

CHAPTER 3

RESULTS

During a six-week period of investigation from June to August 1998, a total of 214 milk samples were purchased and analysed in the Department of Animal and Community Health's food laboratory. One hundred and thirty-five of these samples originated from five "milk-shops". The remaining 79 samples were purchased at three different supermarkets who all sold the same brand of commercially available milk from a large national distributor. Milk-shop 3 and Supermarket 3 are the same shop which sold both milk from a bulk tank and milk from a large commercial national distributor.

The results obtained from the following tests are described:

- 1. Temperature of the milk at purchase
- 2. Aschaffenburg and Mullen phosphatase test
- 3. Standard aerobic plate count
- 4. Psychrotrophic bacterial count
- 5. Coliform count
- 6. Presence of *Escherichia coli* in $1 \text{ m}\ell$
- 7. Presence of *Staphylococcus aureus* in 1 ml and production of *S. aureus* enterotoxins
- 8. Presence of inhibitory substances
- 9. Somatic cell count
- 10. Presence of Brucella abortus antibodies
- 11. Isolation of Salmonella spp. from 1 ml E. coli positive milk
- 12. pH of the milk



Temperature of the Milk at Purchase

Origin	Number of samples tested	Mean temp (°C)	Standard deviation	Range (°C)	Number ≤ 5°C	Percent ≤ 5°C
Milk-shop 1	27	6.3	1.9	3.5 - 10.5	13	48.1
Milk-shop 2	27	5.9	1.1	4.0 - 7.5	9	33.3
Milk-shop 3	27	7.0	1.3	5.0 - 10.0	1	3.7
Milk-shop 4	27	8.6	1.2	6.5 - 11.0	0	0 ^b
Milk-shop 5	27	6.8	1.3	4.5 - 9.0	3	11.1
Supermarket 1 * National distributor X	25	4.0	1.3	1.5 - 7.0	20	80.0ª
Supermarket 2* National distributor X	27	5.7	1.5	2.5 - 8.5	9	33.3
Supermarket 3* National distributor X	27	6.4	1.0	4.5 - 8.0	2	7.4
Totals	214				57	26.6

Table 4:Temperature of the milk (°C) at the time of purchase

* milk from the same national distributor, purchased at three different outlets

^a differed significantly from all other outlets

^b differed significantly from all other outlets

The Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) stipulates that the temperature of milk should immediately be brought down to 5°C or less after pasteurisation (processing), and maintained at this temperature to retard bacterial growth. Retailers are obliged to keep milk at a temperature of less than 5°C. Table 4 and Figure 1 show that this target was only met in 26.6% of samples purchased. Only one of the larger retailers (Supermarket 1) consistently kept the temperature of their milk under 5°C, averaging 4°C during the six-week sampling period. The temperature of the milk from this supermarket differed significantly (p < 0.05) from all the other outlets. All the other shops had averages greater than 5°C, with one milk-shop (Milk-shop 4) never attaining a temperature below 5°C. This shop also differed significantly (p < 0.05) from all the other shops selling milk.



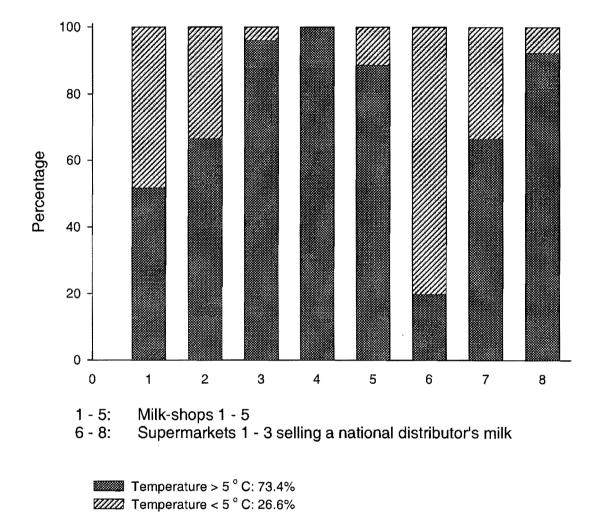


Figure 1: Percentage of milk samples with a temperature greater than or less than 5°C at each of the different outlets





Aschaffenburg and Mullen Phosphatase Test

Origin	Number of samples tested	Labelled as pasteurised	Alkaline phosphatase positive
Milk-shop 1	27	19 (70.4) ^a	27 (100) ^a
Milk-shop 2	27	20 (74.1)	0 (0)
Milk-shop 3	27	27 (100)	0 (0)
Milk-shop 4	27	27 (100)	25 (92.6)
Milk-shop 5	27	0 (0)	0 (0)
Supermarket 1 * National distributor X	25	25 (100)	0 (0)
Supermarket 2 * National distributor X	27	27 (100)	0 (0)
Supermarket 3 * National distributor X	27	27 (100)	0 (0)

Table 5:Indication of the alkaline phosphatase status of the milk, and whether it was
labelled as pasteurised

* milk from the same national distributor, purchased at three different outlets

^a indicates percentage of total

Of the 135 milk-shop milk samples tested, 52 (38.5%) were alkaline phosphatase positive (Table 16). These samples all originated from two of the five milk-shops, one of which had no negative alkaline phosphatase results in the samples taken over the six-week period (Table 5). The national distributor's milk was always alkaline phosphatase negative (Tables 5 and 16).

