African Journal of Rural Development, Vol. 2 (1) 2017: pp. 135-142 ISSN 2415-2838

Date received: 16 June, 2016 Date accepted: 19 December, 2016

Microbiological and compositional quality of raw milk delivered by small scale dairy farmers to the milk collection centres in northern Tanzania

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ABSTRACT

Milk samples were collected from small scale dairy farmers who brought their milk to milk collection centres (MCCs) and vendors in the study area in Tanzania. A total of 310 milk samples were collected. The bacteriological quality, physico-chemical parameters and milk adulteration with sulphonamides and tetracycline drug residues were assessed. Microbiological quality evaluation was done based on TBC, TCC, *Escherichia coli* and *Escherichia coli* pathogenic (O157: H7). Milk samples from Turiani and Mlandiz were added for anti-microbial residue analysis. In addition, a survey with semi-structured questionnaires was conducted to assess the knowledge of farmers on clean milk production in the study area. Microbiological analyses were undertaken using Automatic Milk Analyser and Charm EZ technique. Results revealed that 91% of respondents dried cow udders after washing, 75% cleaned milk containers with hot water and 86.7% were using plastic containers to store and transport milk. The mean bacterial counts were within acceptable limits as per EAC standards. *E.coli* was detected to a level of 54% but no *E.coli* pathogenic detected. Physico-chemical properties of milk were within acceptable ranges for processing. Tetracycline and sulphonamides drug residues were found in farmers' milk samples but not vendors. More quality tests such as iodine and alcohol tests are recommended to ensure freedom of milk from adulteration with other materials exeception of water.

Key words: Adulteration, drug residues, *Escherichia coli*, farmers, Tanzania, vendors

RÉSUMÉ

Des échantillons de lait ont été recueillis auprès de petits producteurs laitiers qui ont apporté leur lait dans les centres de collecte du lait (MCCs) et les fournisseurs dans la zone d'étude. Un total de 310 échantillons de lait a été collecté. La qualité bactériologique, les paramètres physico-chimiques et le frelatage du lait avec les résidus de comprimés de sulfamides et de tétracycline ont été évalués. L'évaluation de la qualité microbiologique a été effectuée sur la base de TBC, TCC, *Escherichia coli* et *Escherichia colii* pathogène (O157: H7). Des échantillons de lait de Turiani et Mlandiz ont été ajoutés pour l'analyse antimicrobienne. De plus, une enquête à l'aide de questionnaires semi-structurés a été menée pour évaluer les connaissances des agriculteurs sur la production de lait saine dans la zone d'étude. Les analyses microbiologiques ont été effectuées enutilisant la technique 'Analyser and Charm EZ'. Les résultats ont révélé que 91% des enquêtés sèchent la mamelle après le lavage, 75% nettoient les contenants de lait avec de l'eau chaude et 86,7% utilisent des contenants en plastique pour stocker et transporter le lait. Les chiffres moyens de bactéries étaient dans des limites acceptables conformément aux normes de l'EAC. *E. coli* a été détectée à un niveau de 54%, par contre aucun E. coli pathogène n'a été détecté. Les propriétés physico-chimiques du lait étaient dans des limites acceptables pour la transformation. Des résidus de tétracycline et sulfamides ont été trouvés dans les échantillons de lait provenant des producteurs laitiers contrairement à ceux des fournisseurs. Plus de tests de qualité tels que les tests d'iode et d'alcool sont recommandés pour préserver le lait du frelatage avec des matériaux autre que l'eau.

Mots clés: Frelatage, résidus de médicaments, *Escherichia coli*, producteurs, Tanzanie, vendeurs

INTRODUCTION

Milk is a highly perishable commodity, vulnerable to spoilage and consequently becomes a health risk to consumers if is not adequately handled from the point of production along the marketing channel (Schooman and Swai, 2011). Owing to the centrality of milk in the diet of children and expectant mothers, its quality is important for the welfare of the community (Sudhasaravanan and Binukumari, 2015). Microbial contamination of milk is the major

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cause of milk spoilage as well as source of health hazards to consumers (Ngasala *et al*., 2015). Presence of high bacteria loads in raw milk reduces the keeping quality of milk as well as the products that will be made from such milk (Minj and Behera, 2012). In order to protect health of consumers, a number of analyses can be undertaken to ensure health safety of consumers including total bacterial count (TBC),total coliform count (TCC), *Escherichia coli* and a pathogenic *E.coli* (Shojaei and Yadollahi, 2008).

