Area

Type of Organism	Number Isolated	% of Total
Micrococci	2855	51.44
Gram -ve rods	1111	20.02
Streptococci	1066	19.21
Gram +ve asporogenous rods	151	2.72
Coliform	125	2.25
Staphylococci	112	2.02
Bacilli	10	0.18
Lactobacilli	3	0.05
Unclassified	117	2.11
Total	5550	100.00

TABLE 6

Incidence of Milk Samples in which Various Bacterial Types were Detected (a) and in which these Types Contributed 50% of More of the Bacterial Flora (b)

Type of Organism	% of samples (a)	(b)
Micrococci	100	55.8
Gram -ve rods	89.2	16.2
Streptococci	88.3	10.8
Gram +ve asporogenous rods	44.1	0.0
Staphylococci	36.0	0.0
Coliform	27.0	0.0
Lactobacilli	2.7	0.0
Bacilli	4.5	0.0
Unclassified	37.8	0.0

rods were found in 44.1% of the samples but were frequent in none. Those organisms were mainly coryneform types in morphology but no distinction was made between the heat labile diphtheroid types and the thermoduric corynebacteria (microbacteria), as no heat resistant tests were done. Such a study may be of interest to health authorities. Coagulase +ve staphylococci were detected in 36% of the milk samples but did not form the dominant group in any of the samples. Coliform organisms were found in 2.7%. They did not constitute an appreciable proportion of the colonies picked from any of the samples. As was previously mentioned, lactobacilli and bacilli contributed very little to the total bacterial flora. Lactobacilli were found in only 2.7% of the samples and bacilli in 4.5%. Neither type was dominant in any of the samples. Unclassified organisms appeared in 37.8% of the samples.

The distribution of bacterial types in relation to colony count is given in Table 7. Farm milk supplies with colony counts of $< 2 \times 10^4$ are considered to have been produced and handled under satisfactory conditions according to Griffiths *et al* (9) and counts $< 5 \times 10^3$ are not uncommon where milking equipment is properly cleaned and sanitized before use.

TABLE 7

Incidence of Different Types of Bacteria According to Range of Colony Count of the Milk Sample

Type of Organism	Nos. of organisms and % (in brackets) isolated from samples with colony counts/ml within the ranges: $<5\times10^3$	6×10^3 - 2×10^4	2.1×10^4 - 5×10^4	$>5\times10^4$	Total
Micrococci	790 (65.8)	1096 (46.6)	588 (49.0)	391 (48.9)	2855 (51.4)
Streptococci	182 (15.0)	418 (17.7)	319 (26.6)	147 (18.4)	1066 (19.2)
Gram -ve rods	151 (12.6)	590 (25.1)	191 (15.9)	184 (23.6)	1111 (20.0)
Gram +ve asporogenous rods	11 (0.9)	61 (2.5)	39 (3.3)	17 (2.1)	151 (2.7)
Bacilli	2 (0.2)	2 (0.1)	3 (0.3)	3 (0.4)	10 (<0.1)
Lactobacilli	3 (0.3)	0 (0)	0 (0)	0 (0)	3 (<0.1)
Staphylococci	27 (2.3)	44 (1.9)	20 (1.7)	24 (3.0)	112 (2.0)
Coliform	11 (0.9)	92 (3.9)	9 (0.8)	13 (1.3)	125 (2.3)
Unclassified	25 (2.1)	46 (1.9)	31 (2.5)	21 (2.6)	117 (2.1)
Total	1200 (21.6)	2350 (42.3)	1200 (21.6)	800 (14.4)	5555

When colony counts range from $2x10^4$ to $2x10^5$ it is generally recognized that cleaning methods could be improved, whereas counts > $2x10^5$ are termed unsatisfactory. Since the farms chosen to participate in this study were limited to those consistently producing milk with a SPC < $5x10^4$, there was no need for the high count classes suggested by Griffiths et al. The ranges reported in Table 7 are, to some extent, a modification of those used by the previous worker.

The bacterial flora of the 24 milk samples produced under very strict hygenic conditions (SPC < $5x10^3$) was dominated by micrococci which constituted 65.8% of the 1200 cultures. Streptococci (15.0%) and Gram -ve rods (12.6%) also made significant contributions.

The cultures identified from the next group of 47 milk samples with reasonably low colony counts ($6x10^3$ - $2x10^4$) differed somewhat from those of the first group. Micrococci were not so dominant, forming only 46.6% of the cultures. Gram -ve rods were much more common, showing an increase from 12.6% in the first colony count range to 25.1% in the second. The percentage of streptococci remained almost constant while that of Gram +ve asporogenous rods showed an increase from 0.9% of the cultures isolated in the first colony count range

to 2.5% in the second range. The figure for this type of organism remained almost constant through the second, third, and fourth colony count ranges. Coliform organisms made up only 0.9% of the bacterial flora in the milk samples with colony count $< 5x10^3$ but their contribution to the flora of the samples in the second range was 3.9%.

The series of 24 samples of milk of doubtful hygenic quality with colony counts between $2x10^4$ and $5x10^4$ showed some changes in distribution of bacterial flora from the second group. Micrococci remained almost constant in contribution while the percentage of Gram -ve rods dropped from 25.1% in the previous series to 15.9% and the percentage of streptococci increased from 17.7% to 26.6%.