Milk-shops did not always indicate whether the milk was pasteurised or not. Milk-shop 5's milk was never labelled at all, and Milk-shops 1 and 2 only labelled their milk 70.4% and 74.1% of the time respectively over the six-week period (Table 5).



BACTERIAL COUNTS

Standard Aerobic Plate Count

Origin	Number tested	Median count (CFU/ml)	Geometric mean (CFU/mℓ)	Range (CFU/mℓ)
Milk-shop 1	25	15 600	22 929	2 200 - 990 000
Milk-shop 2	27	900	1 524	100 - 43 000
Milk-shop 3	27	52 000	36 434	1 200 - 131 000
Milk-shop 4	23	303 500	101 109	100 - 26 600 000
Milk-shop 5	27	103 000	117 809	7 900 - 1 140 000
Supermarket 1 * National distributor X	25	1 900	2 357	900 - 6 500
Supermarket 2 * National distributor X	27	2 200	2 219	700 - 5 600
Supermarket 3 * National distributor X	27	2 300	2 707	800 - 8 700

Table 6:Standard aerobic plate counts (CFU/mℓ) over the six-week sampling period

* milk from the same national distributor, purchased at three different outlets

Pasteurised milk may not contain more than 50 000 CFU/ml of milk as measured by the standard aerobic plate count (Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972). Tables 6 and 14, and Figures 2, 3 and 4 show that the standard aerobic plate count for milk-shop milk (n = 129) varied tremendously over the six-week sampling period. Counts ranged from 1.0 x 10² to 2.66 x 10⁷ CFU/ml, with a median value of 41 000 CFU/ml (the median is the middle value when a sample of observations are arranged in order of magnitude, and is insensitive to extreme values). Standard aerobic plate counts for the national distributor's milk always ranged below 9 000 CFU/ml (Tables 6 and 15).



Standard aerobic plate counts showed no significant (p > 0.05) difference between each week over the trial period of six weeks. There was however, a significant (p < 0.05) difference between milk purchased on Mondays compared with milk purchased on Fridays, with milk purchased on Fridays having a lower standard aerobic plate count than milk purchased on Mondays. No significant (p > 0.05) differences were found between the other days of the week.

There was no significant (p > 0.05) difference in standard aerobic plate count between the national distributor's milk purchased at the three different supermarkets, and also no significant (p > 0.05) difference between the national distributor's milk and the milk purchased from Milk-shop 2. There was however a significant (p < 0.05) difference between the national distributor's milk, and milk purchased from the remaining four milk-shops. After excluding the national distributor's milk samples, there was also a significant (p < 0.05) difference between Milk-shop 2 and all the other milk-shops. Milk-shops 1, 3, 4 and 5 did not differ significantly (p > 0.05) from each other.

There was also no significant (p > 0.05) difference in the standard aerobic plate count between those milk-shops which pasteurised correctly and those that did not.

There was no significant (p > 0.05) difference in the standard aerobic plate count between milk which contained inhibitory substances and milk which did not contain any.



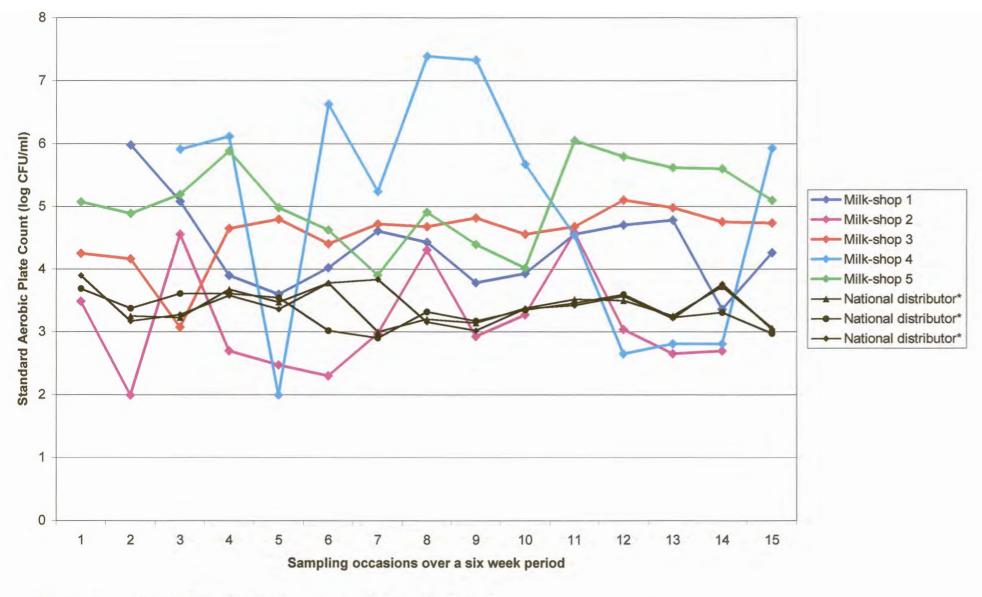


Figure 2:
 Standard aerobic plate counts over the sampling period

 * the same brand of a national distributor's milk was purchased at three different supermarkets