Milk composition forms an important aspect of milk quality proir to consumption and for further processing into different milk products. This also is used as criteria when processors are developing a quality payment scheme to farmers with butter fat and protein being most important content as they are determinant for butter and cheese making. Adulteration of milk by dishonest farmers interferes with milk composition and so it is important to measure this parameter for ensuring that the physico-chemical properties of milk are maintained to qualify milk for further processing and also ensure the quality of the processed products from such milk. Studies have revealed that the presence of tetracycline residues at higher level than it is accepted in food is a serious public health concern because of its harmful effects to the consumers (Gaurav *et al.,* 2014). Presence of antibiotic residues in food is not accepted and this creates need for research to be carried out to establish the status of drug residues in milk from small scale dairy farmers in Tanzania. This study assessed the residue level of sulphonamides and tetracyclines in milk samples from two townships (Turiani, Mlandizi) and one district (Hai) of Tanzania. The quality of milk is an important determinant in keeping quality and the shelf life of the dairy products made from milk and hence disqualifying milk with high load of bacteria for further processing. The study aimed to establish the microbiological and physico-chemical milk quality as well as presence of antibiotic residues in milk produced by small scale dairy farmers in the study areas.

MATERIALS AND METHODS

Study site

This study was carried out in Hai District in Kilimanjaro Region which is located in the northern part of Tanzania (Figure 1). The study area was purposively selected because it has a high proportion of small scale dairy farmers in comparison to other Districts (Huka *et al.,* 2014). The area lies between latitude 2°50’ and 3°29’ South and longitude 30°30’ and 37°10’ East. The district is divided into three zones namely: upper zone, middle zone and lowland zone. The upper zone receives 1250-1750 mm of rain per annum. The lowland and middle zones receive 500-700 mm and 700-1250 mm rainfall, respectively. There are two rainy seasons. The long rainy season in March and June whilst the short rains occur in November to December.

The major agricultural activity is mixed farming. Farmers keep livestock as well as cultivate food and cash crops. Apart from dairy, farmers keep local chickens, sheep and goat. The main crops grown include maize, beans, bananas and coffee. Coffee is a cash crop while banana is both food and cash crop.

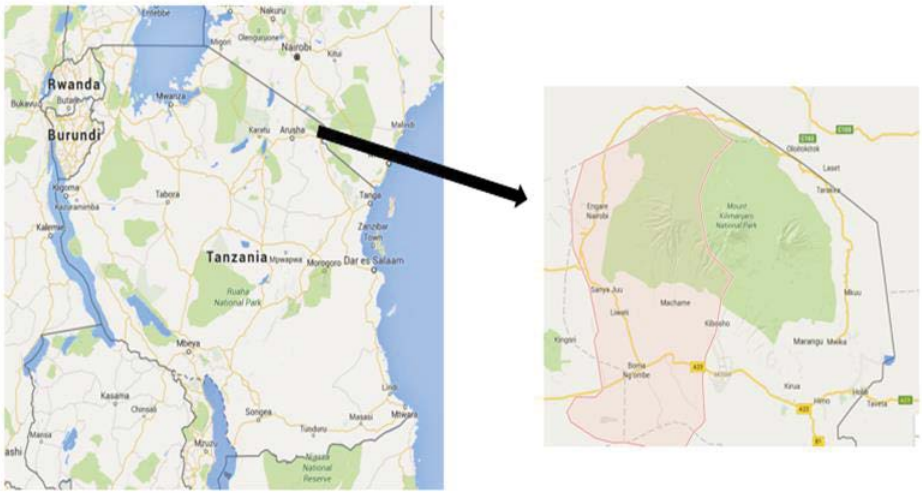

Figure 1 Map of Tanzania showing the location of Hai district
Source https://www.google.co.zw/maps/place/Tanzania