Micrococci (48.9%) and Gram -ve rods (23.6%) together with streptococci (18.4%) were dominant among the 800 bacteria isolated from 16 milk samples with high SPC ($> 5x10^4$). Micrococci and Gram -ve rods were dominant in 5 samples, making up more than 50% of the cultures isolated. Gram -ve rods were dominant in 4 samples, and streptococci dominated only 1 sample although this type of organism occurred in a large number of the samples.

At the time of collecting milk samples from the farm

bulk tank, a visual examination of all equipment with which the milk had come in contact was carried out. The farms were classified "good", "fair", and "poor" on the basis of this visual examination (see Appendix p. 66). Table 8 shows the distribution of bacterial types in the raw bulk tank milk from the farms classified as "good", "fair", and "poor".

Three farms qualified for the "good" category on the basis of techniques used in cleaning their equipment and the cleanliness achieved. Of the 1100 bacterial cultures identified 54.4% were micrococci, 27.0% Gram -ve rods, and 8.2% streptococci.

Six farms were classed "fair" and the remaining five "poor". There was a gradual decrease in the percentage of Gram -ve rods from 27.0% for good farms to 22.9% for fair farms and 12.5% for poor ones, while the percentage of micrococci remained almost constant throughout.

Streptococci made up only 8.2% of the cultures identified from milk samples of good farms; the percentage increased to 20.7% for fair farms, and showed a further increase to 24.4% of the cultures isolated from milk samples of poor farms.

TABLE 8

The Bacterial Flora of Raw Bulk Tank Milk from 14 Farms in the Winnipeg Area Classified as Good, Fair, and Poor by Visual Examination.

Type or Organism	Good Farms (3)	Fair Farms (6)	Poor Farms (5)
Micrococci	598 (54.4)*	1150 (47.9)	1105 (53.9)
Gram -ve rods	298 (27.0)	551 (22.9)	257 (12.5)
Streptococci	90 (8.2)	497 (20.7)	500 (24.4)
Gram +ve asporo-genous rods	21 (1.9)	61 (2.5)	47 (2.3)
Coliform	49 (4.5)	47 (1.9)	35 (1.7)
Staphylococci	30 (2.7)	38 (1.6)	47 (2.3)
Bacilli	2 (0.2)	4 (0.2)	5 (0.2)
Lactobacilli	3 (0.3)	0 (0)	0 (0)
Unclassified	9 (0.9)	52 (2.1)	54 (2.6)
Total	2050	2400	1100

*Percentages are in brackets.

In an attempt to show how variations in the bacterial flora of milk samples from individual farms can effect the results reported in Tables 5 to 8 the flora of three farms, one from each of the categories good, fair, and poor as classified for Table 8, are reported in Table 9. The colony counts of all milk samples from the good farm were $< 5x10^3$. The proportion of micrococci (85.7%) was much higher for the milk of this particular farm than for the average milk sample with colony count $< 5x10^3$ reported in Table 7 where micrococci made up 65.8% of the cultures identified. Micrococci made up 54.4% of the bacteria isolated from milk samples of good farms in Table 8. Only 4.0% Gram -ve rods, and 4.8% streptococci were identified. Surprisingly, 3.7% of the bacteria isolated from the milk of this farm were staphylococci.

The dominant type of organism in the farm classed "fair" in Table 9 is Gram -ve rods which make up 53.5% of the organisms isolated. The incidence of streptococci (14.9%) was close to the overall results reported in Table 5, but micrococci (19.5%) were much less prevalent. Coliform organisms (4.4%) and Gram +ve asporogenous rods (2.3%) were the only other types of organisms making significant

TABLE 9

No.'s and % (in Brackets) of the Various Bacterial Types Isolated from the Raw Bulk Tank Milk of Three Farms, One from Each of the Categories Poor, Fair, and Good as Classified for Table 8.

Type of Organism	Poor	Fair	Good
Micrococci	165 (36.6)	88 (19.5)	300 (85.7)
Gram -ve rods	44 (9.7)	241 (53.5)	14 (4.0)
Streptococci	190 (42.2)	67 (14.9)	17 (4.8)
Gram +ve asporo-genous rods	14 (3.1)	13 (2.3)	3 (0.9)
Coliform	4 (0.8)	20 (4.4)	1 (0.3)
Staphylococci	12 (2.6)	6 (1.1)	13 (3.7)
Bacilli	2 (0.4)	3 (0.6)	0 (0)
Lactobacilli	0 (0)	0 (0)	0 (0)
Unclassified	19 (4.2)	12 (2.2)	2 (0.6)
Mean SPC x 10^3	30	32	3
Total Cultures	450	450	350

contributions.

Streptococci (42.2%) were the dominant type of organism in the milk samples of the farm classed "poor". The percentage of Gram -ve rods was quite low at 9.7% while micrococci made up 36.6% of the 450 cultures identified. Gram +ve rods (3.1%) and staphylococci (2.6%) were present in smaller numbers.