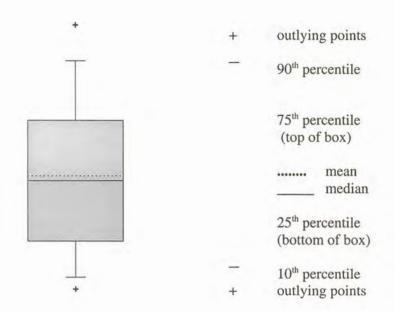


Figure 3: Explanation of a box plot representation of colony counts



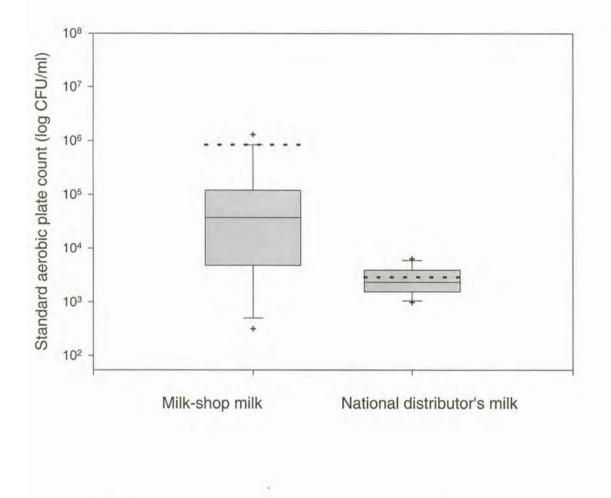


Figure 4: Total aerobic colony counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor



Psychrotrophic Bacterial Count

Origin	Number tested	Median count (CFU/mℓ)	Geometric mean (CFU/mℓ)	Range (CFU/mℓ)
Milk-shop 1	22	65 500 000	68 731 417	15 500 000 - 221 000 000
Milk-shop 2	22	19 700 000	14 055 862	500 000 - 101 000 000
Milk-shop 3	22	5 400 000	5 198 318	300 000 - 183 000 000
Milk-shop 4	22	20 750 000	17 257 115	960 000 - 150 000 000
Milk-shop 5	22	25 350 000	31 028 908	3 200 000 - 212 000 000
Supermarket 1 * National distributor X	25	8 400	8 606	1 600 - 43 000
Supermarket 2 * National distributor X	27	13 600	16 731	3 300 - 87 000
Supermarket 3 * National distributor X	27	8 000	13 817	1 900 - 480 000

Table 7: The psychrotrophic bacterial count (CFU/mℓ) over the six-week sampling period

* milk from the same national distributor, purchased at three different outlets

Psychrotrophic bacterial counts in milk-shop milk (n = 110) differed significantly (p < 0.05) from those of the national distributor, and were extremely high, ranging from 3×10^5 CFU/ml to 2.2 x 10^8 CFU/ml, with a median count of 2.4 x 10^7 CFU/ml (Tables 7 and 14, Figures 5 and 6). The national distributor's milk had a median psychrotrophic bacterial count of 9 200 CFU/ml, ranging from 1 600 CFU to 480 000 CFU/ml (Tables 7 and 15, Figure 5). The milk-shops differed significantly (p < 0.05) from each other with respect to the psychrotrophic bacterial count, but the national distributor's milk did not differ significantly (p > 0.05) between outlets.

There was no significant (p > 0.05) difference in the psychrotrophic bacterial count between the different days of the week or between each week over the trial period of six weeks.



There was a significant (p < 0.05) difference in the psychrotrophic bacterial counts between milk containing inhibitory substances and milk not containing any, as well as between milk which was pasteurised correctly (alkaline phosphatase negative) and milk which was not pasteurised correctly (alkaline phosphatase positive).

After excluding the national distributor's milk, there was a significant (p < 0.05) difference in the psychrotrophic bacterial count between Milk-shop 3 and all the other milk-shops. Milkshop 1 also differed significantly (p < 0.05) from Milk-shops 2 and 3.

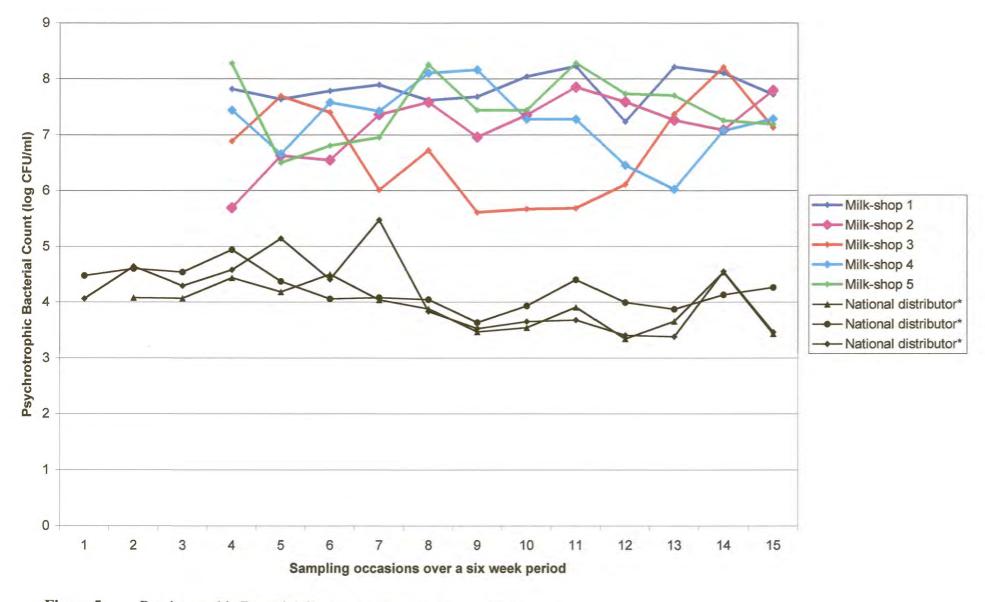
There were correlations between the aerobic standard plate count and the psychrotrophic bacterial count in Milk-shops 2 and 4, as well as in the national distributor's milk purchased from Supermarket 1 (Table 8).