Sampling strategy. Sampling technique of choice was purposive random sampling in which wards with highest milk production were selected. Villages with milk collection centres were subsequently selected and the milk vendors and milk selling points were included in the study. Sample size determination was based on http://www.raosoft.com/samplesize.html. With the assumptions of 5% margin error, 95% confidence interval, 3654 population size and 85% response of distribution, this resulted in a sample size of 180 farmers. Vendors were selected according to their availability in the study area with 30 of them participating.The total sample size for the study was therefore 210.

Collection of milk samples. A total of 310 individual animal milk samples were collected from five villages in Hai District: Nkuu Ndoo, Foo, Wari, Nronga and Lyamungo Kati. In addition milk samples were also collected from vendors that buy the milk from the collection centres in the participating villages. The collected samples were transported to the Microbiology Laboratory of the Department of Animal Science, Sokoine University of Agriculture (SUA) at a temperature of 4-5°C. The samples were frozen upon arrival to SUA and microbiological analysis was performed the next day.

Statistical Model. The following statistical model was used in analysing data for the microbiological quality of the milk

$Yij=\mu + \beta i + eij$

Where Yij =individual observation for each test

βi =the ith milk source effect (i=1,2)

Eij = the error term

Microbiological analysis of milk samples. The collected milk samples were analysed for TBC TCC *E.coli* and *E.coli* pathogenic (0157:H7). These samples were analysed for microbiological quality using the standard procedures as described by USDA (2011). In the analysis of TBC the media used was Plate Count Agar (HIMEDIA REF M091). For TCC the media used was Violet Red Bile Agar (HIMEDIA REF M049). For identification of *E.coli* from the collected milk samples MacConkey Agar (HIMEDIA REF M081) was used. For identification of pathogenic strain of *E.coli* (O157:H7), Sorbitol MacConkey Agar (HIMEDIA REF M298) was used. Standard procedures were followed for plating, incubating of the samples and counting of the grown bacteria.

Confirmation of *E.coli* and *E.coli* pathogenic (O157:H7) The biochemical tests for confirmation were done to confirm presence of *E.coli* in the milk samples.

Gram staining test. This test was done on the sterile glass slides and a drop of normal saline was firstly added on a slide then well isolated colonies were spread on the prepared slides to make smears. Smears were air dried followed by gentle fixing and flaming. Fixed smears were soaked in a crystal violet stain for two minutes then rinsed under running tap water. Slides were then soaked in Lugol's iodine for two minutes and rinsed. Thereafter these slides were decolorized by using acetone alcohol and washed under running tap water followed by covering of these fixed smears with neutral red for two minutes and rinsed. Slides were dried on a draining rack, and a drop of oil immersion was added to examination under light microscope with 100X objective prior to bacterial morphology. Gram positive were cocci with pale to dark purple colour while Gram negative were rod shaped with pale to dark red colour.

Indole Test. Peptone water solution was prepared by diluting 15.23 grams of peptone water powder into 1 litre of the distilled water and then 3mls of this solution was added in bijou tubes by using a sterile plastic disposable pippete. A fresh sterile plastic loop was used to inoculate the colonies to bijou tubes before incubation at 37°C for 48 hours. After incubation 0.5mls of Kovac's reagent (Loba Chemie Pvt. Ltd, Lot LM 01131303) were added to the incubated tubes. Tubes were gently shaken and examined for red coloured ring formation on the surface of the tube. Formation of this red ring is an indication of positive Indole reaction.

Statistical Analysis. The TBC and TCC in milk samples were compared with standards (EAC, 2007). The data were transformed into log10 and the means analysed by the use of SAS at the 95% confidence interval and 5% level of significance.

Table 1 shows the milk grading system practised in East Africa with respective legal limits. TBC and TCC for milk samples collected from farmers and vendors were compared using the standards shown in this table.

