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Full length Research Paper

Microbial quality of milk, produced by small scale farmers in a peri-urban area in South Africa

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Milking practices have improved with the development of technology and have transformed both small and large-scale production methods, however, some producers in rural and peri-urban areas have not adopted these new methods and hand milking is still the most frequently used method. This study was conducted in a typical South African peri-urban area, where the state of environmental health is still developing. The objectives were to determine the presence of contaminating organisms in the milk produced by the small-scale farmer as well as to make suggestions regarding the improvement of the milk quality to these farmers. By considering the total viable counts, coliforms and *Escherichia coli,* it was evident that undesirably high numbers of micro-organisms were ubiquitous, exceeding the SANS by far. Results furthermore indicate that the counts of the coliforms and *E. coli* also differed significantly during the summer and winter months. The high presence of *E. coli* found in the milk samples points to the fact that faecal contamination was unavoidable and unnoticed cow illnesses are likely to be one of the causes of the alarmingly high microbial counts. Traditional practices are likely to contribute to the contamination of the milk and proliferation of the micro-organisms.

Key words: Coliforms, cows milk, environmental health, *Escherichia coli,* microbial quality, milk quality, total viable counts.

INTRODUCTION

Milk is one of the most common food sources in the human diet and is also a product that is directly available for consumption (Grimaud et al., 2009). Milk do have distinct physical, chemical and biological characteristics and its colour, odour, taste, consistency, freezing point (-0.55°C), pH (6.6) and specific gravity (1,032) are characteristics that remain particularly constant. These characteristics present a favourable environment for the multiplication of several bacteria of various genera. It is well known that freshly obtained milk contain some bacteria and somatic cells, which constitute the biological constituents of the milk, which easily change depending on production conditions, such as the health status of the cattle and hygiene practices during milking as well as keeping and transportation of milk and milk products (Turner and Veary, 1990). Authors agree that these micro-organisms do play an integral part in the spoilage and contamination of the milk as well as the milk products (Gilmour and Rowe, 1981). It is also eminent that tem-perature control is critical to prevent milk spoilage due to microbial growth (Frazier and Westhoff, 1988). In South Africa the extent of the microbiological contamination of informal or deregulated dairy products is not always very clear (Manhanta, 1984).

However there have been a studies amongst others, quality of milk in bulk tanks and microbial composition of milk and associated milk practices amongst small-scale farmers in the informal sector (Prinsloo, 2001; O'Ferrall-Berndt, 2003; Jansen, 2003) and on milk- and food quality in developing urban areas in South Africa (Lues et al., 2003). All agreement that the microbiological quality of the product is in principal ensured by the control of the products source.

Unfortunately a number of pathogens do grow readily at refrigeration temperatures, and a place where milk is normally stored, even in the informal sector (Jansen, 2003). One such organism is *Escherichia coli* which are

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fairly often found in raw milk. This bacterium is seen as indicator organism of faecal contamination, along with bacteria such as *Yersinia enterocolitica, Listeria monocytogenes* and many others, which are killed by heat treatments like boiling and pasteurisation (Holt et al., 1994; Muir, 1996; Bell and Kyriakides, 1998). Regrettably milk and milk products still have been incriminated in food borne disease outbreaks. The numerous food borne diseases outbreaks in humans that is related to milk, were mainly caused by pathogens such as *Campylobacter* sp., *E. coli, Salmonella* sp. and *Staphylococcus aureus*. General infections such as typhoid fever, diphtheria, scarlet fever and mastitis-related entero-toxaemia are also often transmitted in milk, whilst the most severe zoonoses transmitted from animals to humans via milk are tuberculosis and brucellosis (Foster, 1990; Willshaw et al., 1993; Orr et al., 1994; Heuvelink et al., 1998; Ruegg, 1999).

