Full Length Research Paper

Quality control of raw milk in the smallholder collection and bulking enterprises in Nakuru and Nyandarua Counties, Kenya

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Kenya has one of the largest dairy industries in sub-Saharan Africa. Most of the milk marketed by small-scale farmers in Kenya has been reported to be of poor quality and does not meet national and international standards due to high bacterial load, high somatic cell count, adulteration and antibiotic residues. This study was designed to assess status of microbiological and physico-chemical quality of raw milk from two smallholder dairy farmer' groups at four sampling levels. Three hundred and eight raw milk samples were collected and analyzed along the value chain. Microbiological analysis for total bacterial count and coliform count was carried out using 3M^{TM} Petrifilms plates. The average total bacterial and coliform counts Log_{10} per ml at the processing factory was 8.462 and 6.770 for Ngorika and Olenguruone, respectively. The antibiotic residues especially β- lactam was prevalent with 44.5% of all the analyzed samples being positive. Likewise, 60% of the samples had a range of 150,000 to 500,000 somatic cells/ml. Average water adulteration level for the two collecting and bulking enterprises was 30.3%. TVBC and CC should be used instead of resazurin while freezing point determination should be used for adulteration.

Key words: Raw milk safety, Adulteration, Antibiotic residues Resazurin test.

INTRODUCTION

Kenya produces an estimated volume of 5 billion liters of milk annually and is therefore the leading milk producer in the East Africa region (Muia et al., 2011). Kenya's dairy industry, the single largest livestock production sub-sector contributes 14% of the agricultural gross domestic product (GDP) and 3.5% of the total GDP (Muriuki et al., 2003).

Kenya's dairy industry is a dynamic and plays an important economic and nutrition role in the lives of many people ranging from farmers to milk hawkers, processors,

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and consumers. Kenya is generally self-sufficient in milk and dairy products. However, the demand for milk and dairy products in developing countries is estimated to increase by 25% by 2025 (Delgado et al., 1999), mainly due to human population growth, further urbanization, increased disposable income, greater diversity of food products to meet nutritional needs, and increased opportunities for domestic and external trade. Indeed, dairy imports in developing countries may reach 38.9 billion litres of milk equivalent by 2030 (Food and Agriculture Organization (FAO) and International Dairy Federation (IDF), 2004). Fortunately, Kenya has the potential to increase milk production from the current 4.2 billion litres in 2009 to over 5.0 billion litres in 2014 (Cherono, 2005).

Kenyan milk production systems can be divided into two general categories: large-scale and small-scale. The small-scale or smallholder dairy production system dominates. The differences between the two dairy systems are in their sizes of operation, level of management and use of inputs. Dairy cattle in smallholdings feed mainly from forage and very small quantities of concentrate, but some small-holder dairy farmers are highly commercial and well versed in dairy production, with high-quality management.

Dairy production is dominated by smallholders who own about 98% of the total dairy herd (Peeler and Omore 1997). Smallholder dairying households estimated to number over 1.5 million households, account for more than 85% of the annual total milk production and 80% of the 1.8 billion litres of milk marketed annually (MoL & FD, 2003; Staal et al., 2001). Over the years, significant changes in the traditional dairying have occurred resulting in a major shift towards market-oriented smallholder production. Farmers' groups handle only about 40 percent of marketed milk production and about 20 percent of total milk (Muriuki, 2003).

Over 800,000 smallholder farmers in Kenya depend on dairy farming for their livelihoods. Small-scale farmers account for 80% of the total milk production and 70% of the total marketed milk in the country. This has positive implications on food security and nutrition and has the potential to reduce poverty, particularly in the rural areas (Chesterman and Neely, 2015).

Milk is synthesized by cells within the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, bacterial contamination can generally occur from within the udder, outside the udder, and from the surface of equipment used for milk handling and storage. Cow health, environment, milking procedures and equipment sanitation can influence the level of microbial contamination of raw milk. Equally important is the holding temperature of milk and the length of time milk is stored before testing and processing that allow bacterial growth. All these factors will influence the total bacteria count and the types of bacteria present in raw milk bulk tank (Murphy and Boor, 2000). Raw milk safety in Kenya has been disputed over a decade but no measurable data exists despite the fact that it requires monitoring from production to consumption. The regulatory institutions are constrained by lack of resources in terms of personnel and equipment (Muriuki et al., 2003) even though, the Kenya Bureau of Standards developed a Hygienic Code of Practice for milk production to assist farmers in producing hygienic milk.

Rejection at market is a result of poor handling and the time taken to reach markets (long distances and bad roads). Rejections are higher during the wet season, when production is high and roads are impassable. Losses at the farm level can be more than 6 percent of total production, which means that at current production levels, national annual losses may reach 60 million kg (Muriuki, 2011). Consequently, most of the milk marketed by small-scale farmers in Kenya has been reported to be of low quality and does not always meet national and international standards due to high bacterial load (Mwangi et al., 2000), antibiotic residues (Omore et al., 2005; Shitandi and Sternesjo, 2004) and water adulteration. The greatest limitations in the whole raw milk collection chain are proper ways to maintain cold collection due to the high investment costs demanded. This particularly affects the informal sector but also relatively in the formal sector (Orregård, 2013).