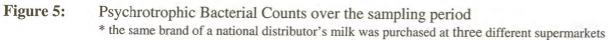
Table 8:Correlation between the Aerobic Standard Plate Count $(log_{10}CFU/m\ell)$ and the
Psychrotrophic Bacterial Count $(log_{10}CFU/m\ell)$ over the six-week sampling
period

Origin	Pearson correlation coefficient (r ²)
Milk-shop 1	0.1
Milk-shop 2	0.54
Milk-shop 3	0.001
Milk-shop 4	0.95
Milk-shop 5	0.37
Supermarket 1 [*] (National distributor X)	0.61
Supermarket 2 [*] (National distributor X)	0.27
Supermarket 3* (National distributor X)	0.12

* milk from the same national distributor, purchased at 3 different outlets









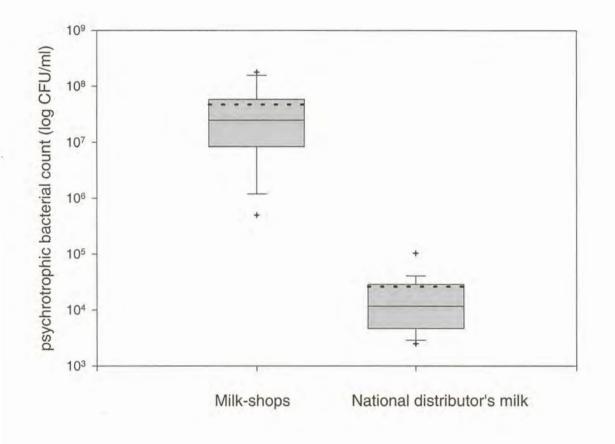


Figure 6: Psychrotrophic bacterial counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor



Coliform Count

Origin	Number tested	Median count (coliforms/ml)	Geometric mean (coliforms/ml)	Range (coliforms/ml)
Milk-shop 1	26	180	233	51 - 9 000
Milk-shop 2	26	16	20	0 - 1 110
Milk-shop 3	27	0	2	0 - 50
Milk-shop 4	23	130	184	0 - 34 000
Milk-shop 5	27	20	25	0 - 1 090
Supermarket 1* National distributor X	25	0	-	0
Supermarket 2 [*] National distributor X	27	0	-	0
Supermarket 3* National distributor X	27	0	-	0

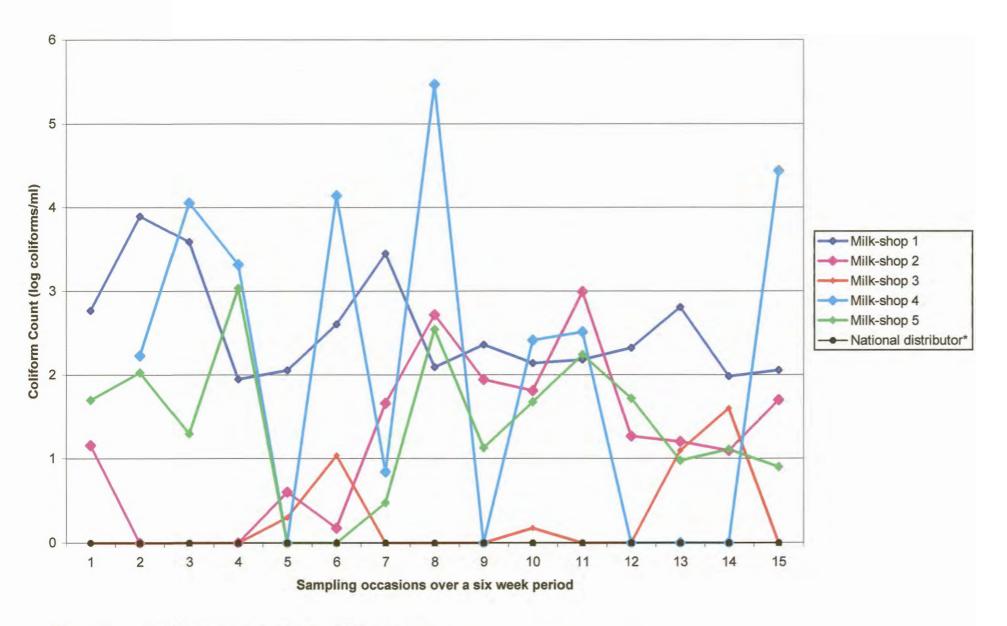
 Table 9:
 Coliform counts over the six-week sampling period