The percentages of bacterial types found in raw bulk tank milk of individual farms are given in Table 10. Percentages of micrococci given in Table 10 range from 85.7 (farm 10) to 19.5% (farm 11) with an average of 51.4%. The average Gram -ve rod content of all milk samples examined was 20%. Farm 11 had the highest proportion of Gram -ve rods (53.5%) while farm 5 had the lowest (1.5%). There was much less variation in the percentages of streptococci in the milk of the fourteen farms. The greatest percentage was reported from farm 13 (42.2%) while farm 2 had the least with 5.4%. The overall average streptococci content of the milk samples was 19.2%. Gram +ve asporogenous rods, and staphylococci made small contributions to the total bacterial flora of most farms. Coliform organisms made a significant contribution to the flora of four farms. Bacilli and lactobacilli

TABLE 10

Percentages of Bacterial Types Found in the Raw Bulk Tank Milk of Individual Farms Classified Good, Fair and Poor, in the Winnipeg Area.

Farm	Micrococci	Gram -ve rods	Streptococci	Gram +ve asporogenous rods	Coliform	Staphylococci	Bacilli	Lactobacilli	Unclassified	Visual Classification
1	56.0	16.8	20.6	1.2	2.3	2.2			1.2	Fair
2	38.3	48.6	5.4		3.9	2.4	0.3	0.6	0.9	Good
3	43.4	30.8	20.6	1.1	1.8	1.1	0.3		1.0	Fair
4	74.5	8.3	10.5	2.6	1.5				2.5	Poor
5	75.0	1.5	15.0	3.1		2.3			3.0	Poor
6	47.0	32.0	17.0	1.0		0.5			2.5	Poor
7	54.0	23.7	13.7	2.5	1.0	1.0			3.7	Fair
8	43.5	27.5	13.5	3.2	8.8	2.3		0.2	0.9	Good
9	58.5	8.0	25.2	3.5	1.0	1.0			2.7	Fair
10	85.7	4.0	4.8	0.9	0.3				0.6	Good
11	19.5	53.5	14.9	2.3	4.4	1.1	0.6		2.2	Fair
12	58.6	4.8	23.7	5.7	1.5	2.8			2.4	Fair
13	36.6	9.7	42.2	3.1	.8	2.6	0.4		4.2	Poor
14	38.7	11.3	35.5	3.0	4.1	5.9	0.8		0.8	Poor

were negligible in every case.

The percentage of bacterial types isolated from raw milk and reported by workers cited in this thesis appear in Table 11. The results of this study agree quite closely with the mean results of all workers in the three main bacterial types, micrococci, Gram -ve rods, and streptococci. The greatest variation occurs in the results of Schalm and Lasmanis who worked with a single herd. There appears to be a considerable difference between the percentages of the various types of organisms identified by Garvie and Rowlands, who used 22° C for incubation, and the remainder of the results which were obtained at higher temperatures. The results of Thomas et al, Jackson, and Ashton each include a study of in excess of 2000 cultures, while other workers made fewer isolations.

The results of the identification of the bacterial flora of each milk sample taken from each farm are reported in the Appendix pp. 52-65.

TABLE 11

Percentage of Bacterial Types Isolated from Raw Milk

Type of Organism	Workers										
	McKenzie & Bowie		McKenzie & Booker	Schalm & Lasmanis	Carreira et al	Garvie & Rowlands		Thomas et al	Jackson	Ashton	Mean
	Poor	Good				22°C	37.5°C				
Micrococci	33	23	68.8	70.8	68	11.6	20.0	38.4	46.2	51.9	43.2
Gram -ve rods	43	16	3.0	1.1	9	36.9	27.3	20.9	11.8	20.0	18.9
Streptococci	12	54	19.0	8.5	15	23.9	28.0	15.2	26.5	19.2	22.0
Gram +ve asporogenous rods	12	7	9.4	.2	5	18.0	9.0	11.0	6.9	2.7	8.1
Coliform				11.2		1.1	1.6	6.5	13.0	2.2	6.0
Staphylococci						2.5	10.0			2.0	5.0
Bacilli								5.4	3.5	.2	3.0
Lactobacilli										.05	
Unclassified				8.2	3	5.6	3.3	2.6	4.6	2.1	4.5
Cultures Identified	78	61	137.0	--	--	284	275	2065	2820	5555	

DISCUSSION

Only those farms consistently producing raw milk with a SPC $< 5x10^4$ under conditions of machine milking were considered by Dr. Johns in 1961. The experiments were designed to assess existing laboratory tests, (particularly PI), for raw milk quality in an attempt to find a bacteriological test which would indicate sanitary conditions of production even when milk was stored cold in a bulk tank on the farm. It was felt that existing laboratory tests were capable of indicating unsatisfactory conditions of production on the farm when raw milk had a SPC $> 5x10^4$. However, many shippers, with the aid of bulk tank cooling, were producing milk with a SPC $< 5x10^4$ under conditions of cleanliness which were considered unsatisfactory by visual examination. Therefore, a new test, in order to be of practical value, would have to be able to detect unsatisfactory conditions of sanitation on the farm even when the SPC of raw milk was $< 5x10^4$.

The cleanliness of all pieces of equipment with which the milk had come in contact was determined by visual examination at the time of sampling. Unfortunately the inside surfaces of milk pipelines are rather difficult to inspect

therefore all farms with pipelines were excluded from the experiment.

With these restrictions in effect, the fourteen farms chosen to participate in the experiment can hardly be regarded as representative of all the farms in the Winnipeg area producing raw milk and using bulk tank storage. Therefore, the results obtained from a determination of the bacterial types present in the raw milk of these fourteen farms do not necessarily apply to all raw milk produced in this area.