The formal milk trade is the market segment licensed by KDB. The informal markets controls an estimated 70 percent of the total milk marketed in Kenya (Kenya Dairy Board (KDB), 2009; Government of Kenya, 2006). This sector is important and is driven by among other factors the traditional preferences for fresh raw milk and its relatively lower cost. Raw milk markets offers both higher prices to producers and lower prices to consumers but with several challenges relating to quality control and standards, and the associated health and safety concerns. Other players in milk marketing include informal traders, distributors and retailers. The existence of informal trade results from a combination of the formal system's failure or inefficiency, consumer habits/preferences, and price differences between raw and processed milk (Muriuki, 2011).

Additional factors like unhygienic milking and handling practices, results in poor raw milk quality. According to Orregård, (2013), plastic jerry cans are impossible to clean and are often used for transporting milk by most motor bike transporters. This result in a less hygienic handling compared with the use of aluminum cans whose only limitation is the acquisition cost. Plastic jerry cans which could contribute to milk quality deterioration. This is in line with Gemechu, (2015), who found out that milk producers use plastic containers which are difficult to clean and disinfect and thus it might contribute to poor quality of the milk. The collection and bulking enterprises (CBE's) critical quality control challenges in line with milk bulking are; adulteration (both water and preservatives),

Table 1. The number of samples collected and analyzed from the four sampling levels in each CBE per replicate.

CBE	NGORIKA	Olenguruone
Can	44	24
Collection routes	6	6
Cooling tank	1	1
Tanker	1	1

high bacterial load due to warm collection, potential for contamination with coliforms due to handling, presence of anti-microbial residues and zoonotic diseases like Tuberculosis and Brucellosis (Muriuki, 2011). Owing to the large amount of milk that is marketed unprocessed, and to weak monitoring of the market, public health risks are a concern. The main public health concern is the potential risk of diseases such as brucellosis and tuberculosis (TB). Drug residues are also of concern, even in the processed milk channel.

Nyandarua County produces the highest amount of milk due to its higher population of dairy cows as compared to the other regions in Central Kenya (Muia et al., 2011). However, reports for Central Kenya indicates that dairy production potential for Nyandarua County is the least exploited (Romney, 2004; Staal et al., 2001; Schreiber, 2000; Baltenweck et al., 1998). Nakuru County had many districts; it has an area of 166 square km and human population of 25,800 people (GOK, 2009). The area has now settled down as a productive area with a high potential for dairy farming. The division has a total of 8925 cattle producing 7.5 million litres annually (District livestock production annual report, 2012).

This study was designed to monitor microbiological and physico-chemical quality of raw milk from two smallholder dairy farmer' groups at four sampling levels according to the requirements of the Kenya Bureau of Standards (KEBS).

MATERIALS AND METHODS

Study site

The study was carried out in New Ngorika Milk Producers Limited in Nyandarua County and Olenguruone dairy farmer's cooperative society in Nakuru County. For both CBE's, milk from individual farmers was collected and bulked into milk-cans while warm and transported in the same condition to the CBE cooler. The mode of transport per CBE varied from truck, tractor with trailer, tricycles, donkeys, individual farmer's delivery and motor bikes. Milk collection was only done once in the morning with some few farmers offering their evening milk separately along the routes. Laboratory tests were carried out at the Happy Cow Ltd laboratory and the food chemistry laboratory at the Egerton University department of dairy and food science. The Table 1 shows the number of samples carried out in every CBE during the analysis.

Milk sampling

Milk was initially stirred using a plunger to obtain a homogenous sample. The samples were obtained aseptically per the farmers group cans after acceptance at the reception platform, composite sample per route, CBE cooler and processor tanker. Additional samples were collected from the milk delivered late in the afternoon in Olenguruone, bulk samples from the rejected milk at the CBE platform, composite samples at the dump tank for the farmers that delivered individually to the cooling plant and extra samples for the freezing point determination. Antibiotic residues analysis involved only the bulk samples at the routes and cooler/ tanker levels due to the cost of the analytical method applied.

Microbial analysis

Total viable bacterial count and coliform count were done according to AOAC (2005) methods 991.14 and 990.12, respectively using $3M^{TM}$ petrifilms plates. Serial dilution was done up to 10^{-5} and 10^{-6} for coliform count and total viable bacterial count respectively. Incubation was done at $37^{0}C$ for 24 hr and at $32^{0}C$ 48 hr for coliform count and total bacterial count, respectively. After the growth, the colony counting was done using the 3M plate reader (6499, 3M health care, Germany) and the total colony forming units (CFU) /ml recorded in an excel sheet.

Adulteration

The freezing point determination was carried out to assess adulteration according to Draaiyer et al. (2009). When it is adulterated with water or other materials are added the density and freezing point of milk change from its normal value causing a detectable elevation of the freezing point of milk from its normal values of -0.54°C. This was done using a cryoscope according to the manufacturers operating instructions. After calibrations, 2.5 ml of the sample were put in the sample vial and placed at the measuring point of the cryoscope. The start measure of the machine was selected and the results presented as a percentage on the display of the machine and as well as printed.

Antibiotic residues

The presence of the antibiotic residues was detected using the delvo test (SP NT and BLF) according to Delvotest technical bulletin (2011). Delvo test is easy to use and covers the broadest spectrum of antibiotic residues in the industry. Moreover, it is reliable and accurate with detection levels closest to maximum residue levels and safe tolerance levels (Hillerton et al., 1999). The milk sample (0.15 ml) was added to the ampule and incubated at 64°C in a delvo incubator for 3 h to observe colour changes (SP NT). The other delvo test (BLF) that involved use of ampules together with strips, was carried out. The incubator was set and the ampules with 0.15 ml milk sample inserted in it for 2 min. The milk sample was swirled again before inserting the strips in the ampules for 3 min and the results recorded.