A national survey, by the South African Department of Health (1995) to determine the hygiene of fresh milk offered to consumers in the market place, indicated that only 25% of a total of 918 samples, included in the survey, complied with all the legislative requirements for raw and pasteurized milk (South Africa, 2001). Thirty six percent of the total number of samples represented raw milk samples in the survey (Dept. of Health 1995), of which only 4% complied to the regulations. Unfortunately no registration system for informal farmers exists in the country and this hinders the transmission of information between farmers and local authorities (Jansen, 2003). It is thus difficult to not only determine the quality status of the milk but also the economic impact, due to the fact that most of the farmers consume their own milk and seldom sell it (Jansen, 2003; Lues et al, 2003; Dovie et al., 2006). It therefore became the aim of the study to determine the presence of contaminating micro-organisms in the milk produced by small scale dairy farmers in a typical South African peri-urban area where milking is done by hand.

MATERIAL AND METHODS

Study population and sampling protocol

The study population consist of small scale farmers of a previously disadvantage population as described in the study of Jansen (2003), 51 farmers were identified who participated in this study. The milk samples were aseptically collected during the period January to July (summer to winter seasons) and 15 consecutive sampling runs were performed. During sampling the samples were kept on ice and transported to the laboratory for immediate analysis. All analyses were performed at least in duplicate and the significance level for statistical analysis was $P \leq 0.05$.

Microbiological analysis

Standard plate count agar (PCA, Merck, RSA) was used to enumerate total aerobic colonies in the milk and for the enumeration of total coliforms and *E. coli*, violet red bile - mug agar (VRBMA, Merck, RSA) was used in accordance with the specifications of South African legislation R1555 of 1997 as amended in R.489 in 2001, Annex A 4 and 7 respectively (South Africa, 2001). The plate loop method was used to quantify the various microbial groups and all plates were incubated at 32°C for 24 h before further investigation according to standard protocol (Chirsten et al., 1993; Houghtby et al., 1993).

Evaluation of results according to South African legislation

Evaluation of results was carried out in accordance with standards set in paragraph 7 of Annex A in the regulations R.489 of 2001 that state standard plate counts may not exceed 5×10^4 CFU.ml^{-1} (raw milk intended for consumption) and 2×10^5 CFU.ml^{-1} (raw milk for further processing). The mentioned legislation further states that, for both the purpose of direct consumption and further processing, coliforms must be below 20 CFU.ml^{-1}. Additionally, no *E. coli* is expected in 1 ml of milk intended for direct consumption as well as no colonies must be present in 0.01 ml of milk intended for further processing (South Africa, 2001).

Recording of environmental temperatures

The temperature at the point of sampling was also taken, by means of a sterile mercury thermometer (Lasec, RSA) and a temperature probe (Envirocon Instrumentation, RSA). All other environmental information was collected from the South African Weather Services in Pretoria and updates were received throughout the sampling period.

RESULTS AND DISCUSSION

Enumeration of micro-organisms found in milk samples

The distribution of the total viable micro-organisms (Figure 1a) ranged from $>10^4$ CFUml^{-1} to 10^7 CFUml^{-1} with the highest recorded count at 6.08×10^7 CFUml^{-1} which is much higher than the legislative standard of 5×10^4 CFUml^{-1} for raw milk intended for consumption. Although 6.1% of the samples complied with the 2×10^5 CFUml^{-1} guideline set by the regulations (R1555 of 2001 as amended by R.489 of 2001) for raw milk intended for further processing, milk from the study area is mainly used for direct consumption, however this is mentioned to shed light especially for the small-scale farmers planning to grow their production and partake in further processing of their milk as it has been noticed during sampling period that some farmers intend to further process the milk for local people. It has been noticed that it is a common practice by some farmers to use the milk to produce sour milk known as Amasi hence more focus on direct consumption. These high counts found in the study suggested probable contamination via aesthetic conditions, public health aspect and economic conditions (Lück and Gravon, 1987) . Lück and Gravon (1987) further elaborates that milk should not con-tain aesthetically objectionable products that can affect the quality of milk, such as the exterior of the udder, the presence of infection within the udder and /or poor storage practices. Although the study did not focus on somatic cell