Colonies were picked from SPC agar into enriched litmus milk (Y.D. litmus milk) containing 100 gm. dehydrated litmus milk (Difco), 5 gm. yeast extract (Difco), and 5 gm. bacto-dextrose (Difco) per liter of distilled water. Although the reactions of enriched litmus milk to growing bacterial cultures were of limited value for identification purposes, it was necessary to add yeast extract and dextrose to the media to approximate the composition of SPC agar so that all bacterial colonies growing on SPC agar and picked into Y.D. litmus milk would continue to grow. Bergey's Manual does not contain reactions of Y.D. litmus milk to bacterial growth, but with some experience it was possible to

use the Y.D. litmus milk reactions as an aid in identification. For example, most organisms placed in the bacterial type "Gram -ve rods" produced either no change, or an alkaline reaction in Y.D. litmus milk, while most organisms classified as "Coliforms" caused acid coagulation and gas production in Y.D. litmus milk.

The scheme for differentiation of the main types of bacteria isolated from raw bulk tank milk given in Fig. 2 does not include all the microorganisms that are likely to be present in milk, nor does it classify all organisms to the same extent. However, it does distinguish between the main bacterial groups present in raw milk. Microorganisms not included in this scheme, and isolates which failed to grow were placed in a group termed "Unclassified".

There are several reasons why some isolates failed to develop in Y.D. litmus milk:

(i) Very small sub-surface colonies on SPC agar may become obscured when the surface of the agar is deformed by the platinum loop during picking and it is possible that the colony is not transferred.

(ii) The colony, when picked from SPC agar, may be surrounded by a small block of agar and during incubation in

Y.D. litmus milk may fail to grow out of the agar.

(iii) Essential growth substances may be lacking in Y.D. litmus milk.

An idea of the types of bacteria likely to be found in raw milk, and the contribution of each type to the total flora can be gained from the Review of Literature. There is general agreement that when milk leaves the udder of a healthy cow the bacterial flora consists mainly of micrococci together with smaller numbers of staphylococci and coryne-bacteria. Other types of bacteria found in milk are the result of contamination from equipment coming in contact with the milk, and from the air. It would appear, therefore, that milk leaving the udder contains a minimum population of bacteria dominated by micrococci. Milk taken from the farm bulk tank contains counts of bacteria which range upward in size from the minimum supplied by the udder; the actual colony count being dependent upon (i) the cleanliness of milking equipment, and (ii) the technique used in the milking operation. One would expect the organisms derived from dirty equipment to be primarily saprophytic because of the compo-sition of the residues on poorly cleaned milking utensils, and because of the temperature (50-75° F) at which such

equipment is stored when not in use. The types of organisms making up this saprophytic group would probably vary from farm to farm, depending upon the habits of the individuals involved, the bacterial flora of the water supply, the type of cleaning and sanitizing chemicals used, and the cleanliness and nature of the area used for housing and milking the cows.

The results of Thomas et al which are summarized on page 11 of this thesis support the above reasoning. These workers reported a predominance of micrococci in milk of low count, a co-dominance of streptococci, corynebacteria, and micrococci in milk with colony counts between $2x10^4$ and $2x10^5$, and the dominance of non-pigmented Gram -ve rods and streptococci in the high count milks. Several milk samples examined by them had a characteristic individual flora, irrespective of colony count level, dominated by either corynebacteria, aerobic spore formers, coli-aerogenes organisms, fluorescent pseudomonads, or flavobacteria. These results were confirmed by Jackson as shown in Table 4, and by the distribution of main types of bacteria found in raw bulk tank milk of fourteen farms in the Winnipeg area as shown in Table 5.

The milk samples examined in this study contained low

18 hr. cultures and therefore many bacilli could have been placed in the classification "Gram -ve rods" and thus never tested for spore production.

The distribution of main types of bacteria among all the 5550 cultures reported in Table 5, provides an interesting comparison with the results obtained by Garvie and Rowlands (6) for cultures picked from 22° C yeastrel milk agar plates poured with 14 hr. old milk. These workers found a much lower incidence of micrococci (14% compared with 51%), and a somewhat higher incidence of streptococci (24% compared with 19%). Gram +ve rods were also common (18% compared with 3%) and were nearly all corynebacteria. Gram -ve rods constituted 31% of the bacterial flora compared with 20% in the present investigation. The low incubation temperature (22° C) may have caused some significant differences since, micrococci originate largely in the cow's udder and have a higher optimum growth temperature while Gram -ve rods may be favoured at 22° C.

Table 6 indicates that only the three types of organisms, micrococci, Gram -ve rods, and streptococci were frequent or dominant in the milk samples examined. In this connection, it is interesting to note the point made by

Thomas et al (23) who suggested that in surveys designed to identify pure cultures picked as colonies from agar plates most of the types of bacteria selected by the colony picking technique must constitute an appreciable proportion of the microflora of milk able to form colonies on the media used and at the temperature of incubation. That is, only the most abundant types are selected.