Somatic cell count

The somatic cell count was done on all the samples using California Mastitis Test (CMT) according to Mellenberger and Roth (2000). An equal amount of commercial CMT reagent was added to each cup and a gentle circular motion applied to the mixture in a horizontal plane. A positive gelling reaction and colour change occurred in 10 s with the positive samples. The gel formation and colour changes

Table 2. Means for Log10 TVBC and CC (CFU /ml), Lactic acid (LA, %) and Resazurin test (RT) for Ngorika samples.

Sampling levels test	Can	Route	Cooler	Tanker	Rejected
TVBC	8.396[b]	8.818[a]	8.708[a]	8.828[a]	8.889[a]
CC	6.785[b]	7.240[a]	6.953[a]	7.150[a]	7.590[a]
LA	0.151[c]	0.156[b]	0.150[c]	0.150[c]	0.178[a]
RT	3.971[a]	3.647[a]	3.846[a]	4.111[a]	1.778[b]

Means within a row marked with different letters are significantly different at (p< 0.05).

Table 3. Means for Log10 TVBC and CC (CFU/ml), Lactic acid (LA, %) and Resazurin test (RT) for Olenguruone samples.

Sampling levels test	Can	Route	Cooler	Tanker	Rejected
TVBC	6.455[c]	6.276[c]	6.369[c]	7.138[b]	8.222[a]
CC	4.137[c]	4.683[bc]	5.322[ab]	5.390[ab]	6.422[a]
LA	0.148[b]	0.152[b]	0.151[b]	0.153[b]	0.167[a]
RT	4.817[a]	5.000[a]	5.000[a]	4.889[a]	3.222[b]

Means within a row marked with different letters are significantly different at (p< 0.05).

were observed and compared using the colour and viscosity comparison table.

Ten minute Resazurin test

The Resazurin test was done as per Draaiyer et al. (2009) where a resazurin tablet was completely dissolved in 50 ml of sterile distilled water according to the manufacturer's instructions. One milliliter of the resulting solution was added into 10 ml of the milk sample in a test tube, mixed and then incubated at 37°C in a water bath for 10min in a water bath. The samples were then read using a Lovibond comparator (14 20 00, Tintometer ltd, England) from a good source of light for colour change and numerical score value ranging from 1 to 6, assigned. A milk sample without the resazurin dye was similarly treated and used as the blank in the comparator. Samples with comparator readings ranging from 4 to 6 were acceptable based on the Kenya Bureau of Standards (KEBS) on milk quality.

Titratable acidity

The titratable acidity test was done as per Draaiyer et al. (2009). This measured by titration whereby 3 to 4 drops of 0.5% phenolphthalein were added in 9 ml of milk sample in a beaker on a white tile and titrated against 0.1 equivalents/litre NaOH with constant shaking of the milk until a permanent colour change (pink) was observed. By recording the volume of base used and the volume of the milk sample, the amount of developed lactic acid was calculated and expressed as a percentage lactic acid.

Statistical analysis

The laboratory experimentation employed a randomized complete block design with a 2×5 factorial arrangement having three replications. The blocks of the experiment were the two CBE while the treatments were the four different sampling levels. Analysis of variance (ANOVA) was used to analyze the results obtained. This was done using PROC general linear model (GLM) procedure of the statistical analysis system (SAS) version 9.0 (SAS, 1999). Means were separated using least significant difference (LSD).

RESULTS AND DISCUSSION

Total viable bacterial count (TVBC) and coliform count (CC)

According to KS 05-1552, bacteriological grades for raw milk grade III for total bacterial count and coliform count are 2,000,000 and 1,000 (CFU/ml), respectively. This study realized results that were generally higher compared to the standards. Mean $\log_{10}$ for total bacterial count and coliform count per ml (CFU/ml) in Ngorika (Table 2) was not significantly different (p ≤ 0.05) among the milk samples collected from the route composite, cooler and tanker but it was significantly different (p ≤ 0.05) for the composite cans. This could have been contributed by the warm collection and the much time spent during transportation favouring the bacterial multiplication. Warm collection in the dairy value supply chain creates an optimum environment for microbial growth consequently causing milk quality deterioration (Mwangi et al., 2000; Orregard, 2013). The rejected milk analysis results represent the plates that had countable colonies and it had no significant difference (p ≤ 0.05) from the route, cooler and tanker samples. Moreover, plates with Too Many To Count (TMTC) microbial colony growth were 44.4 and 33.3% for TVBC and CC, respectively for both locations. These uncountable results were not included in the statistical analysis of the data.

In Olenguruone (Table 3), the means $\log_{10}$ for TVBC were significantly different (p ≤ 0.05) at the tanker level

Table 4. Means for Log_{10}TVBC and CC CFU/ml for all sampling points in the two locations.

Sampling point	TVBC	CC
Rejected	8.889[a]	7.590[a]
Tanker	8.462[c]	6.770[ab]
Cooler	8.305[bc]	6.694[ab]
Route	8.187[bc]	6.474[b]
Can	8.078[b]	6.226[b]

Means within a column marked with different letters are significantly different at (p< 0.05).

compared to the can route and cooler levels. Additionally, significant difference (p ≤ 0.05) was observed between the tanker and the rejected milk. The means log_{10} CC count CFU/ml for the can and route sample were not significantly different (p ≤ 0.05) similarly to the means for the route, cooler and tanker.