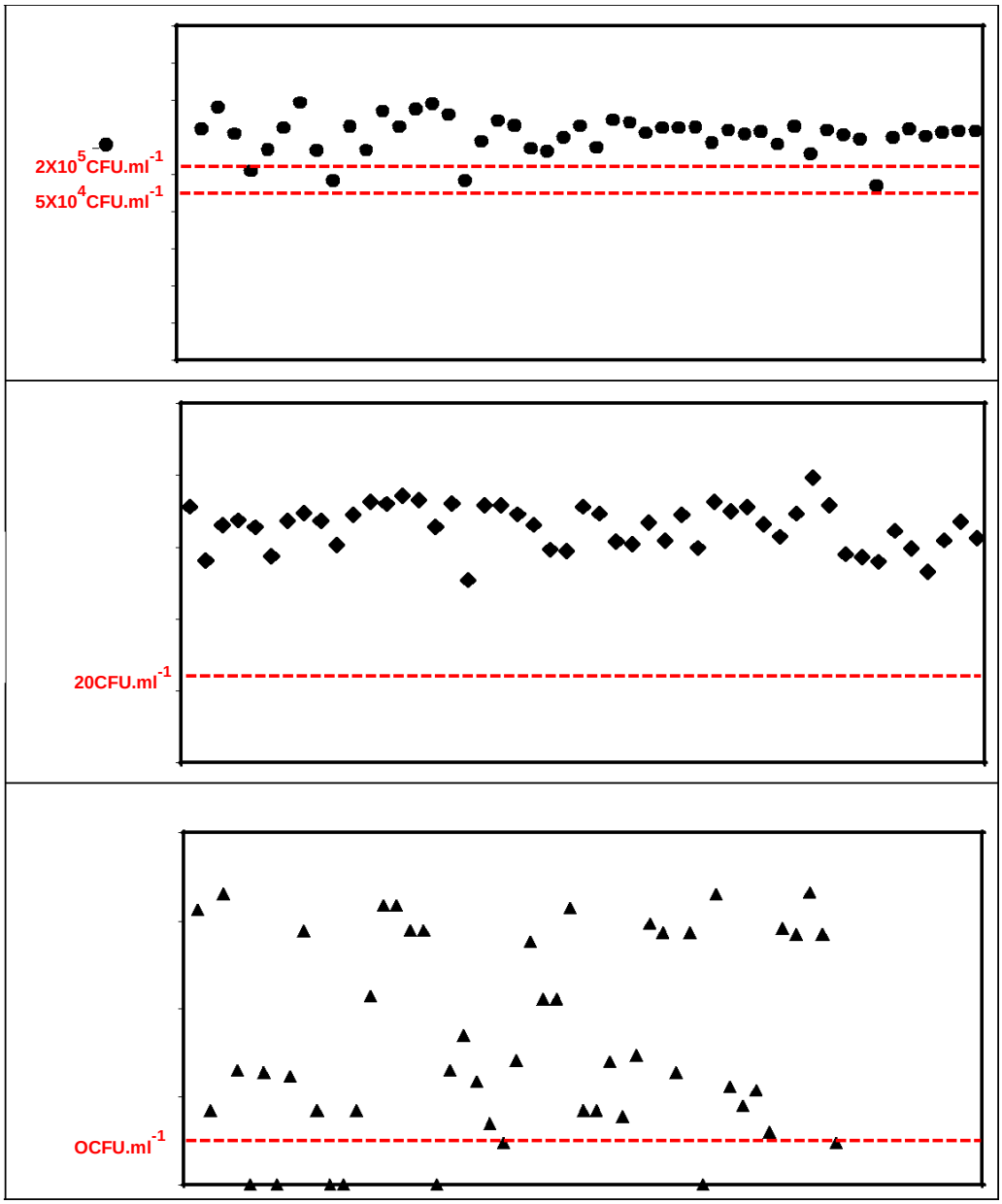

Figure 1. Distribution of the (A) total viable micro-organisms, (B) coliforms and (C) *E. coli* in milk samples from the small-scale farmers of a peri-urban