The incidence of the different bacterial types according to the colony count of the milk sample is reported in Table 7. As a result of preceding evidence and discussion, one would expect that the flora of milk samples falling into the first colony count range ($< 5x10^3$ bacteria per ml) would be dominated by micrococci together with smaller numbers of Gram -ve rods and streptococci. As the colony count increased through the other three ranges one would expect a decrease in the contribution of micrococci to the total flora, with a corresponding increase in the incidence of both streptococci and Gram -ve rods. This trend cannot be expected to be as pronounced as that described by Thomas et al because of the limited number of milk samples with high colony counts examined in this study.

Of the bacterial flora of the 24 milk samples with

colony counts < $5x10^3$, 92.5% consisted of micrococci (65.8%), streptococci (15.0%), and Gram -ve rods (12.6%). The corresponding percentages reported by Jackson (Table 4) for samples of raw bulk tank milk with colony counts in this range were, micrococci 67.8, streptococci 16.3, and Gram -ve rods 4.1 for a total contribution of 88.2% to the flora of this category. He found that asporogenous Gram +ve rods contributed 5.9% and bacilli 3.1% as compared to 0.9% and 0.2% respectively in Table 7. Reasons for the low incidence of bacilli have already been discussed (p. 35). The differences in incidence of asporogenous Gram +ve rods could be due to laboratory technique, or to unknown factors prevailing on the farms used in the two studies.

The next group of 47 milk samples in Table 7, with colony counts between $6x10^3$ and $2x10^4$ differ somewhat in the percentages of micrococci and Gram -ve rods from the samples of the first range, while the percentage contribution of streptococci increased only slightly. The decrease of micrococci from 65.8% to 46.6% and the increase of Gram -ve rods from 12.6% to 25.1% was to be expected with a corresponding increase in colony count of the milk sample. The percentage of coliform organisms present increased from 0.9 in the first

range to 3.9 in the second, and then declined again as colony count increased through the third and fourth ranges. The higher incidence of coliform organisms in the second category is probably due to the influence of Farm 8 (see Appendix 1, p. 59) which had two milk samples, containing 33 coliform organisms, in this colony count range. The cows were kept in a loose housing barn on this farm and milked in a three-unit parlour. It is possible that the cows' udders and flanks carried a considerable amount of refuse, containing coliform organisms, into the milking parlour from the loose housing environment where a manure pack was allowed to build up. Fewer coliform organisms were found in the milk samples from the other farms where the cows were housed and milked in a stanchion barn which was cleaned several times daily.

There were 24 milk samples with colony counts in the range 2.1×10^4 and 5×10^4 and 16 with counts 5×10^4. The distribution of bacterial flora amongst the various types of organisms fails to follow the predicted pattern as colony count increases. For example, the percentage of micrococci is almost constant around 48% in the three upper ranges while it was predicted that it should show a gradual decline. The

percentage of streptococci shows a gradual increase up to the end of the third range, then drops again in the fourth range while the percentage of Gram -ve rods increases from the first range to a maximum in the second, then shows a considerable decrease, finally increasing again in the fourth range. These inconsistencies can be explained by an examination of the flora of individual farms which could, by placing several milk samples in a given colony count range, greatly influence the results of that range. This point is developed more fully in the discussion of Table 9.

Table 8 shows the bacterial flora of the 14 farms used in this study classified "good", "fair" and "poor" on the basis of a visual examination of the cleanliness of the milking equipment (Appendix 2, p. 66). The results for good farms reported in Table 8 differ considerably from those reported by McKenzie and Bowie (Table 1), whose percentages were, micrococci 23, Gram -ve rods 16, and streptococci 8.2. If the results in Table 8 for good farms are compared with the overall results reported in Table 5 it is seen that there is little difference between the percentages of micrococci. Gram -ve rods increased from 20% of the total bacterial flora reported in Table 5 to 27% of the bacterial flora in the milk

from good farms reported in Table 8. At the same time, there was a marked decrease in the percentage of streptococci to 8.2% of the bacteria in the milk of good farms from 19.2% of the total bacterial flora identified. McKenzie and Bowie found that streptococci made up only 12% of the bacterial flora of milk of farms classed "poor" by visual examination. In the present study the percentage of streptococci showed an increase from 8.2% of the bacteria identified in the milk of good farms to 20.7% for fair farms, and 24.4% for poor farms. If the classification of farms by visual examination was accurate in the present study, it would appear from these results that the percentage of streptococci in raw bulk tank milk increases as the sanitary conditions on the farm deteriorate while at the same time the percentage of Gram -ve rods decreases. These results contradict the trend shown in Table 7 where it was observed that as raw milk colony count which is often used as a measure of sanitation on the farm increased, the percentage of Gram -ve rods also increased.

A possible explanation for these inconsistencies may be found in an examination of the results of individual farms (Table 9). Several farms, irrespective of the colony count of the milk samples, had a characteristic individual flora

dominated either by micrococci, Gram -ve rods, or streptococci. Gibson and Abd-el Malek (8) showed that udder flora is composed mainly of micrococci; other bacterial contaminants of raw milk being picked up from dirty equipment and from the air. This information can be used to explain the high percentage of micrococci in the good farm of Table 9 since visual examination showed that techniques used in cleaning milking equipment were excellent, eliminating bacterial contamination from utensils and the air. However, it fails to explain the big difference between the proportion of micrococci in milk samples from one good farm and the average micrococci content of three good farms considered together in Table 8. Perhaps the visual examination was inaccurate and the other two farms included in the "good" category in Table 8 are misplaced.