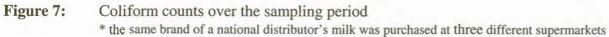
milk from the same national distributor, purchased at three different outlets

Coliform counts in milk-shop milk (n = 129) ranged from 0 to 3.4 x $10^5/m\ell$ (Table 9 and Figure 7), with 88 (68%) samples having counts lower than 20 coliforms per m ℓ , which is the maximum number allowed when the Petrifilm method of counting is used. The median value for milk-shop milk was 30 coliforms per m ℓ (Table 14). Coliform counts for the national distributor's milk were always zero (Tables 9 and 15).

Coliform counts in Milk-shop 1 ranged from 51 to 9 000/ml and in Milk-shop 4 they ranged from 0 to 34 000/ml (Table 9). The other milk-shops which pasteurised correctly had variations between 0 and 1 100 coliforms per ml.









There was a significant (p < 0.05) difference in the coliform count between the different days of the week with Mondays differing from Wednesdays and Fridays.

The national distributor's milk never contained any coliform bacteria, and differed significantly (p < 0.05) from all the milk-shops except Milk-shop 3 (Figure 8). After excluding the national distributor's milk, Milk-shop 3 also differed significantly (p < 0.05) from all the other milk-shops. Milk-shop 1 and Milk-shop 4 did not differ significantly (p > 0.05) from each other with respect to the coliform count, but they differed significantly (p < 0.05) from all the other milk-shops.

There was a significant (p < 0.05) difference in coliform counts between milk containing inhibitory substances and milk not containing any, as well as between milk which was pasteurised correctly (alkaline phosphatase negative) and milk which was not pasteurised correctly (alkaline phosphatase positive).



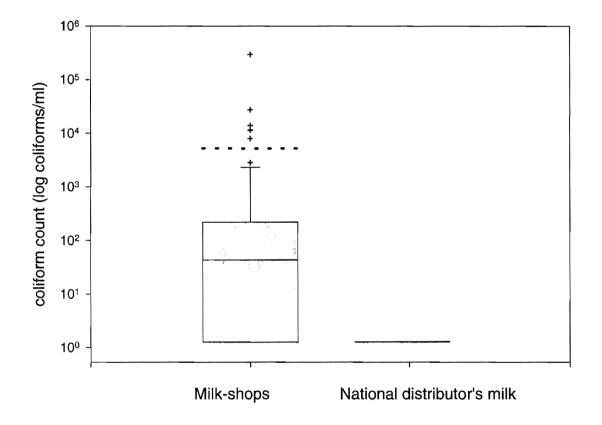


Figure 8: Coliform counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor



Escherichia coli (E. coli)

Origin	Number tested	<i>E. coli</i> positive in 1 mℓ	% positive
Milk-shop 1	27	21	77.8
Milk-shop 2	27	1	3.7
Milk-shop 3	27	0	0
Milk-shop 4	27	2 (11) ^a	7.4 (40.7) ^a
Milk-shop 5	27	0	0
Supermarket 1* National distributor X	25	0	0
Supermarket 2* National distributor X	27	0	0
Supermarket 3* National distributor X	27	0	0

Table 10: Presence of *E. coli* in the milk purchased over a six-week sampling period.

* milk from the same national distributor, purchased at three different outlets

^a indicates suspect *E. coli*.

Out of 135 milk-shop milk samples tested for *E. coli*, 24 (17.7%) were positive in 1 ml, and a further 11 (8.1%) were suspect for the organism (Tables 10 and 16). Over 95% of isolates originated from milk which was alkaline phosphatase positive. Of the 27 samples of milk purchased from Milk-shop 1, 21 (77.8%) were *E. coli* positive. Milk-shop 4 sold 14 (51.9%) samples which were *E. coli* negative. The remaining thirteen (48.1%) samples were either positive or suspect positive for *E. coli*. Excluding the suspect samples, only 7.4% of Milkshop 4's milk was *E. coli* positive, but if one includes the suspect samples then 40.7% were positive for *E. coli*. Milk-shop 2 sold one sample which was positive for *E. coli* (Table 10). The milk originating from Milk-shops 3 and 5 never contained any *E. coli* organisms.

The national distributor's milk was always negative for *E. coli* in 1 ml (Tables 10 and 16).



Staphylococcus aureus (S. aureus) and S. aureus Enterotoxins

Origin	Number tested	S. aureus positive in 1 ml
Milk-shop 1	27	26 (96.3) ^a
Milk-shop 2	27	4 (14.8)
Milk-shop 3	27	5 (18.5)
Milk-shop 4	27	8 (29.6)
Milk-shop 5	27	11(40.7)
Supermarket 1* National distributor X	25	0 (0)
Supermarket 2* National distributor X	27	0 (0)
Supermarket 3* National distributor X	27	0 (0)

 Table 11:
 Presence of S. aureus in the milk purchased over a six-week sampling period

* milk from the same national distributor, purchased at three different outlets

^a indicates percentage of total

Fifty-four (40%) of all milk-shop milk samples purchased contained the organism *S. aureus* in 1 m ℓ (Tables 11 and 16, and Figure 9). One third of these organisms was found in correctly pasteurised milk and the other two thirds in milk which was not correctly pasteurised. Analysing the two milk-shops which sold milk which was alkaline phosphatase positive, *S. aureus* was found in 96% of Milk-shop 1's, and 30% of Milk-shop 4's milk.