counts, related studies indicated that a cow with mastitis has the potential to shed large numbers of micro-organisms (up to 10^7) into the milk supply and the influence of mastitis on the total bacteria count of milk depends on the strains of the infecting micro-organism, the stage of infection as well as the percentage of the herd that is infected (Murphy and Boor, 2000). Luck and Gavron, 1987 also stated that intravital infections of the cow such as mastitis, tuberculosis, brucellosis as well as the envir- onment (excretion of cattle, dust, water and equipments) can be possible contaminants of the milk.

Keeping all this in mind it could be suggested that infections in the udder together with unhygienic practices in general and poor storage practices could be responsible for these marked high counts. Jansen (2003), Bodman and Rice (2002) and Frazier and Westhof (1998), highlighted the influence of personal hygiene of the milkers as well as status of containers used during milking process and storage. The authors reported that good hygiene standards are required during milking and as a result clean milking cloths and hooded milking buckets are necessary to prevent dust, dirt and udder

hairs from falling into the milk. The udders and tails of cows need regular clipping before milking begins. Moreover, the foremilk should be drawn and examined and all visible dirt should be removed from the udder and teats, through washing and drying off with disposable towels must be done. Milking should commence with clean, dry hands, using the full hand in preference to just a finger and thumb, which could lead to misshapen udders and teat injuries. It is best to milk the rear quarters first as they contain the higher proportion of milk. Whether you are utilizing hand or machine for milking, the cow should be adequately prepared for all the milk to be removed from the udder; the milk should then be cooled within 3 h or transported to a cooling facility. Because ill animals have been identified in the study and it was found in previous studies that hand-milked milk had higher total counts, ranging between 10^4 to 10^5 CFU.ml^{-1} it could be concluded that the milking practice contributes to the microbiological outcome of milk as far as the total viable counts (TVC) are concerned (Murphy, 1997; Department of Health, 1999; Blowey and Edmondson, 2000; Murphy and Boor, 2000). Lower counts obtained in some loca-lities as seen in Figure 1a on the other hand indicated that more acceptable counts, even with the limited resources at their disposal are possible.

Results indicated the distribution of the coliform organisms (Figure 1b) averaging 1.9 x 10^3 CFUml^{-1} over the sampling period. These counts were found higher when referenced with the national standard of 20 CFUml^{-1} (South Africa, 2001) and it is clear that none of the samples tested conformed to this standard, presenting a definite cause for concern especially to immuno-compromised individuals.

Wessels et al. (1988) proposed that this type of distribution could be expected because raw milk is usually contaminated with coliform organisms that contribute significantly to the bacterial count of the milk and these organisms are predominantly associated with the environment and unhygienic practices (Boor et al., 1998; Murphy and Boor, 2000). Coliform bacteria in dairy products are associated with taste and texture failure and their presence can thus affect the quality of the final product intended for selling or immediate consumption (Wessels et al., 1988). In a previous study by the South African Department of Health (1995) only 28% of samples collected complied with the hygiene require-ments, thus suggesting that there is still a great lack of implementation of proper measures by local authorities towards improving hygienic production of milk practises. O'Ferrall-Berndt (2003) further stated that more stringent control and public education are required to strengthen the legislation as it cannot survive on its own. Agenbag and Lues (2009) also reported that although there are some inspections done, there is a need to better manage and increase manpower to control the informal milk producing sector in order to improve the service delivery. Moreover, there is a need to have quaified and registered Environmental Health Practitioners (EHP's) in respective municipalities and metros to be able to manage and control all activities relating to environmental health such as informal milk producing sector (Agenbag et al., 2009).