The farm classed "fair" in Table 9 has an unusually high proportion of Gram -ve rods in its bacterial flora. It was noticed, while making the visual examination of milking equipment on the farm, that this producer used a Quaternary Ammonium Compound (Q.A.C.) for sanitizing surfaces of equipment with which the milk was to come in contact. One of the properties of Q.A.C. is selectivity against Gram +ve organisms.

This fact is offered as a probable explanation for the dominance of Gram -ve rods in the cultures isolated from raw milk samples of the "fair" farm in Table 9.

The proportion of streptococci in the milk samples of the farm classed "poor" in Table 9 is very high (42.2% of the cultures identified) when compared to the overall results of Table 5 (19.2% streptococci) and the average streptococci content for poor farms (24.4%) given in Table 8. The sanitary conditions under which milk was being produced on this farm were unsatisfactory. It is interesting to note that Reinbold (20) suggested that with well-cooled milks the enterococci are valuable as an index of sanitation. Since no attempt was made in this study to separate the various groups which make up the species of the Genus Streptococcus it is possible that large numbers of bacteria of the Enterococcus Group were present in the raw milk of this particular farm, confirming Reinbold's belief.

In Table 10, the percentages of bacterial types found in raw bulk tank milk of individual farms are reported. It is interesting to note that while the percentages of streptococci showed little variation between farms, there was a great deal of variation in the percentages of both micrococci

and Gram -ve rods. It was suggested in the Review of Literature that in raw milk of high colony count, i.e. milk produced under unsatisfactory conditions of the farm, there is a dominance of Gram -ve rods and streptococci. In low count milk Thomas et al (23) reported a predominance of micrococci. It is possible to support these generalizations with the results of some of the farms reported in Table 11. However, when one considers the overall results reported in Tables 7 and 8 it is difficult to recognize such trends. The one generalization which may be valid from this study is that as colony count of milk increases the percentage of streptococci tends to increase. However, it is necessary to bear in mind that one farm with high colony counts also had a particularly high percentage of streptococci, and may have influenced the trend.

The results of studies of the bacterial flora of raw milk by all workers cited in this thesis are reported in Table 12. The wide variations in conditions supplied by the workers for the cultures isolated prevent the drawing of comparisons. For example, Garvie and Rowlands (6) used both 22° C and 37.5° C while Thomas et al (23) used 30° C. Also, some workers reported very small numbers of isolates since

the identification of flora of raw milk was only a small part of a larger investigation. It is interesting to note, however, that the results obtained in this study are fairly close to those obtained by workers identifying in excess of 2000 cultures, and also closely approximate the mean results of all workers.

CONCLUSIONS

The fourteen farms chosen to participate in the experiment were not representative of all the farms in the Winnipeg area producing raw milk and using bulk tank storage. Therefore, the results obtained from a determination of the bacterial types present in the raw milk of these fourteen farms do not necessarily apply to all raw milk produced in this area.

1. Micrococci was the predominant type of organism in the raw bulk tank milk of the fourteen farms studied in the Winnipeg area. Gram -ve rods and streptococci also made significant contributions to the total flora. Asporogenous Gram +ve rods, coliform organisms, and staphylococci each made small contributions. The incidence of bacilli and lactobacilli was negligible.

2. The incidence of streptococci was greater on farms classed poor by visual examination than on those classed fair and good.

3. Milk samples from individual farms possessed characteristic flora dominated by either micrococci, Gram -ve rods, or streptococci.

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APPENDIX 1

Distribution of Bacterial Types Selected from the Raw Bulk Tank Milk of Individual Farms in the Winnipeg Area

Farm	Micrococci	Gram -ve rods	Streptococci	Gram +ve asporogenous rods	Coliform	Staphylococci	Bacilli	Lactobacilli	Unclassified	Total
1	194	59	72	8	8	7			25	350
2	134	170	19		13	8	1	2	3	350
3	152	108	72	4	6	4	1		3	350
4	298	33	42	11	6				10	400
5	300	6	60	13		9			12	400
6	188	128	68	4		2			10	400
7	212	94	55	11	4	4			15	400
8	174	110	54	13	35	9		1	4	400
9	231	32	101	19	4	62			11	400
10	300	14	17	3	1	13			2	350
11	88	241	67	13	20	6	3		12	450
12	264	22	107	26	7	13			11	450
13	165	44	190	14	4	12	2		19	450
14	155	45	142	12	17	23	3		3	400
Total	2855	1111	1066	151	125	112	10	3	117	5550

APPENDIX 1 cont'd

Appendix pp. 52-65 . The Numbers of Bacteria of Each Type Isolated from Each Sample of Raw Bulk Tank Milk for Each of the Fourteen Farms.