Milk composition and drug residues analysis. Milk composition analysis was carried out using an Ultrasonic Milk Analyser Model LM 2, serial number 11673 IndiaMART Company Limited. The milk constituents measured were butterfat content and solids not fat. The same equipment was also used to measure milk density and freezing point. Sulphonamide and tetracycline drug residues in milk were analysed using Charm EZ technique according to the manufacturers' instruction. Tetracycline and sulphonamides are the most commonly used antibiotics in Tanzania. For tetracycline, incubation time was 8 minutes, and 4 minutes for sulphonamides.

RESULTS

The results on the grades of the milk from two sources based on EAC legal limit standards shows that the large proportion of milk were within grades I (37.6%) farmers, (33.3%) vendors and II (41.4%) farmers, (36.7%), vendors (Table 2).

There were significant differences in TCC and TBC from milk samples collected from farmers and vendors with farmers having a significantly lower TBC and TCC ($p \leq 0.05$),(Table 3), Implying that milk from farmers was of better quality than those from vendors.

Table 1. Proposed raw milk grading system for EAC based on microbiological quality

Parameter	Grading	Range (cfu/ml)
TBC	I	<200,000
	II	>200,000 – 1,000,000
	III	>1,000,000 – 2,000,000
	Reject	More than 2 000 000
TCC	I	0-10 000
	II	10 000- 50 000
	Reject	More than 50 000

Source (EAC, 2006)

Table 2. Classification of Microbiological quality of raw milk samples collected from farmers and vendors in Hai District

Parameter	Grading	Range (cfu/ml)	Farmers	Vendors
TBC	I	<200,000	68 (37.6%)	10(33.3%)
	II	>200,000 – 1,000,000	75 (41.4%)	11(36.7%)
	III	>1,000,000 – 2,000,000	16 (8.8%)	4 (13.3%)
	Reject	More than 2 000 000	21(11.6%)	5 (16.7%)
TCC	I	0-10 000	114 (63%)	24 (82.8%)
	II	10 000- 50 000	48 (26.5%)	4(13.8%)
	Reject	More than 50 000	18 (9.9%)	1 (3.4%)

Table 3. Total Coliform Count and Total Bacterial Count for farmers and vendors

Variable	Farmer	Vendor	p-Value
TCC	$1.5\pm0.9x10^{4a}$	$1.8\pm0.8x10^{4b}$	0.03
TBC	$7.\pm0.2x10^{5a}$	$1\pm0.2x10^{6b}$	0.0001

LSM ± SE with different superscripts in the same row under the same variable differs statistically ($p \leq 0.05$).

Table 4 shows the mean Total bacterial and coliform counts of milk from farmers in target villages. The highest and lowest counts for TCC were found in Nronga and Lyamungo Kati villages respectively. For TBC mean counts, the highest and lowest counts came from samples collected from Foo and Wari villages.

Table 5 presents the mean bacterial counts of milk from vendors in the villages participated in the study. Table 6 shows that milk samples from both farmers and vendors tested positive for the presence of E.coli all samples tested were negative for the pathogenic strains of *E.coli.* (Table 6)

Physicochemical properties of milk. Levels of milk fat were found to be 3.5% and 3.1% for farmers and vendors, respectively. Milk samples from vendors had no added water but some samples from farmers had added water. Results for freezing point of milk samples were not significantly different ($p>0.05$) between farmers and vendors. (Table 7).

Antimicrobial Residues. Results showed that the highest frequency of positive samples of Sulphur residues was in milk samples from Turiani (53.1%) and lowest in mlandizi (25%). Tetracycline highest level was in milk samples from Turiani (25%), and lowest (6.2%) in samples from Mlandizi. Milk samples from vendors tests negative for the two antibiotic residues. (Fig 2).