Of the 51 *S. aureus* positive cultures which were tested for the production of enterotoxins, four (7.83%) produced heat stable staphylococcal enterotoxins A (SEA), B (SEB), D (SED) or a combination of them (Table 16). All the toxins isolated originated from Milk-shop 1. SEA/SEB was produced by two *S. aureus* strains and SEA/SEB/SED by the other two strains.

The national distributor's milk did not contain any *S. aureus* or enterotoxin in 15 milk samples tested (Table 16 and Figure 9).



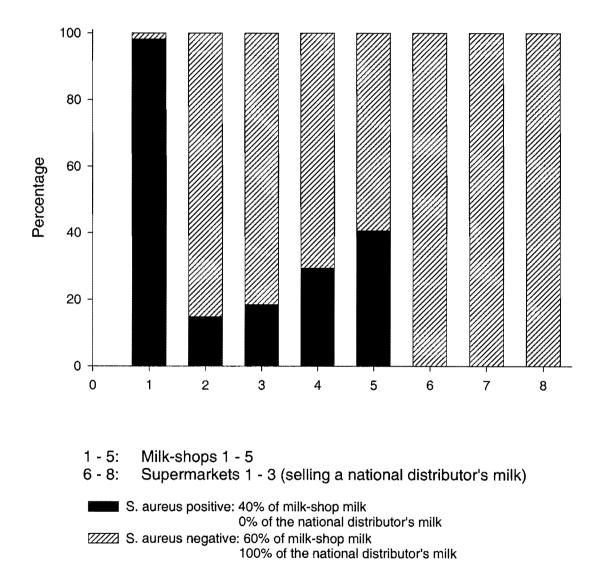


Figure 9: Presence or absence of *S. aureus* in the milk



Thermo-resistant Inhibitory Substances

Table 12:	The presence of inhibitory substances in the milk purchased over a six-week
	sampling period

Origin	Number tested	Inhibitory substances present
Milk-shop 1	27	15 (55.6) ^a
Milk-shop 2	27	25 (92.6)
Milk-shop 3	27	10 (37.0)
Milk-shop 4	27	14 (51.9)
Milk-shop 5	27	9 (33.3)
Supermarket 1* National distributor X	25	0 (0)
Supermarket 2* National distributor X	27	0 (0)
Supermarket 3* National distributor X	27	0 (0)

* milk from the same national distributor, purchased at three different outlets a indicates percentage of total

The occurrence of inhibitory substances in milk-shop milk was high, with 73 out of 135 (54%) samples containing some sort of inhibitory substance (Tables 12 and 16, and Figure 10). Inhibitory substances present in the milk ranged from 33.3% in milk-shop 5 to 92.6% in milk-shop 2. The milk was not analysed further to determine which substances were present.

The results showed that the national distributor's milk never contained any inhibitory substances (Table 16 and Figure 10).