FARM 1. Fair

Type of Organism	SAMPLES 1	2	3	4	5	6	7
Micrococci	34	22	30	32	19	35	24
Gram -ve rods		4	2	13	31	8	1
Streptococci	11	20	15			2	24
Gram +ve asporo-genous rods		1		2		1	
Coliform	1	1		1		4	
Staphylococci	4		2	1			
Bacilli							
Lactobacilli							
Unclassified		2	1	1			1
SPC x 10^3	3	4	8	14	8	5	3

APPENDIX 1 cont'd

FARM 2. Good

Types of Organism	SAMPLES 1	2	3	4	5	6	7
Micrococci	33	35	19	10	8	8	11
Gram -ve rods	2	4	15	36	39	39	39
Streptococci	10	3	3		1	2	
Gram +ve asporo-genous rods		4		1			
Coliform			8	3	2		
Staphylococci	1	2	5				
Bacilli	1					1	
Lactobacilli	2						
Unclassified	1						
SPC x 10^3	5	12	8	15	14	11	17

APPENDIX 1 cont'd

FARM 3. Fair

Type of Organism	SAMPLES 1	2	3	4	5	6	7
Micrococci	28	16	13	34	24	4	33
Gram -ve rods	19	28	30	3	21	1	6
Streptococci		1	7	9	5	42	8
Gram +ve asporo-genous rods	2			1		1	
Coliform	1	4				1	
Staphylococci						1	3
Bacilli		1					
Lactobacilli							
Unclassified				3			
SPC x 10^3	4	4	3	13	19	25	40

APPENDIX 1 cont'd

FARM 4. Poor

Type of Organism	SAMPLES 1	2	3	4	5	6	7	8
Micrococci	35	30	28	43	43	47	22	50
Gram -ve rods	9	1	2		6	2	19	
Streptococci	5	11	17	1		1		
Gram +ve asporo-genous rods	1	6	3				1	
Coliform				6				
Staphylococci					1			
Bacilli								
Lactobacilli								
Unclassified		2					8	
SPC x 10^3	4	2	9	10	18	250	300	330

APPENDIX 1 cont'd

FARM 5. Poor

Type of Organism	Samples 1	2	3	4	5	6	7	8
Micrococci	38	31	18	43	34	47	43	46
Gram -ve rods	2	1	1			1	1	
Streptococci	8	16	17	6	3	2	5	3
Gram +ve asporo-genous rods			11				1	1
Coliform								
Staphylococci			1	1	7			
Bacilli								
Lactobacilli								
Unclassified	2	2	2		6			
SPC x 10^3	9	15	100	290	50	47	30	23

APPENDIX 1 cont'd

FARM 6. Poor

Type of Organism	Samples 1	2	3	4	5	6	7	8
Micrococci	14	30	29	7	38	20	22	27
Gram -ve rods	28	1	1	43	1	19	17	19
Streptococci	7	17	11		11	9	10	3
Gram +ve asporo-genous rods			3			1		
Coliform								
Staphylococci		1					1	
Bacilli								
Lactobacilli								
Unclassified	1	1	6			1		1
SPC x 10^3	16	6	68	200	60	50	22	27

APPENDIX 1 cont'd

FARM 7. Fair

Type of Organism	Samples 1	2	3	4	5	6	7	8
Micrococci	29	33	44	14	17	35	19	25
Gram -ve rods	1	2	1	35	20	9	19	8
Streptococci	13	11	4	1	11	4	3	8
Gram +ve asporo-genous rods	2						6	3
Coliform		1					3	
Staphylococci		1	1					2
Bacilli								
Lactobacilli								
Unclassified	5	2			2	2		4
SPC x 10^3	4	3	4	18	6	7	14	25

APPENDIX 1 cont'd

FARM 8. Good

Type of Organism	Samples 1	2	3	4	5	6	7	8
Micrococci	29	42	36	8	32	12	10	5
Gram -ve rods	7	4	5	19	6	17	13	39
Streptococci	4	4	8	20	9	2	5	2
Gram +ve asporo-genous rods	3		1	1	1	3	3	
Coliform	2					15	18	
Staphylococci	4			2	2			1
Bacilli								
Lactobacilli	1							
Unclassified						1	1	2
SPC x 10^3	4	1	2	5	1	7	6	7

APPENDIX 1 cont'd

FARM 9. Fair

Type of Organism	Samples 1	2	3	4	5	6	7	8
Micrococci	17	25	43	32	41	27	11	38
Gram -ve rods	22	12	1	7	2	1	3	2
Streptococci	1	6	3	10	5	19	26	10
Gram +ve asporo-genous rods	2	5	1		1		5	
Coliform	4	2	2					
Staphylococci					1		2	
Bacilli								
Lactobacilli								
Unclassified	4			1		3	3	
SPC x 10^3	5	9	19	8	18	25	25	25

APPENDIX 1 cont'd

FARM 10. Good

Type of Organism	Samples 1	2	3	4	5	6	7
Micrococci	49	48	44	33	44	43	39
Gram -ve rods	1	2	3	3	4	1	
Streptococci			1	13		2	1
Gram +ve asporo-genous rods						3	
Coliform			1				
Staphylococci			1	1	2		9
Bacilli							
Lactobacilli							
Unclassified							1
SPC x 10^3	2	3	3	5	3	3	2

APPENDIX 1 cont'd

FARM 11. Fair

Type of Organism	Samples 1	2	3	4	5	6	7	8	9
Micrococci	5	9	6	19	23	4	2	8	12
Gram -ve rods	26	37	32	6	6	40	35	40	19
Streptococci	4	3		24	15	6	9	2	4
Gram +ve asporo-genous rods					1				12
Coliform	15		4	1					
Staphylococci		1	2		3				
Bacilli									3
Lactobacilli									
Unclassified			6	2	4				
SPC x 10^3	13	14	13	20	15	55	100	25	30