Nonetheless, significant difference (p ≤ 0.05) for the rejected milk was observed for both TVBC compared to all the sampling levels (Table 3). These samples were collected from the longest routes with poor infrastructure and took longer time for milk collection in Olenguruone. The microbial quality of the rejected milk samples in Olenguruone was similar to the samples at the tanker level in Ngorika.

In Table 4, the overall mean log_{10} for total bacterial count in both locations was 8.078, 8.187, 8.305 and 8.462 TVBC log_{10} CFU/ ml for milk samples collected directly from the can composite, route composite, cooler (bulk) and upon arrival at the processing plant (tanker), respectively. An increasing trend of TVBC was observed at the different points considered from the can to the tanker and consequently the rejected milk samples. However, the means log_{10}TVBC for the two locations indicated that the can level, route level and cooler level were not significantly different (p ≤ 0.05) unlike the tanker level, but significant difference (p ≤ 0.05) for TVBC between all the levels and the rejected milk samples was observed. Coliform count indicated no significant difference (p ≤ 0.05) between can, route composite, cooler and tanker levels. Furthermore, no significant difference (p ≤ 0.05) was observed between the rejected milk, cooler and the tanker levels. Accordingly, the TVBC increased by 4.75% (0.384 log_{10} CFU/ml) from the can to the tanker milk sampling points. Increase in TVBC observed along the value chain may be due to several factors like contamination at the farm, storage and transport using improperly cleaned milk cans, and lack of controlled temperature during transportation (Doyle et al., 2015).

The difference between the two CBES might be due to the fact that the Olenguruone was sampled from the smallest radius to the cooling plant and the milk was transported using motor bikes that are very fast in terms of delivery time. An increase in microbial growth was observed between the cooler and the tanker which could have been majorly contributed by the cooler efficiency that is taking over three hours to cool the milk from 18 to 4°C (which was the required temperature for the processor), the use of the plastic containers in the collection of milk, the milking practices and the handling hygiene through the chain. Additionally, the sample was taken immediately after the cooler filled up. In Ngorika, the sampling was done in all the routes, and collection The raw milk microbial quality was very poor at all levels for this CBE. For both CBE, this may be due the contribution of insufficient pre-milking udder preparation, insufficient cleaning of milk handling equipment, use of poor quality water for cleaning, the storage time and lack of cold chain facility starting from the production site (Doyle et al., 2015). As reported by Van Kessel et al. (2004), the use of insufficient and poor quality water for cleaning of milk handling equipment can result in milk residues on equipment surfaces that provide nutrients for the growth and multiplication of bacteria that can then contaminate the milk. Murphy and Boor (2000) noted that ineffective cleaning, use of water without heat treatment and the absence of sanitizers tend to fasten growth of less heat resistant organisms. Similarly, mastitis infected cows can also contribute to high TVBC.

Generally, the presence of coliforms in milk confirms that the milk has been contaminated with fecal materials and it is an indicator of the sanitary conditions in the production and handling of the milk starting from production (Orregard, 2013) Accordingly, poor herd/farm hygiene, use of contaminated water, unsanitary milking practices, and use of improperly washed equipment for storage and distribution can all lead to elevated coliform count (CC) in raw milk (Gemechu et al., 2015). The fact that high proportion (90%) of the milk samples taken from all levels had coliform counts more than the upper limit of KEBS standards accepted for CC in raw milk, provides irrefutable evidence that the udder of the cows have been soiled with fecal materials and/or the udder is improperly washed; that is, milk contamination in the study area happened starting from milking of the cows. In addition, the presence of coliform in an aseptically collected sample of raw milk shows the use of bacteriological low quality water, either for washing utensils or mixing in raw milk (Farhan and Salik, 2007). Apart from safety and public health concerns, high contaminations by coliforms results in off-flavours in milk and reduced shelf life of dairy products (Reta and Addis, 2015: Kaindi et al., 2011). Generally, the bacterial generation (doubling) time is between 10 to 15 min depending on the conditions. According to Orregard (2013), aluminum cans allows better hygienic handling unlike plastic jerry cans. In this study, milk was transported while warm and in plastic jerry cans which could contribute to milk quality deterioration, unlike the recommended containers that do not have adhesive properties and are easy to clean when
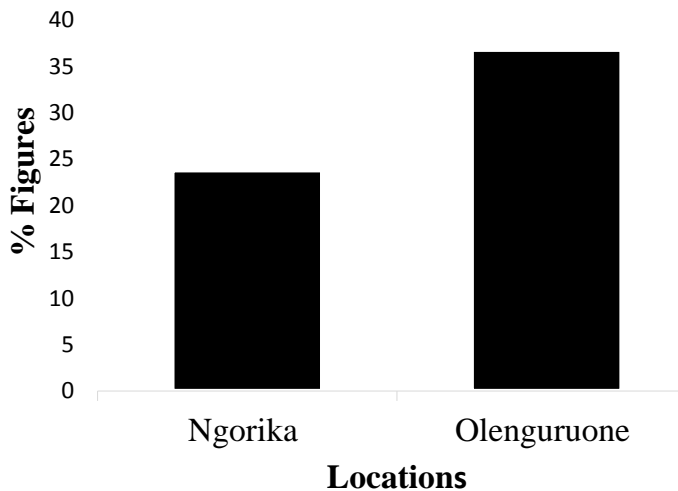
Figure 1. The average percent water adulteration in both CBE's.

compared with plastic containers. Moreover, more than 3 h, where natural lactoperoxidase enzyme could sustain the milk quality, were surpassed in some routes before refrigeration could take place. Cooling of milk is advocated to help in significantly reducing the multiplication of bacteria and in turn reduce spoilage (Kurwijila, 2006).

