Screening of milk contaminants at critical control points of the milking machine in dairy parlor: case of Molelwane dairy farm, North West Province, South Africa.

Mwanza M[1], Segwagwa OM[1], Ngoma L[2], Moratei Mefane[1]

1. Department of Animal Science, Faculty of Agriculture and Technology, Mafikeng Campus, North West University, Private Bag X2046 Mmabatho2735, South Africa
2. Department of Biology, Faculty of Agriculture and Technology, Mafikeng Campus, North West University, Private Bag X2046 Mmabatho2735, South Africa
mulunda.mwanza@nwu.ac.za

Abstract: Demand for milk and dairy products has increased around Mafikeng areas where people's incomes have been growing. However, despite milk's contribution as a food, raw cow milk is a suitable growth medium for different microorganism either desirable or undesirable. Consumer safety is a matter of increasing concern, and is subject of continuous media attention as well as the general public attention. To assure that food products of animal origin collected from Molelwane dairy farm are safe, milk samples were evaluated to determine whether it fell within the parameters laid down by the South African legislation. A total of 60 samples were obtained over a 6-week period from May to October 2012. Out of the 60 samples collected, 30 were from the bulk tank unit (50%) and 30 from the transfer line (50%). By considering the total viable counts, it was evident that undesirably high numbers of microorganisms were present in the samples. The total viable count of milk samples over 5 days have shown significant high bacterial contamination on the transfer line ($P \leq 0.0001$) as compared to the milk obtained from the bulk tank. The average values of contamination in the transfer line were 483.3 CFU/ml, registered on first day and 1150 CFU/ml on the fifth day of collection. While in the Bulk tank unit contamination was 483. CFU/ml on the first day increased to 883.3CFU/ml on the second day and dropped to 316.7CFU/ml on the fifth day. These results highlighted the need to design appropriate mechanical systems/equipment and hygienic measures at each critical point in order to safeguard consumers from foodborne pathogens. [Mwanza M, Segwagwa OM, Ngoma L, Moratei Mefane. **Screening of milk contaminants at critical control points of the milking machine in dairy parlor: case of Molelwane dairy farm, North West Province, South Africa**. *Life Sci J* 2013;10(2):2562-2568] (ISSN: 1097-8135). http://www.lifesciencesite.com. 356

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1. Introduction

Raw cow milk is a highly nutritious and valuable human food, but in its natural state is a suitable growth medium for microbes because its pH is neutral , higher water content within which a wide range of nutrients such as carbohydrates, proteins, fats, vitamins and minerals are suspended (Frank and Hassan 2003; Perko, 2011). Microorganism may contaminate milk at various stages of procurement, processing and distribution. This contamination could arise from the cow's udder, barn, milk collection materials, various ingredients added to dairy products and dairy farm workers (Garedew et al., 2012). Therefore milk has been incriminated in food borne diseases outbreaks in human mainly caused by pathogens such as *Salmonella* species, *Campylobacter, Staphylococcus aureus* and *Listeria monocytogenes* (Early, 1998; Yagoub et al., 2005). Infection such as typhoid fever, diphtheria, scarlet fever and mastitis related entero-toxaemia, tuberculosis and brucellosis are also often transmitted in raw cow milk (Ruegg, 2003). Preventing the growth of contaminating bacteria in milk involves good hygienic practice during milking as well as storage and transportation of milk plays a major role in reducing the pathogens or contamination (Perko, 2011). Teat disinfection before milking is an important factor that reduces somatic cell count in bulk milk (Ingawa et al., 1992). Teat disinfection after milking has not been adapted universally. It has not been considered necessary in some countries and doubts have been raised on whether residues may contaminate milk (Hillerton, 1996). Blowey (1999) reported that the effect of pre-dipping with an iodophore disinfectant reduces the occurrence of mastitis by 57% whereas the total bacterial count was reduced by 70%. A common procedure to judge the hygienic quality of milk is to determine its bacterial content. Milk with low bacterial count is generally regarded as a better and safer product than milk with a higher bacterial count. The dairy industry regards the somatic cell count as an indicator of an outstanding importance among the qualification parameters of raw milk; therefore, dairy producers should make every effort to ensure that the somatic cell count of the milk produced by their herd is constantly at the lowest possible level which will meet with the qualification limits in place This