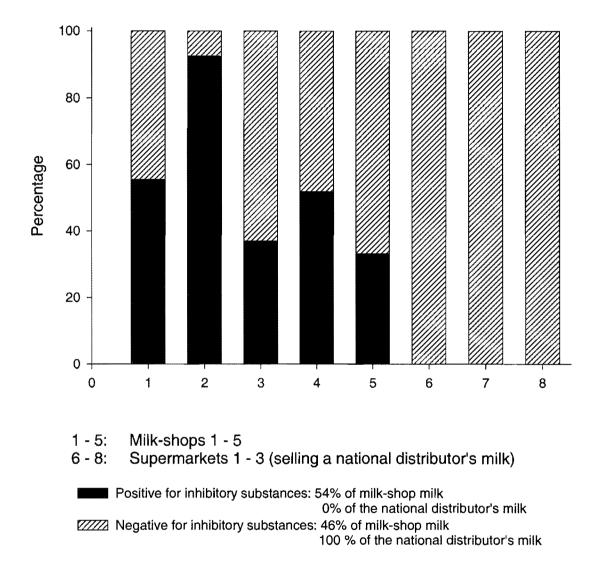


Figure 10: Presence or absence of inhibitory substances in the milk



Somatic Cell Count

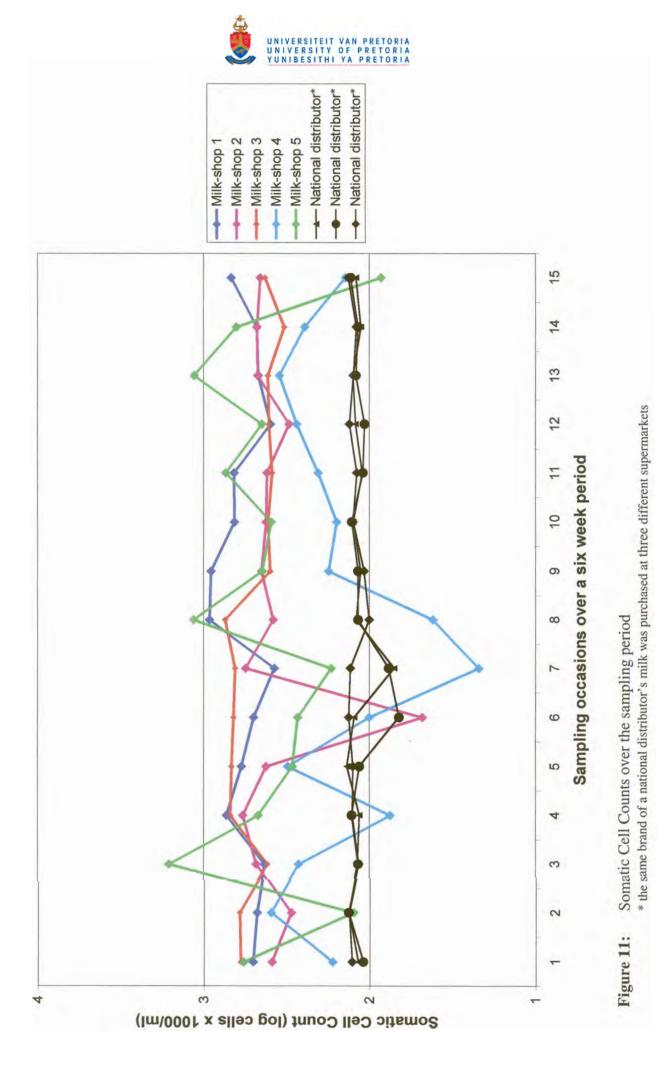
Origin	Number tested	Median count (cells/mℓ)	Geometric mean (cells/mℓ)	Range (cells/ml)
Milk-shop 1	27	500 000	564 000	372 000 - 961 000
Milk-shop 2	27	423 000	371 000	32 000 - 582 000
Milk-shop 3	27	425 000	503 000	324 000 - 778 000
Milk-shop 4	27	192 000	155 000	12 000 - 502 000
Milk-shop 5	27	455 000	429 000	80 000 - 1 623 000
Supermarket 1* National distributor X	25	120 000	116 000	64 000 - 137 000
Supermarket 2* National distributor X	27	117 000	111 000	63 000 - 138 000
Supermarket 3* National distributor X	27	127 000	123 000	80 000 - 145 000

Table 13:The somatic cell counts over the six-week sampling period

* milk from the same national distributor, purchased at three different outlets

Milk-shop milk somatic cell counts varied between 1.2×10^4 and 1.6×10^6 cells per ml, with a median count of 4.2×10^5 cells (Tables 13 and 14, and Figure 11). Only 18.7% (25 out of 135 samples) of somatic cell counts were above the legal limit of 500 000 cells/ml.

The national distributor's milk always had somatic cell counts of less than 150 000 cells per m ℓ (Tables 13 and 15, and Figure 11) and differed significantly (p < 0.05) from all the milk-shops except for Milk-shop 4 (Figure 12). The national distributor's milk purchased at the three different supermarkets did not differ significantly (p > 0.05) from each other with respect to the somatic cell count over the trial period.





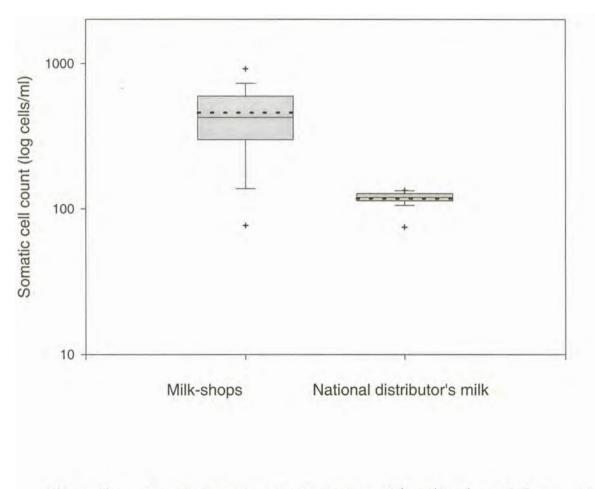


Figure 12: Somatic cell counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor



Table 14:	Summary of the bacterial and somatic cell counts of pasteurised milk obtained
	from milk-shops

	Legal limit	Median (Milk-shop mil Geometric mean	
Standard plate count (CFU/mℓ)	< 50 000	41 000	55 961	100 - 26 600 000
Coliform count/ml	< 20	30	93	0 - 344 000
Psychrotrophic bacterial count (CFU/ml)	EU limit: < 100 000	24 000 000	27 254 324	300 000 - 221 000 000
Somatic cell count/ml	< 500 000	424 000	404 400	12 000 - 1 623 000

Table 15:Summary of the bacterial and somatic cell counts of pasteurised milk obtained
from a large national distributor

	Legal limit	Nat Median	tional distributo Geometric mo	
Standard plate count (CFU/ml)	<50 000	2 200	2 428	700 - 8 700
Coliform count/ml	<20	0	-	0
Psychrotrophic bacterial count (CFU/ml)	EU limit: <100 000	9 200	13 051	1 600 - 480 000
Somatic cell count/ml	<500 000	120 000	116 668	63 000 - 145 000



Brucella abortus

All samples tested were negative for antibodies to B. abortus (Table 16).