Adulteration

Freezing point of milk is its most constant property. According the Kenya Standard (KS 05-1552), approximately 0.545°C; but not less than 0.525°C is the freezing point of milk. Results indicated that adulteration incidences in Ngorika and Olenguruone were 23.8% and 36.8%, respectively (Figure 1). Adulteration in Ngorika was lower than in Olenguruone. This could have been contributed by the effect of the penalty attached to a farmer when caught in this act. Added water can occur in milk due to both unintentional (e.g., poor system drainage) and intentional addition (Kurwijila, 2006). Agents in some areas add water to increase the volume to make a larger profit, especially during the dry period when milk supplies are low and prices are high (Orregård, 2013). This can be detected using a cryoscope by measuring its freezing point. Normally, the freezing point of milk is slightly less than that of pure water and is relatively constant. Typical milk generally has a freezing point below minus 0.542 degrees Hortvett but when water is added to milk, the freezing point increases approximately 0.005°H for every 1% water addition. The lactometer test could not detect water adulteration especially when done at minimal levels. The freezing point determination as a standard gauge for water adulteration (Draaiyer et al., 2009) was applied to

compliment the lactometer test. This study corresponds a study carried out by Orregård, (2013), where the results showed that about 33% of the samples from farmers were not within the acceptable density limit. According to (KS 05-1552), density of milk of 20°C shall be within the following range: 1.026-1.032 g/ml.

Antibiotics

Antibiotics in milk are a major concern due to the risk of allergic reactions and the development of antibiotic resistant pathogen and inhibition of dairy starter cultures used to develop acid (e.g., lactic acid bacteria), which can result in the loss of significant amounts of product and milk (Popelka et al., 2004). The delvo test was carried out for the composite samples at the route level and the cooler. In total, 74 samples were analyzed in both CBE and out of these, 54 and 35% from Olenguruone and Ngorika, respectively were positive (Figure 2). This study corresponds to another study carried out by Aboge et al. (2000) on antimicrobial residues detected on marketed milk in Kenya. According to Shitandi, (2004), eighteen percent (18%) of the samples from small-scale producers in his study area were β-lactam positive significant (p<0.001) while other studies carried out within Kenya showed that many animal products in Kenyan market have high level of drug residues which is unacceptable (Muriuki, 2001). According to Gallagher (2015), consumption of food with antibiotics residues can lead to bacteria becoming completely resistant to treatment in human beings, a situation reffered to as antibiotic apocalypse.

Somatic cell count

Somatic cell count should not exceed 300 000 per ml when tested in accordance with ISO 13366. The California mastitis test (CMT) has been used for more than 50-years and continues to be the most accurate farm screening test for subclinical mastitis (Ruegg and Reinemann, 2002). The heavier the gel the higher the somatic cells in the milk and vice versa, indicative of the leukocyte count (Quinn et al., 1994). Most of the samples were in the range of 150,000 to 500,000 cells/ml which is an indicator of presence of clinical mastitis in the farms. In Ngorika, no sample was within the 0 to 200,000 cells/ml which reflects absence of somatic cells in milk suggesting that it has higher incidences of mastitis compared to Olenguruone which had 5% samples being mastitis negative.

Additionally, 65% of the samples collected Ngorika were lying under the range150,000 to 500,000 cells/ml which is higher than Olenguruone (55%) (Figure 3). The farmers in Olenguruone have a low average production per cow compared to Ngorika which could be a contributing factor to the high incidences of mastitis observed. *S. agalactiae* is known to be an occasional
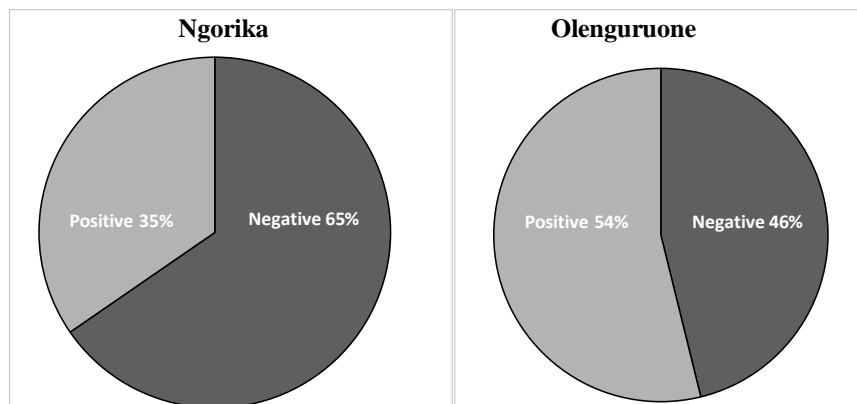
Figure 2. Detection percent of the antibiotic residues in both CBE's.
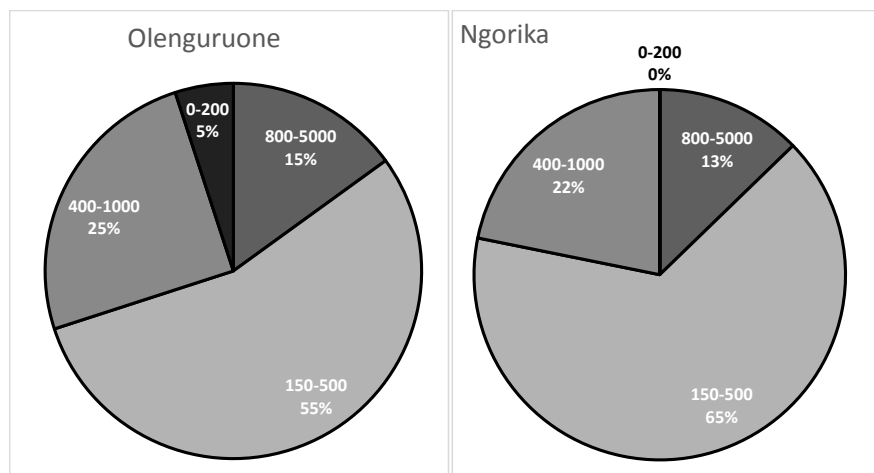