practically means regular check-up of herd level mastitis control (Baltay and Janosi, 2001). Buelow et al. (1996) reported that cows producing individual bulk milk with low (<400 thousand per ml) somatic cell count are considered healthy. However, even cows producing individual bulk milk of relatively low somatic cell count may include animals affected by sub-acute or chronic subclinical mastitis in one or two of their udder quarters. They continued to say that in such animals the somatic cell count of the individual bulk milk samples remains below the limit because of the diluting effect of milk from healthy udder quarters which may pose a serious risk to healthy herd mates, especially if it is a contagious pathogen like *Staphylococcus aureus*. Bulk milk somatic cell count is used worldwide as a measurement for quality of milk. Hayes et al. (2001) studied daily variation in bacterial counts over 14 days and suggested that analysis of differentiated bacterial counts would aid in the identification of sources of bacterial contamination. Quality management on dairy farms becomes more and more important regarding the different areas of animal health, animal welfare and food safety. In order to provide safe and healthy milk products, the Hazard Analysis and Critical Control Points (HACCP) system should be implemented starting from milk collection, through processing and storage (Cannas and Noordhuizen, 2008). The applicability of this kind of program on the dairy farm is very important on farm strategy. This allows dairy farm to control the quality of the food produced as well as the production process on the areas of animal health and animal welfare (Lievaart et al., 2005). In developed countries like United Sates of America, Unite kingdom, France etc., directives have been issued to ensure that the whole food chain is under control, and that food products from animal origin are safe for consumers (Cannas and Noordhuizen, 2008). Persistent observation of such fundamental requirements facilitates not only low bacterial count in milk but also reduces the risk of new udder infections and mastitis (Kuang et al., 2009). Therefore, the present study was initiated to provide base-line information on the quality of raw cow milk from Molelwane farm in order to identify critical control points, from production to consumption by the public.

2. Material and Methods

2.1 Site description

This study was carried out in Mafikeng, Capital city of North West province of South Africa. Mafikeng is located between 25 and 28 degrees south of the equator and 22 and 28 degrees longitude east of the Greenwich meridian. The city shares an international border with the Republic of Botswana in the North and 260 km West of Johannesburg. It is built on the open veld at an elevation of 1.500 m, by the banks of the Upper Molopo River. Climatic conditions vary significantly from West to East. The Western region receives less than 300mm per annum, the Central region around 550mm p.a., while the Eastern and South-Eastern region receives over 600 mm per annum (De Villiers and Mangold , 2012).

2.2 Collection of milk samples

Raw milk samples were collected from different critical points of the production process. A total of 60 samples were collected at the critical control points in the morning for duration of 6- week. Out of the 60 samples collected, 30 were from the bulk tank unit (50%) and 30 from the transfer line (50%). The study was conducted from May to October 2012. Laboratory test were conducted at the bacteriology laboratory at the Department of Animal Health, Faculty of Agriculture Science and Technology at the North West University, Mafikeng Campus. Sterile cotton swabs were used to take samples from the liners and rubbers of the clusters of the milking machine considered as critical control points. About 100 ml of individual raw milk sample were collected at morning from each cow aseptically to avoid any contamination using sterile sampling bottles. One hundred and twenty (120) swab samples were taken from the teat liners and rubbers of the clusters. They were taken before milking (30) and after milking (30), before cleaning (30) and after cleaning the milking machine (30). After collection, the samples were transported to the laboratory in thermos cool boxes for processing at the laboratory. Bacteriological qualities of each sample were analyzed including enumeration of total viable count, morphology and biochemical for the determination of sanitary quality.