R1555 of 1997 as amended by R.489 of 2001 stipulate unambiguously that no *E. coli* are allowed in raw milk as well as in any milk product (South Africa, 2001). The presences of this organism in milk pose not only a considerable threat of food-borne disease (World Health Organization, 1997) but various outbreaks associated with this organism occurred in the past, globally (Effler et al., 2001; Foodhaccp, 2007). However, Figure 1c demonstrate the alarmingly high presence of *E. coli* in the milk sampled for this study. It is noticeable that considerable fluctuations occurred during the sampling period with the mean value being 1.6 x 10^1 CFUml^{-1}, which ranges from a minimum of 10 CFUml^{-1} and a maximum value of 8.3 x 10^3 CFUml^{-1}. Only 12.2% of the milk samples conformed to legislation (absence of *E. coli* in 1 ml milk). Luck and Gavron (1987) stated that the presence of *E. coli* can be associated with faecal contamination during milking and the high distribution noted in Figure 1c indicates that a considerable degree of faecal contamination occurred.

The results found in this study corresponded with a survey done by the department of Health in 1998 showing total plate counts of raw milk in excess of 2x10^5 CFUml^{-1}, coliforms above 110 CFUml^{-1} and a 51.3% *E. coli* positive detection rate (Department of Health: Food Control, 1999).

The influence of environmental temperature on the distribution of micro organisms

The changes in the average distribution of the TVC, coliforms and *E. coli* as well as the variation in the environmental temperature and milk sample temperature are illustrate in Figure 2. It is clear that the *E. coli* counts decreased from ± 10^3 to almost undetectable quantities during the later stages of sampling, the coliforms decreased by approximately 1 log phase and the TVC remained between 1 x 10^6 CFUml^{-1} and 1 x 10^7 CFUml^{-1}. The drop in the counts of these organisms is concomitant with a drop in sample (24 to 18°C) and environmental (29 to 5°C) temperatures.

Coliform and *E. coli* as all micro-organisms is significantly affected by temperature and theoretically micro-organisms can grow at all temperatures, however each micro- organism has an optimum temperature where it multiplies best (O' Connor, 1994) . Reports suggested that the keeping of milk is affected by microbial counts exceeding 2 x 10^5 CFUml^{-1} and the temperature of the milk should not exceed 5.5°C. Milk should preferable been chilled, from 35 to below 5°C, within 30°C 3 h (R1266 of 1987) after milking in order to slow down bacterial growth (Du Preez and Kowalski, 1987) to deliver a quality product. In a study by Hankin et al. (1977) the