APPENDIX 1 cont'd

FARM 12. Fair

Type of Organism	Samples 1	2	3	4	5	6	7	8	9
Micrococci	29	25	30	24	20	25	38	29	44
Gram -ve rods	1	2	2	1	2	1	1	6	6
Streptococci	12	3	16	21	19	17	4	15	
Gram +ve asporo-genous rods	5	8			7	4	2		
Coliform						3	4		
Staphylococci		12					1		
Bacilli									
Lactobacilli									
Unclassified	3		2	4	2				
SPC x 10^3	4	8	9	16	9	7	11	30	22

APPENDIX 1 cont'd

FARM 13. Poor

Type of Organism	Samples 1	2	3	4	5	6	7	8	9
Micrococci	23	19	15	35	20	4	15	11	23
Gram -ve rods	10	1		13	13	3		4	
Streptococci	2	30	30	2	14	36	30	32	14
Gram +ve asporo-genous rods	5						5	2	
Coliform					3				
Staphylococci	8		1			2			
Bacilli	2								
Lactobacilli									
Unclassified		4				3		1	11
SPC x 10^3	8	6	20	95	31	33	28	25	28

APPENDIX 1 cont'd

FARM 14. Poor

Type of Organism	Samples 1	2	3	4	5	6	7	8
Micrococci	24	22	13	27	15	10	31	13
Gram -ve rods		6	12	7	9	5	4	2
Streptococci	19	20	18	10	24	34	15	1
Gram +ve asporo-genous rods	2		6					4
Coliform	5			6	1			6
Staphylococci			1			1		21
Bacilli								3
Lactobacilli								
Unclassified		2			1			
SPC x 10^3	20	8	33	35	100	180	200	440

APPENDIX 2

FARM RECORD SHEET

Center [ ] 1 Farm [ ][ ][ ] 2 3 4 Day [ ][ ][ ] 5 6 7 Year [ ] 8 Card No. [ ] 9

Milkhouse temperature [ ][ ] 10 11 Daily milk production [ ][ ][ ][ ] 12 13 14 15 No. cows milked [ ][ ][ ] 16 17 18

A. BULK TANK

Cleanliness:

Walls	[ ]	19
Covers	[ ]	20
Bridge	[ ]	21
Agitator	[ ]	22
Outlet valve	[ ]	23

1. Good
2. Fair
3. Poor
4. Not inspected

Type of detergent [ ] 24

1. Alkaline, 2. Chlorinated alkaline, 3. Iodophor,
4. Alternating alkaline and acid, 5. QAC, 6. Other

B. MILKING MACHINE

Type	[ ]	25
Make	[ ]	26
No. of units	[ ]	27

Type: 1. Long tube, 2. Short tube
Make: 1. DeLaval, 2. Surge, 3. Universal, 4. Rite-way, 5. Other

Rubberware

Type of inflations	[ ]	28
Type of long milk tube	[ ]	29

Type of inflations: 1. Natural, 2. Synthetic, 3. Blend
Type of long milk tube: 1. Rubber, 2. Plastic

Physical condition

Inflations	[ ]	30
Long milk tubes	[ ]	31
Short milk tubes	[ ]	32

1. Good
2. Fair
3. Poor

Cleanliness

Inflations	[ ]	33
Vacuum hose	[ ]	34
Pailhead gasket	[ ]	35

1. Clean, 2. Milkstone, 3. Milk residue, 4. Other

Pulsator

Cleanliness [ ] 36

Cleaning

Type of detergent [ ]
1. Alkaline, 2. Chlorinated alk., 3. Iodophor, 4. Alternating alk. & acid,
5. QAC detergent-sanitizer, 6. Other (specify), 7. None

Rubber storage [ ]
1. Dry, 2. Wet: lye, 3. Iodophor, 4. QAC, 5. Chlorine, 6. Other (specify)

Inflations alternated [ ]
1. Weekly, 2. Fortnightly, 3. Monthly, 4. No.

Treatment of resting set [ ]
1. Boiled, 2% lye, 2. Stored, 5% lye, 3. Other (specify)

Sanitizing [ ]
1. Hypochlorite, 2. Chloramine-T, 3. Iodophor, 4. QAC, 5. Acid
6. Other (specify), 7. None

C. PAILS & STRAINERS

Physical condition [ ]
1. Good, 2. Fair, 3. Poor

Cleanliness [ ]

D. COWS' UDDERS

Cleanliness [ ]
1. Good, 2. Fair, 3. Poor

Disinfectant in wash water [ ]
1. Hypochlorite, 2. QAC, 3. Iodophor, 4. Other (specify), 5. None

APPENDIX 2

LABORATORY REPORT SHEET

Center No. ☐ Farm No. ☐☐☐ Day No. ☐☐☐ Year No. ☐

Milk Temps.
In bulk tank ☐☐
On arrival at lab. ☐☐
Next a.m. ☐☐

S.P.C. Fresh ☐☐☐☐00 P.I. ☐☐☐☐☐☐000

Isolate No.	Day 7		Day 8	Day 9		Day 11	Day 17	Misc. obs.	CLASSIFICATION									
	LM	Morph.	Gram.	Cat.	Coag.	BGB	Spore		Bacil.	Lactob.	Microb.	Arthro.	Colif.	Gm – rod	Streptoc.	Microc.	Staph.	Unclassif.
1																		
2																		
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Grand Total								Totals										