2.3 Sample analysis

2.3.1 Enumeration of Bacterial Populations

Isolation of bacterial pathogens was determined following aseptic sampling techniques as described by Quinn et al. (2002) and Barrow and Feltham, (2004). Briefly: 25 ml of each sample were thoroughly mixed by shaking and added to 225 ml of buffered peptone water (Sigma, South Africa) and serial dilutions were prepared in the same solution and aliquots of 1 ml of the appropriate dilutions (10^{-6}) were plated in triplicate onto selective agar including plate count agar supplemented with 1 % skim milk powder, blood agar and MacConkey (Merck, South Africa) and incubated aerobically at 37ºC for 48 hrs. Following incubation, plates exhibiting 30-300 colonies were counted. The

average number of colonies in a particular dilution was multiplied by the dilution factor to obtain total viable count. The total viable count was expressed as the number of colony forming units per ml (CFU/ml) of samples according to ISO (1995). Subsequently, the swabs were prepared under sterile conditions and put in the 5 ml of buffered peptone water and incubated at 37ºC for 24 hrs. Serial dilutions were prepared as described above. To further identify organisms, single colony from a pure culture was subjected to Grams' stain and other tests like morphology, catalase assays (Vasanthakumari, 2009).

2.4 Evaluation of results according to South African legislation

The results of this study were evaluated in accordance with the National Regulation on diary product processing and consumption as stipulated in Annexure A, paragraph 7, R 489 0f 2001. According to the sited regulation, plate counts may not exceed 5 x 104 CFU.ml-1 (raw milk intended for consumption) and 2 x 105 CFU.ml-1 (raw milk for further processing) and 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).

3. Results and discussions

This was conducted to assess the general hygienic quality of raw cow milks from Molelwane farm and the extents of possible microbial contamination. The results of microbiological quality of the 60 analyzed raw milk samples obtained in this study are presented in tables 1-6 and figures 1-6. The total bacterial count of milk samples collected over 5 days have shown significant high bacterial contamination on the transfer line ($P \leq 0.0001$) as compared to the milk obtained from the bulk tank (Figure 1). The average values of contamination was 1150 CFU/ml, registered on day 2 in the transfer line and 316.7 CFU/ml registered on day 5 of collection (Table 1). The contamination counts recorded in this study suggest that it is form aesthetic conditions, public health aspect and economic conditions (Lues et al., 2010).

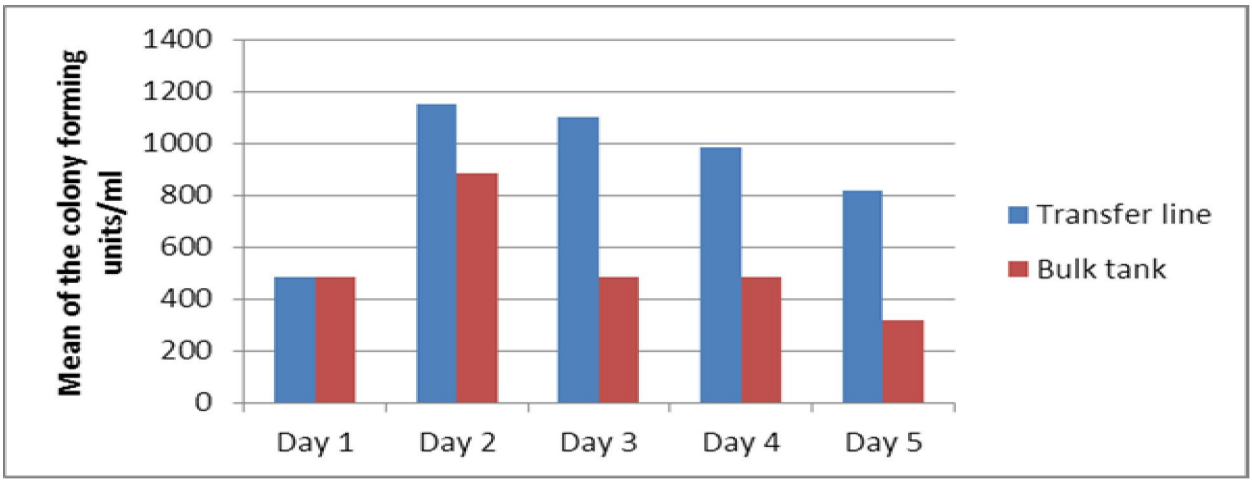

Figure 1: Results of bacterial colony forming unit contamination in milk between the Transfer line and bulk Tank