Figure 3. California mastitis test somatic cell count per 1000 mls per CBE.

cause of high bacterial counts and subclinical mastitis problems, hence it should be considered when both the SCC and TVBC are high (Ruegg and Reinemann, 2002). For that reason, the higher somatic cell counts detected could have contributed to the high levels of the total bacterial counts observed in both locations.

Increased somatic cell numbers are positively correlated with concentrations of plasmin, a heat-stable protease, and of lipoprotein lipase in freshly produced milk (Barbano et al., 2005). Activities of these enzymes can supplement those of bacterial hydrolases, hence shortening the time to spoilage. The major determinants of quantities of these enzymes in the milk supply are the initial cell numbers of psychrotrophic bacteria, their generation times, their abilities to produce specific enzymes, and the time and temperature at which the milk is stored before processing. Several conditions must exist for lipolyzed flavor to develop from residual lipases in processed dairy foods, that is, large numbers ($>10^6$ CFU/ml) of lipase producers (Stead, 1986), stability of the

enzyme to the thermal process, long-term storage and favorable conditions of temperature, pH, and water activity.

Resazurin test

It uses the indicator resazurin to measure the bacteriological quality of milk. The majority of the organisms in milk are capable of reducing and decolorizing the resazurin dye. When bacteria grow in the milk they utilize oxygen, the rate of removal or reduction is proportional to the keeping quality (Draaiyer et al., 2009). The Resazurin dye is more sensitive than the methylene blue and for this reason, this test provides a rapid measure of the keeping quality of milk. There was no significant difference ($p \leq 0.05$) for 10 min Resazurin test at the four sampling levels except for the rejected milk for both CBE's (Table 2 and 3). The correlation of the 10 min resazurin test and TVBC was not

Table 5. Correlation of general quality results evaluated at four sampling levels in smallholder dairy farmers supply systems in Kenya.

	TIME	Route	TVBC	CC	LA	RT
TIME	1.00000	-0.74248***	0.42845**	0.53512***	0.22400	-0.47639**
ROUTE		1.00000	-0.46522***	-0.47591***	-0.17855	0.64902***
TVBC			1.00000	0.67682***	-0.06786	-0.23962
CFU				1.00000	0.02256	-0.21445
LA					1.00000	-0.34558*
RT						1.00000

*,**,***, Indicates significance at p ≤0.05, p ≤0.01 and p ≤0.001, respectively.

significantly different (p ≤ 0.05) at the route level (Table 5). According to KEBS, (2007) raw milk specification, only 20.6% of samples were unacceptable based on this test contrary to the TVBC where all the samples were way above grade III raw milk requirements (2,000,000 CFU/ml) which is unacceptable. The study results agrees with the study carried out by Muliro et al. (2013) on quality assessment of raw camel milk using dye reduction tests that the resazurin test is not reliable as a measure of total viable bacterial count. According to Murphy and Boor (2000), a significant correlation (p ≤ 0.01) between the total bacterial count and time used to deliver milk for cooling was observed. The Plate count test has been reported to be generally accepted as the most accurate and informative method of testing the bacteriological quality of milk (Kurwijila, 2006: Muliro et al., 2013).

Titratable acidity test

The means separation indicated no significance difference (p ≤ 0.05) at the can, cooler and tanker level unlike the route level and the rejected milk in Ngorika (Table 2). This could have been contributed to the dilution factor as milk is being bulked together in the cooler. A different scenario was observed in Olenguruone where no significance difference (p ≤ 0.05) at the can, route, cooler and tanker level but significant difference (p ≤ 0.05) was observed with the rejected milk (Table 3). All the samples at the can, route, cooler and tanker levels were found to have acidity levels within the range of 0.16±0.02 and therefore judged to be of good quality for the titratable acidity test. This was contrary to all the milk samples rejected at the reception platform that were above 0.18% lactic acid, hence rejected as per the KEBS standards.