Table 4. Total Bacterial Count and Total Coliform Count for milk collected from farmers from participating villages in Hai district

	Village					
Parameter	**1**	**2**	**3**	**4**	**5**	**P-Value**
TCC	2.8 ±0.7 x 10^{4a}	0.4 ±9.4 x 10^{4b}	0.1 ±9.4 x 10^{4b}	4.8 ±0.7 x 10^{4a}	1.7 ±9.4 x 10^{3b}	0.03
TBC	8.4 ±1.9 x 10^{5a}	1.5 ±0.2 x 10^{6b}	5.5 ±2.3 x 10^{5a}	x 6.5 ±1.9 x 10^{5a}	9.1 ±2.3 x 10^{5a}	0.01

LSM ± SE with different superscripts in the same row under the same variable differ statistically ($p<0.05$)

Table 5. Total Bacterial Count and Total Coliform Count for milk collected from vendors from participating villages in Hai district

	Village					
Parameter	**1**	**2**	**3**	**4**	**5**	**p-Value**
TCC	1.8 ±0.6x10^{4a}	2.0 ±0.1x10^{4a}	1.7 ±0.7x10^{4a}	7.3 ±2x10^{4b}	2.8 ±2x10^{4a}	0.0009
TBC	9.5 ±0.5x10^{5a}	9.8 ±0.6x10^{5a}	6.9 ±0.5x10^{5a}	1.6 ±0.6x10^{6b}	1.1 ±0.5x10^{6b}	0.02

LSM ± SE with different superscripts within columns and under the same variable are statistically different ($p<0.05$).

Table 6. Proportions of samples with *E.coli* in milk samples collected from farmers and vendors in the Hai District

Actor	**N**	**Samples with *E.coli***	**%**
Nkuu Ndoo	29	5	17.2
Foo	18	5	27.8
Wari	41	1	2.4
Nronga	11	6	54.0
Lyamungo Kati	29	4	13.8
Vendors	9	1	11.0

Table 7: Comparison of physico-chemical properties of milk samples collected from selected villages in the Hai district in Tanzania

Parameter	Village			
	1(n=30)	2(n=43)	3(n=27)	P-Value
Fat (%)	3.4 ± 0.09[a]	3.8 ± 0.11[b]	3.4 ± 0.09[a]	0.003
Temperature(°C)	9.8 ± 0.3[a]	22.8 ± 0.37[b]	21.14 ± 0.3[c]	0.0001
Density	23.8 ± 0.4a	28.9 ± 0.51[b]	28.45 ± 0.42[a]	0.003
Added water	1.3 ± 0.21[a]	0.05 ± 0.3[b]	0.05 ± 0.21[a]	0.0003
SNF (%)	7.5 ± 0.01[a]	6.3 ± 0.02[b]	6.4 ± 0.01[b]	0.0001
Freezing Point	-0.53 ± 0.01[a]	-0.51 ± 0.01[a]	-0.53 ± 0.01[a]	0.42

Figures with different superscript letter in the same row and under the same variable differs ($p<0.05$)

Table 8 Comparison of hysico-chemical properties of milk from farmers and vendors

Parameter	Farmers	Vendors	p-Value
Fat (%)	3.6 ± 0.1[a]	3.2 ± 0.1[a]	0.5
Temperature(°C)	19.9 ± 0.4[a]	21.6 ± 0.4[a]	0.3
Density	28.7 ± 0.5[a]	27.9 ± 0.7[a]	0.4
Water	0.12 ± 0.08[a]	0[b]	0.0001
SNF (%)	7.5 ± 0.02[a]	7.3 ± 0.02[a]	0.7
Freezing Point	-0.54 ± 0.06[a]	-0.53 ± 0.01[a]	0.5

Figures with different superscript letter in the same row means the two groups were statistical different ($p<0.05$).