Table 1: The average value of total bacterial count in raw milk collected in the transfer line and the bulk tank unit of the milking machine

Localizations	Day 1	Day 2	Day 3	Day 4	Day 5
Transfer line	483.3 CFU/ml	1150 CFU/ml	1100 CFU/ml	983.3CFU/ml	816.7CFU/ml
Bulk tank unit	483. CFU/ml	883.3CFU/ml	483.3CFU/ml	483.3CFU/ml	316.7CFU/ml

It was found that the samples collected from transfer line showed significant difference in bacteria population level as compare to one from bulk tank. This difference could be explained by the presence of residues in the transfer line which contaminates milk at the point of collection while the bulk tank was cleaned and disinfected on regular basis. The contamination of milk at the transfer line can be also associated with poorly designed mechanical systems/equipment which makes the milking system difficult as parts such as crevices, joints and blind ends to be cleaned (Murphy and Boor, 2000). In this

respect McKinnon et al. (1990) suggested that the quality of the milking equipment might be responsible for microbial quality of raw milk. The contamination of the milk by microorganisms is often original but can also occur after handling draft in non-hygienic conditions. The analysis of swabs collected before and after the milking of cows revealed the highest bacterial count on day 1 (1633.3 CFU/ml) as compare to the control, while the lowest was on day 5 (483.3 CFU/ml) (Table 2). In addition, it was noted that there was statistically differences ($P \leq 0.0001$) between treatment and control bacterial contamination on swabs samples analyzed (Figure 2).

Table 2: Average bacterial count after culturing on plate count agar in colony forming units per milliliter: treatment on the teat rubbers of the milking machine (swabs collected before milking and after milking) and control

	Day 1	Day 2	Day 3	Day 4	Day 5
Treatment	1150 CFU/ml	850 CFU/ml	850 CFU/ml	566.7 CFU/ml	483.3 CFU/ml
Control	1633.3 CFU/ml	1116.7 CFU/ml	1150 CFU/ml	1150 CFU/ml	966.7 CFU/ml

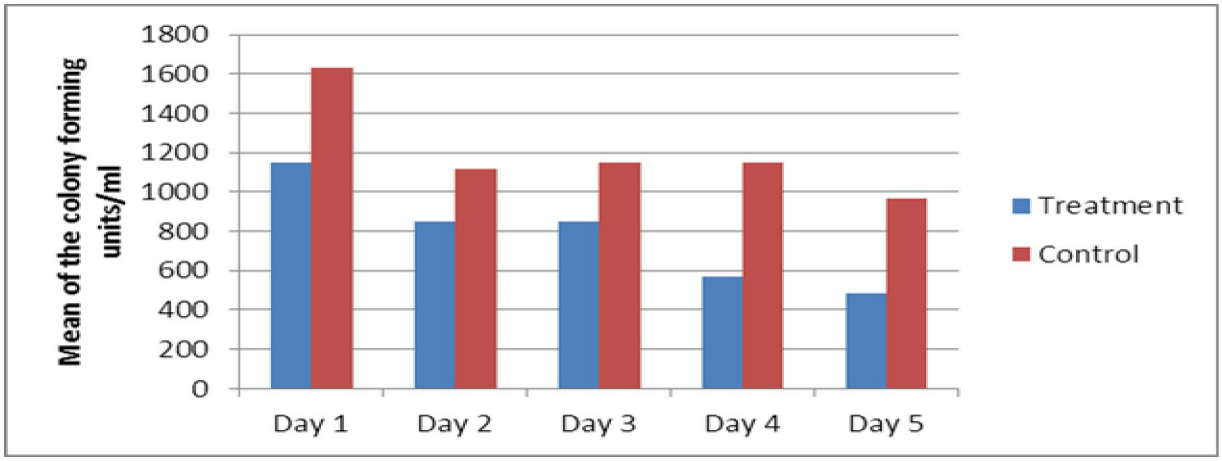

Figure 2: Comparison of bacterial colony forming unit contamination using Swabs collected on teat rubbers of milking machine before and after milking.