CONCLUSION AND RECOMENDATIONS

The study showed that total bacterial count and coliform count were way above the KEBS standards although along the dairy value chain. Factors like famers training

on the clean milk production, milk handling hygiene, use of appropriate containers and reduced delays in milk collection could assist in reducing the microbial load in raw milk. Milk quality tracking and tracing was lacking hence the concept of quality based milk payment system (penalties and premium) would be difficult to introduce. Can labeling and subsequent ownership which was lacking should be enhanced for tracking and tracing. The cooling plants were taking more than 3.5 h to cool the milk to below 5°C which justifies use of plate heat exchangers as an alternative for speedy cooling. The study also revealed higher figures for antibiotic residues which suggests that withdrawal period was not observed. To overcome this, inclusion of the test in quality based payment system and vigorous training to farmers will ensure that the antibiotic residues are within the maximum allowable limit. Adulteration of milk with water was evident which could be due to the fact that the farmers are paid according to supplied quantities. Milk policies should be established and punitive penalties introduced to farmers found to have adulterated the milk unlike the current situation where no action is taken. For raw milk quality based payment system, Resazurin test and lactometer test are not sufficient. TVBC and CC should be used instead of resazurin while freezing point determination should be used for adulteration.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Aboge GO, Kang'ethe EK, Arimi SM, Omore AO, McDermott JJ, Kanja LW, Macharia JK, Nduhiu JG, Githua A (2000). Antimicrobial agents detected in marketed milk in Kenya. 3rd All Africa Conference on Animal Agriculture. 6-9 November 2000, Alexandria, Egypt. Nairobi (Kenya): Smallholder Dairy Project (SDP).

AOAC (2005). Official Methods of Analysis of AOAC International. (15th ed.). Method Nr 990.12., 991.14.3M. USA.

Baltenweck I, Staal S J, Owango M, Muriuki H, Lukuyu B, Gichugu G, Kenyanjui M, Njubi D, Tanner J, Thorpe W (1998). Intensification of dairying in the greater Nairobi milk-shed: Spatial and household analysis. Ministry of Agriculture (MoA), Kenya Agricultural Research

Institute (KARI) and International Livestock Research Institute (ILRI) Collaborative Research Project Report. 26. http://www.smallholderdairy.org/collaborative res reports.htm

Cherono PK (2005). School milk program for Kenya. Kenya Dairy Board (KDB). Paper presented at the 1st African School Milk Conference, 28th September 2005. Kampala, Uganda. 21.

Chesterman S, Neely C (Eds) (2015). Evidence and policy implications of climate-smart agriculture in Kenya. CCAFS Working Paper no. 90. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). Copenhagen, Denmark. Available online at: www.ccafs.cgiar.org.

Delgado C, Rosegrant M, Steinfield H, Ehui S, Courbois C (1999). Livestock to 2020: the next food revolution. Food, Agriculture, and the Environment discussion Paper 28. Washington, DC: International Food Policy Research Institute (IFPRI), Food and Agriculture Organization (FAO) and International Livestock Research Institute (ILRI). 83.

Delvotest Technical Bulletin (2011). DSM food specialties B.V., Netherlands. Cited on 20th February 2016. http://www.agripress.be/_STUDIOEMMA_UPLOADS/downloads/Delvotest.pdf

Doyle MM, Garcia S, Bahati E, Karamuzi D, Cullor JS, Nandi S (2015). Microbiological analysis of raw milk in Rwanda. Afr. J. Food Sci. Technol. 6(5): 141-143.

Draaiyer J, Brian D, Antony B, Jerome M (2009). Milk testing and payment systems. A practical guide to assist milk producer groups. (FAO) Food and Agricultural Organization. Rome, Italy.

Farhan M, Salik S (2007). Evaluation of bacteriological contamination in raw (unprocessed) milk sold in different regions of Lahore, Pakistan. J. Agric. Soc. Sci. 3(3): 104-106.

Food and Agriculture Organization (FAO) and International Dairy Federation (IDF) (2004). Dairy Development Newsletter. October 2004 – Issue No. 8.

Gallagher J (2015). Antibiotic resistance: world on cusp of "post-antibiotic era". www.bbc.com/news/health-34857015. Accessed on November 20, 2015.

Gemechu T, Beyene F, Eshetu M (2015). Physical and chemical quality of raw cow's milk produced and marketed in Shashemene Town, Southern Ethiopia. ISABB J. Food Agric. Sci. 5(2):7-13.

Government of Kenya (2006). Sessional Paper on Dairy Industry Development.

Hillerton JE, Halley BI, Neaves P, Rose MD (1999). Detection of antimicrobial substances in individual cow and quarter milk samples using Delvo-test microbial inhibitor tests. J. Dairy Sci. 82(4):704-711.

Kaindi DWM, Schelling E, Wangoh J, Imungi JK, Farah Z, Meile L (2011). Microbiological quality of raw camel milk across the Kenyan market chain. Food 5. Global Science Books. (Special Issue 1). Pp.79-83

KEBS (Kenya bureau of standards) (2007). Kenya standards East African Standards (KS EAS).Raw cow milk specification, 1st edition, Arusha. Tanzania.

Kenya National Bureau of Statistics (KNBS) (2010) Kenya 2009 Population and housing census results. Ministry of Planning, National Development and Vision 2030. Nairobi, Kenya. United States Agency for International Development (USAID), United Nations Population Fund (UNFPA) and Swedish International Development Agency (SIDA). Nairobi, Kenya. 8.

Kurwijila LR (2006). Hygienic milk handling, processing and marketing. Reference guide for training and certification of small-scale milk traders in East Africa. Vol. 1

Mellenberger R, Roth CJ (2000). California Mastitis Test (CMT). Fact Sheet, Dept. of Animal Sciences, Michigan State Univ. and Dept. of Dairy Sci., University of Wisconsin-Madison.