Salmonella spp.

Seventeen *E. coli* positive samples were further tested for the presence of *Salmonella* spp. in 1 m ℓ , but these samples were all negative for the organism (Table 16).

Table 16:	Potential pathogens or hazards found in pasteurised milk samples over the six-
	week sampling period

Potential Pathogen	Milk-shop milk		National distributor's milk			
or hazard	Nº tested	Nº pos	% pos	N ^o tested	Nº pos	% pos
E. coli	135	24 (35) ^a	17.7 (25.9)ª	79	0	0
S. aureus	135	54	40	79	0	0
S. aureus enterotoxin	51	4	7.8	15	0	0
B. abortus	135	0	0	79	0	0
Salmonella spp.	17	0	0	not tested		
Inhibitory substances	135	73	54.1	79	0	0
Alkaline	135	52	38.5	79	0	0
phosphatase						

^a includes suspect *E. coli* cases



pH of the Milk

Origin	Number tested	Mean pH at sale	Mean pH after incubation
Milk-shop 1	27	6.79 (±0.06)	6.48 (±0.31) ^b
Milk-shop 2	27	6.78 (±0.06)	6.70 (±0.13)
Milk-shop 3	27	6.76 (±0.08)	6.73 (±0.08)
Milk-shop 4	27	6.70 (±0.10) ^a	6.56 (±0.30) ^b
Milk-shop 5	27	6.77 (±0.07)	6.65 (±0.18)
Supermarket 1* National distributor X	25	6.77 (±0.04)	6.78 (±0.06)
Supermarket 2* National distributor X	27	6.75 (±0.06)	6.78 (±0.05)
Supermarket 3* National distributor X	27	6.75 (±0.07)	6.76 (±0.05)

Table 17:	The mean pH at sale, and after incubation at 21°C for 18 hours, over the six-
	week sampling period

* milk from the same national distributor, purchased at three different outlets

^a differed significantly from all other outlets

^b differed significantly from all other milk-shops

The mean pH value at sale for the milk-shop milk and for the national distributor's milk was 6.76. There was no significant (p > 0.05) difference in the pH of the milk at sale except for Milk-shop 4 which differed significantly (p < 0.05) from the other shops (Table 17).

The mean pH value after incubating the milk at 21 °C for 18 hours for milk-shop milk samples was 6.62 and for the national distributor's milk was 6.77. The pH of the national distributor's milk taken after incubating the milk for 18 hours at 21 °C differed significantly (p < 0.05) from the pH of the milk-shops. All milk-shop milk was visibly thicker than the national distributor's milk after incubation. Milk-shops 1 and 4 differed significantly (p < 0.05) from the rest of the milk-shops with respect to the pH after incubation (Table 17).



Fitness for Human Consumption

Table 18:Indication as to whether or not pasteurised milk samples passed all the criteria
laid down by the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of
1972) over a six-week sampling period

Origin	Number tested	Number of samples passed
Milk-shop 1	27	0 (0) ^a
Milk-shop 2	27	9 (33)
Milk-shop 3	27	4 (15)
Milk-shop 4	27	1 (4)
Milk-shop 5	27	4 (15)
National distributor's milk	79	79 (100)

^a Indicates percentage of total

Table 18 and Figure 13 show that of the 135 pasteurised milk samples purchased from milkshops, 117 (87%) were not fit for human consumption on the basis of all the criteria laid down in the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972). Milk-shop 1 never sold milk which was fit for human consumption, whereas the remaining four milk-shops, only complied with the Act between 4% and 33% of the time.

One hundred percent of 79 samples which were purchased from a large national distributor passed all the criteria laid down in the Act (Table 4 and Figure 13).



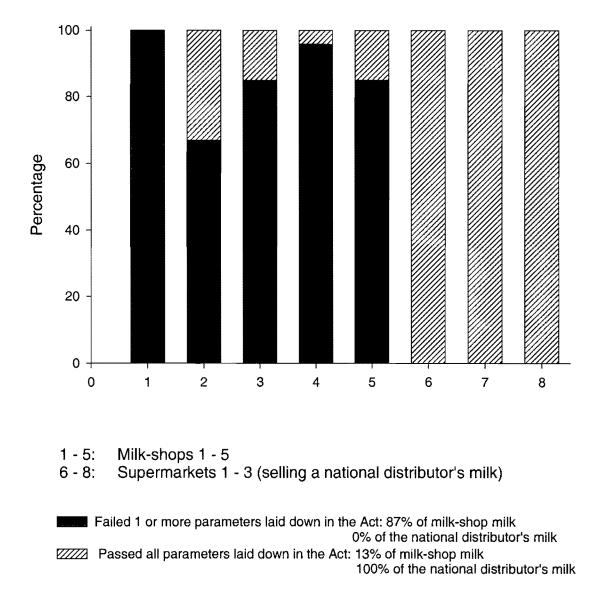


Figure 13: Indication of whether or not milk complied with all the criteria laid down in the Foodstuffs, Cosmetics and Disinfectants Act