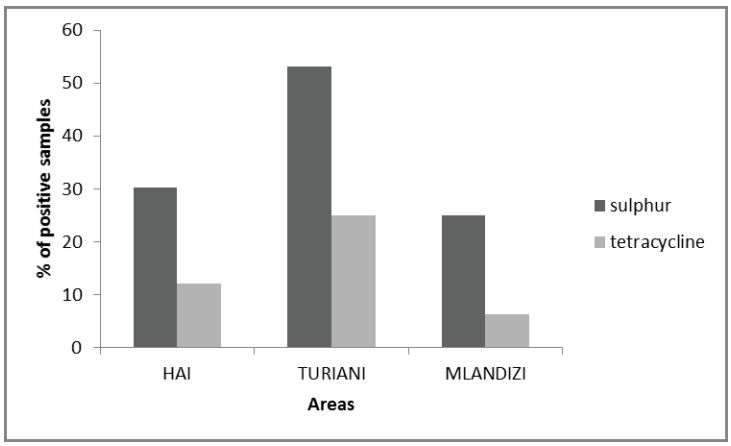

Figure 2. Drug residues in milk samples collected from Hai, Turiani and Mlandizi areas in Tanzania

DISCUSSION

The mean TBC of bacteria was statistically different villages, and also between samples from farmers and vendors in the study area. However the observed level of bacteria load in milk from the study area were in the acceptable ranges as per East African Community Standards as they were below $2x10^6$cfu/ml indicating the improved hygiene milk handling. These results however were in agreement with (Mdegela *et al*., 2009) who reported mean Total Bacteria Count of milk from small scale dairy farmers in Njombe District Tanzania to be within the acceptable ranges. Significantly higher levels of the mean total bacterial count of milk ($p=0.0001$) from vendors compared to the samples from the farmers were contributed by the lack of use of cold chain by

vendors during transportion of milk to the selling points as the rate of microbial notification in milk increases at temperatures above 4 °C.

The majority of the milk samples from this study show a Total Coliform Count levels that were within the accepted range for both farmers and vendors as well as in the target villages. This can be contributed by proper cleaning of milk containers by the use of hot water and reduction of contamination of milk with faecal materials by proper udder cleaning and drying.

The present study reported presence of *E.coli* in milk samples in the target villages for both farmers and vendors which is in agreement with (Ngasala *et al.,* 2015) who conducted the study in Arusha and Meru Districts and also (Karimuribo *et al*., 2016) in Kilosa District in Tanzania. Recovery of *E.coli* from fresh raw milk is an indicator of the possible presence of other pathogenic micro-organisms such as Salmonella which are also of faecal origin. This indicates risk on health of consumers as milk is supposed to be free from *E.coli* according to EAC Milk Standards (EAC, 2006). The study did not find any evidence for the presence of *E.coli* pathogenic strains, which is consistent with the study by Shija (2013) in Tanga Region.

Additionally this study recognised presence of tetracycline and sulphonamide antibiotic residues in the analysed milk samples from Hai, Turiani and Mlandizi. The presence of sulphonamides and tetracyclines in milk samples is an indication of lack of adherence to the drug withdrawal period after administration of antibiotics to sick milking animals. This is an indication of dishonesty because interviewed farmers said that they were not selling milk from animals on treatment. Lack of adherence to the practice can be due to fear of financial losses a farmer incurs due to throwing away the milk.

The physico-chemical properties of milk were found to meet the minimum standards required for processing of dairy products. This observation is based on the results obtained using the lactometer test for the milk brought to Milk Collection Centres. The results were similar to those reported by Séverin (2013) in Côte D'ivoire.

CONCLUSION AND RECOMMENDATIONS

Information generated from this study on milk quality can be used in the development of strategies to boost the small scale dairy sector in Tanzania. The information will also be useful to all stakeholders in dairy sector in Tanzania. There is need to conduct formal training of farmers and vendors for purpose of ensuring clean milk production and handling. Results from the study suggest that milk quality from this study area met the minimum required fat content for processing. However, presence of antibiotics residues in the tested milk is of serious concerns and this needs policy intervention.

Further, good practices such as introduction of platform tests as a basis for accepting milk at MCC's and introducing quality premium payment schemes to reward farmers who supply milk free from drug residues and penalise those who do not, can improve milk quality at sale.

ACKNOWLEDGEMENT

This study was funded by USAID, the Innovative Agricultural Research Intiative (iAGRI) projects and RUFORUM is thanked for its coordination and logistical support.

STATEMENT OF NO CONFLICT OF INTEREST

We the authors of this paper hereby declare that there are no competing interests in this publication.

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