Ministry of Livestock and Fisheries Development (MoL&FD) (2003). Livestock Production Annual Report 2002. Nairobi, Kenya.

Muia JMK, Kariuki JN, Mbugua PN, Gachuiri CK, Lukibisi LB, Ayako WO, Ngunjiri WV (2011). Smallholder dairy production in high altitude Nyandarua milk-shed in Kenya: Status, challenges and opportunities. Livest. Res. Rural Dev. 23(5):108.

Muliro SP, Shalo LP, Kutima MP (2013). Quality assessment of raw camel milk using dye reduction tests. Afr. J. Food Sci. Technol. 4(5):116-121.

Muriuki HG (2001). Smallholder dairy production and marketing in Kenya. In: Smallholder dairy production and marketing-Opportunities and constraints. Proceedings of a South-South workshop held at NDDB, Anand, India. pp. 13-16.

Muriuki HG (2011). Dairy development in Kenya. Food and Agricultural Organization of the United Nation, Rome, Italy.

Muriuki HG, Omore A, Hooton N, Waithaka M, Ouma R, Staal SJ, Odhiambo P (2003). The policy environment in the Kenya dairy sub-sector: A review. Smallholder Dairy (Research and Development) Project, Nairobi, Kenya.

Muriuki HG (2003).Assessment of the level, type and value of post-harvest milk losses in Kenya. Results of a rapid appraisal for a national sub-sector assessment for FAO.

Murphy SC, Boor KJ (2000). Sources and causes of high bacteria counts in raw milk: An abbreviated review. Dairy Food Environ. Sanit. 20(8):1-4.

Mwangi A, Arimi SM, Mbugua S, Kang'ethe EK, Omore AO, McDermott JJ (2000). Assurance of marketed milk quality in Kenya. Paper presented at the Faculty of Veterinary Medicine Biennial Scientific Conference, 30-31 August 2000, University of Nairobi, Kenya.

Omore A, Lore T, Staal S, Kutwa J, Ouma R, Arimi SE, Kang'ethe EK (2005). Addressing the public health and quality concerns towards marketed milk in Kenya. Small Holder Dairy Project Research and Development Report No. 3.

Orregård M (2013). Quality analysis of raw milk along the value chain of the informal milk market in Kiambu County, Kenya. 4:1101-8151

Peeler EJ, Omore A (1997). Manual of livestock production systems in Kenya. 2nd Edition. Kenya Agricultural Research Institute (KARI). Nairobi, Kenya. 138 p.

Popelka PR, Nagy J, Popelka P, Marcincak S, Rozanska H, Sokol J (2004). Comparison of sensitivity of various screening assays and liquid chromatography technique for penicillin residue detection in milk. Bull. Vet. Inst. Pulawy 48(3):273-276.

Quinn J, Carter E, Markey B, Carter R (1994). California mastitis test (CMT). In: Clinical Veterinary Microbiology. 1st ed. London: Wolfe publishing. Pp.333-334.

Reta AM, Addis AH (2015). Microbiological quality assessment of raw and pasteurized milk. Int. J. Food Sci. Microbiol. 2(6):87-91.

Romney DL (2004). Understanding the role of dairy in smallholder systems and the effect of intensification. Proceeding of the 9Th Kenya Agricultural Research Institute (KARI) Biennial Scientific Conference, 8th-12 Th November, 2004, Nairobi, Kenya.

Ruegg PL, Reinemann DJ (2002). Milk quality and mastitis tests. Bovine Pract. 36(1):41-55.

SAS Institute Inc. (1999). SAS/STAT Users' Guide, Version 8 Release 6.03. Statistical Analysis Systems (SAS), Carry, NC. 549-640.

Schreiber C (2000). Sources of innovation in dairy production in Kenya. International Services for National Agricultural Research (ISNAR) briefing paper No. 58. Ministry of Agriculture and Rural Development (MoARD), Kenya Agricultural 53 Research Institute (KARI) and International Livestock Research Institute (ILRI)/ISNAR. The Hague, the Netherlands.

Shitandi A (2004). Risk factors and control strategies for antibiotic residues in milk at farm level in Kenya. Doctoral dissertation. Department of Food Science. Swedish University of Agricultural Sciences. Sweden. 91(3):576-6477.

Shitandi A, Sternesjo A (2004). Factors contributing to the occurrence of antimicrobial drug residues in Kenyan milk. J. Food Prot. 67:399-402.

Staal SJ, Owango M, Muriuki H, Lukuyu B, Njoroge L, Njubi D, Baltenweck I, Musembi F, Bwana O, Muriuki K, Gichugu G, Omore A, Thorpe W (2001). Dairy systems characterization of the greater Nairobi milk shed. Smallholder Dairy (Research & Development) Project. Kenya Agricultural Research Institute (KARI), Ministry of Agriculture (MoA) and International Livestock Research Institute (ILRI) Collaborative Dairy Research Program, Nairobi, Kenya. 73. http://www.smallholderdairy.org/collaborative res reports.htm

Van Kessel SJ, Karns SJ, Gorski L (2004). Prevalence of Salmonellae, Listeria monocytogenes, and fecal coliforms in bulk tank milk on US dairies. J. Dairy Sci. 87:2822-2830.