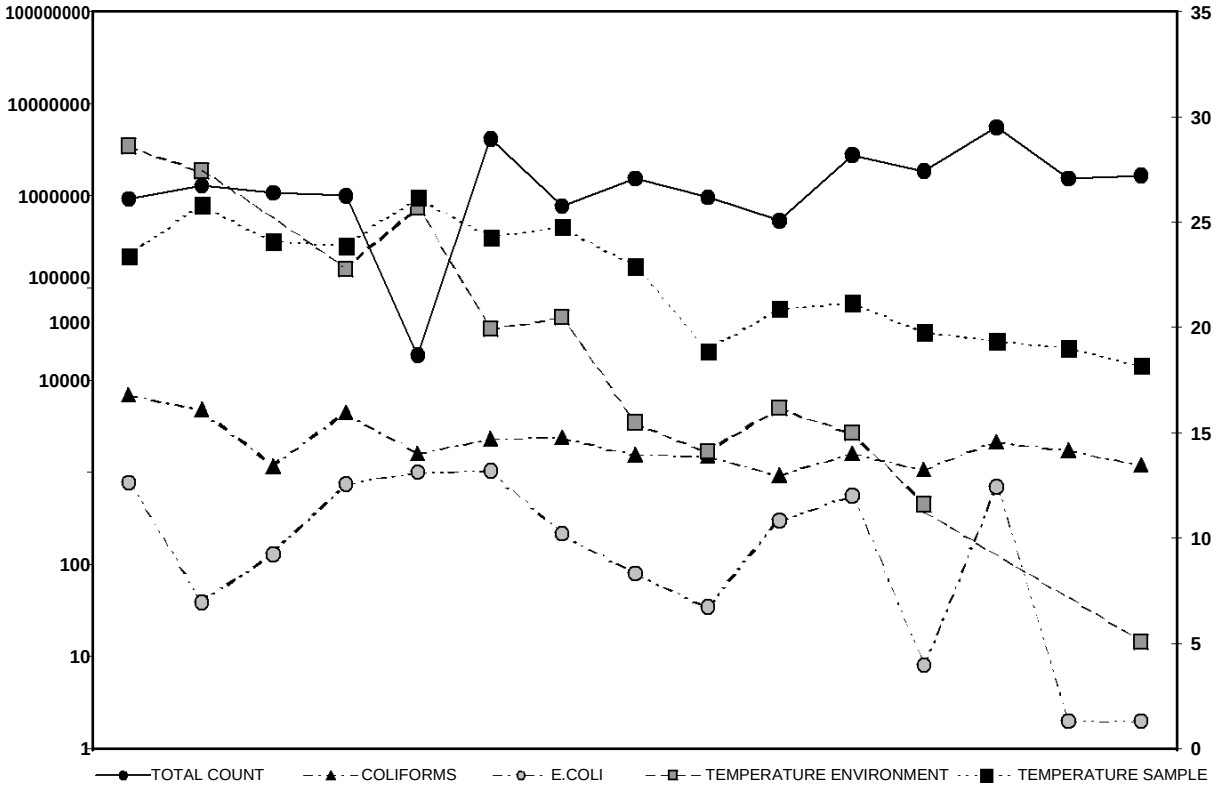

Figure 2. Microbial and temperature changes during the sampling period.

number of storage days and the storage temperature with the number of bacteria present in milk was positively correlated as shown by the temperature influence on microbiological proliferation in the present study as well. In another study on South African mass- contained milk an average psychrophilic count of *circa* 2 x 10^4 $CFUml^{-1}$ were reported and the average shelf-life of these milk samples was 105 h at 4°C, 82 h at 6°C and 57 h at 8°C, illustrating the radical effect that temperature has on shelf-life and microbiological predominance (Swart et al., 1988) . According to the data portrayed in Figure 2, it could be concluded that the temperature of the milk samples and environment had a definite affect on the growth of the bacteria as the study was conducted from summer to mid-winter. As a result, the milkers should be trained and assisted on how to cool the milk during all seasons to minimize any possible microbial proliferation. They may use methods such as the use of refrigerators and/or cool areas.

Inter-relationships between micro-organisms and temperature

In order to determine the exact statistical relationships between the various micro organisms in the samples and the environmental temperatures, Spearman's correlation was used to construct a correlation matrix (Table 1) using the following 5 variables: TVC, coliforms, *E. coli*, sample temperature and environmental temperature. The purpos-e of this evaluation was to ascertain the actual role that temperature plays during informal milking processes, as well as to establish whether the microbial groups (TVC, coliforms and *E. coli*) stipulated in legislation as indicators, in fact present an accurate measure of the true microbial load.

In Table 1 the correlation matrix of the mentioned variables is shown over the whole sampling period. A weak positive correlation of $r^2 = 0.29$ was noted between *E. coli* and the TVC and the counts did not correlate significantly with coliforms (- 0.11), thus emphasizes the fact that this group should be included as a parameter for the evaluation of microbial contamination and cannot necessarily be deduced merely by measuring the coliforms. The most likely reason for this observation is that the total counts comprise many different microbial genera, which exhibit a diverse range of growth conditions and temperature preferences. The correlation between *E. coli* and coliforms ($r^2 = 0.34$) indicate some resemblance between these indicator groups.