The differences noted between the two sampling period might be explained by the fact that treatment swabs collected after the cleaning was done on the teat rubbers while the controls were collected after milking and thus contamination might have occurred through cow teats if not properly cleaned by the parlour workers on the dairy. Add-on, these rubbers use to be replaced after long periods and this could also allow the accumulation of bacteria and cause milk contamination as observed in table 1 and figure 1. McManus and Lanier (1987); Feldman et al. (2006) reported as well the increase in bacterial load in milk after milking due to bovine teat contamination. Feldmann et al. (2006) reported that milk cluster or other parts of the milking system were at the highest risk of microbial contamination of the milking system if kept out of the cluster pick up between milking. The high contamination on day 1 which was a Monday can be explained by the accumulation of organisms over the weekend in the machine. Subsequently on Friday there has been consistent cleaning on a daily basis which helps reduce the microorganisms which had accumulated on the teat rubbers thereby reducing the total Bacterial count to 483.3 CFU/ml as compared to the previous days of the week. Related studies done by Murphy and Boor (2000) and Lues et al. (2010) showed that a cow with mastitis causes considerable compositional changes in milk and has the potential to release large numbers of harmful microbes (up to 107) into the milk supply. Table 3 shows the means of results of bacterial colony contamination obtained from swabs collected from milking machine on the teat rubbers (Cluster) before and after cleaning. The results revealed that there was no significant difference in bacterial colony forming unit before and after cleaning. However, a significant difference was observed on day 1 of collection which was a Monday (Figure 3) due to poor hygiene system (cleaning system without proper disinfectants or the absence of cleaning knowledge by cleaners). In addition, the use of cold water as it is practiced at Molelwane farm might be one of the contributing factor of high bacterial contamination of milk.

Table 3: Mean of the Total Bacterial Count after culturing on the plate count agar in colony forming units per milliliter: Treatment (swabs collected before cleaning) and Control (swabs collected after cleaning) on the teat rubbers of the milking machine

	Day 1	Day 2	Day 3	Day 4	Day 5
Treatment	1633.3 CFU/ml	1116.7CFU/ml	1166.7CFU/ml	1133.3CFU/ml	950 CFU/ml
Control	616.7 CFU/ml	1100 CFU/ml	1050 CFU/ml	1266.7CFUml	1216.7 CFU/ml