Over the entire sampling period, the environmental temperatures correlated moderately ($r^2 = 0.56$) too

Table 1. Correlations (r^2) amongst the various organisms, sample and environmental temperatures in milk collected from the informal settlement of a peri-urban area over a period of 29 weeks.

	Total viable count	Coliform	*E. coli*	Sample temperature	Environmental temperature
Total viable count					
Coliform	-0.1050				
E. coli	0.2895	0.3409			
Sample temperature	-0.1288	0.4323	0.4380		

strongly (r^2 = 0.75) with *E. coli* and coliforms respectively. Strong correlation (0.87) between the sample and environmental temperatures was also observed, proving that the milkers have little or no means available to protect the milk against environmental temperature fluctuations. Grimaud et al. (2009) reported that when temperatures are high such as during summer seasons, microbial contamination was observed. The fact that a negative correlation existed between the sampling temperature and the TVC (-0.13), suggests that the source of contamination was not necessarily from proliferation in the milk itself, but rather from other external sources such as hides, dust, faecal material and/or the milkers, that are not influenced by the temperature of the sample. There is a negligible deviation with regard to the TVC during the winter and summer months while the coliforms and *E. coli* are considerably lower in winter. A significant difference did not occur between the TVC in summer and winter ($P \geq 0.05$). The winter coliform counts, however, differed significantly between summer and winter ($P \leq 0.05$) and a significant difference also occurred between the summer and winter *E. coli* ($P \leq 0.05$) organism counts. Furthermore, according to the results it may be suggested that allowing the sample temperature to more closely resemble the environmental temperature in winter, could be a means of curbing the contamination and the predominance of the, bacteria, especially of faecal origin, that is, coliforms and *E. coli*. It was noted in a study (Shale et al., 2007), for example, that during winter the milkers attempt to keep the milk warm for as long as possible after milking, a probable means of keeping the milk at room temperature and easy to drink. However, as stated before, the milkers should be trained on how to keep milk at low temperatures to avoid any microbial growth and lengthen shelf life.

In conclusion, it was apparent that undesirably high numbers were prevalent for the TVC, coliforms and *E. coli*. None of the TVC, or the coliform counts and only 12.2 % of the *E. coli* counts complied with the national evaluation standard for raw milk for consumption (5×10^4 $CFUml^{-1}$). When evaluated against the 2×10^5 $CFUml^{-1}$ (raw milk intended for further processing) only 6.1% of the TVC complied. Results clearly indicated that the counts of coliforms and *E. coli* were significantly higher in summer than in winter. The alarmingly high incidence of micro-organisms in the milk sampled in this study is of particular interest to the field of environmental health as well as to the community which utilizes this source as a primary element of their daily diet. The fact that throughout the sampling period most of the respondents' milk supplies did not comply with set legislative standards is not only a legislative concern but also a public health concern which requires the relevant authorities, industry and the public attention and interventions. The suitability of the product for human consumption is therefore also questioned from a public health point of view.

In addition, the incidence of coliforms and *E. coli* in raw milk presents a cause for concern due to their association with contamination by faecal matter and pathogens and also partly because of the spoilage that can be produced by their growth in milk at ambient temperatures. Coliforms can build up rapidly in moist conditions and relatively low coliform counts in milk do not necessarily point to clean and sanitary equipment. Local authorities generally consider coliforms in excess of 100 $CFUml^{-1}$ as evidence of unsatisfactory production (Free State Government, 1999). It is thus recommended that the local town council be approached to erect a crush-pen for animal inspection and medical treatment the milkers themselves be educated on correct care for their animals and on what is expected from a milk handler with regard to milking practice and milk quality. All municipalities

should be encouraged to obtain information regarding these small-scale farmers and to compile registers for these farmers who produce milk, regardless whether or not it is only for their own personal use to pin point possible cases of outbreaks that may lead to sicknesses such as diarrhoea normally linked with the poor quality of water in informal settlements.

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