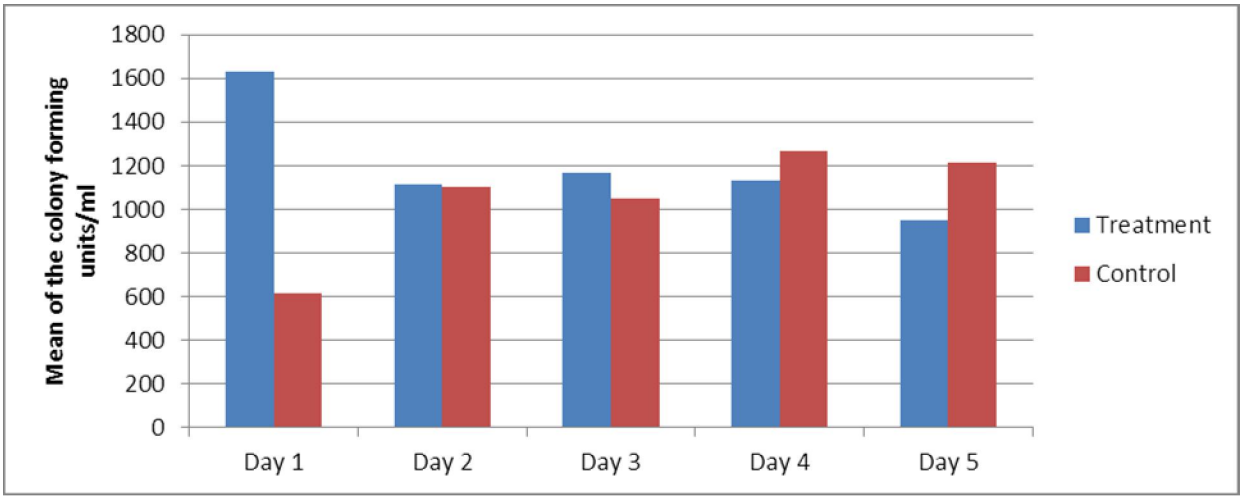

Figure 3: Graph showing a comparison of bacterial colony forming unit (cfu/ml) contamination on the teat rubbers collected using sterile swabs before and after cleaning of the milking machine.

These results are in accord with the findings of Mckinnon et al. (1990); Feldmann et al. (2006) Lues et al. (2010) and Zucali et al. (2011) who 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. Chambers (2002) noticed also in his work that the quality and temperature of water used to clean the milking machine had an important impact on the reduction of bacterial on machine parlor. The author reported also that contaminated water can be a source of *Pseudomonas* spp, *coliforms* and Gram negative bacteria. In all these critical control points' mostly prevalent microorganisms were the Gram–negative bacteria and this corresponded with survey done by Hayes et al. (2001) who reported that Gram-negative bacteria easily multiply in the milk residues left after improper cleaning of milking equipment. High levels of coliforms or Gram-negative bacteria in raw milk usually reflect unhygienic production practices (Murphy and Boor, 2000). To find out the degree of contamination of milk, the plate counts or total colony counts test is required to estimate viable bacterial populations in the cow raw milk. This test gives a crude indication of hygienic practices used in the dairy farm. But in the present study the tested samples for milk quality parameters did not meet the regulatory limit of South Africa which stated that the recommended limit for total bacterial count in raw milk may not exceed 5 x 104 CFU.ml-1 (raw milk intended for consumption) and 2 x 105 CFU.ml-1 (raw milk for further processing), coliforms must be below 20 CFU.ml-1, 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.

Conclusion

In this study, cow milk samples obtained from the transfer line, the bulk tank and swabs collected before and after milking were cultured for bacterial colony forming units in order to assess the point of contamination along the line. Results obtained showed significant differences of bacterial load contamination in milk collected on the line as compared to the one from the bulk tank. The result from swabs showed significant contamination of samples collected from teat rubbers before milking as compared to the one collected after milking. In opposition they were no significance difference in the results of swabs collected before and after cleaning of

the milking machine. However there was a significance difference in the results on Day 1 of collection which was a Monday. The high incidence of microorganisms in the cow 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 food. Most of the milk sample did not comply with set South African legislative standards. The suitability of the product for human consumption is therefore also questioned from a public health point of view. From this study, we can conclude that the cleaning of the teat rubbers, the machine in its entirety, cow udders and teats before milking and after milking, the workers hygiene, the quality of water as well as the temperature and regular cleaning of the machine even over the weekend are among the most important factors which influence the quality of milk at Molelwane Dairy.

In regard to the above mentioned elements, it is therefore recommended that the cleaning system be improved at Molelwane dairy according to milk hygiene regulations as South African department of Health regulation on Pre-milking and post-milking cleaning regulations. The use of hot water during the cleaning of machine and detergents might have impact on microbial survival. In addition there is a need to review the milking machine design to avoid the U-shape which retains milk and favor bacterial growth and contamination. Critical Control Points at the parlor must be identified and workers trained on bio security in order to reduce the contamination risks.

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Corresponding Author:
Dr. Mwanza Mulunda
Department of Animal Science, Faculty of Agriculture and Technology, Mafikeng Campus, North West University, Private Bag X2046 Mmabatho2735, South Africa
E-mail: Mulunda.Mwanza@nwu.ac.za

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