Assessment of coliform bacterial contaminations in raw cow milk from selected dairy farms in Morogoro Municipality, Tanzania

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SUMMARY

Milk quality depends on many parameters including microbial status which mainly originate from the lactating animal itself. Secondary sources of microbial contaminations occur along the milk production chain. Unhygienic harvesting, handling and processing of raw cow milk affect its microbial quality. Microbial contaminations in milk cause spoilage and milk-borne diseases in humans. The current cross-sectional study was conducted to assess the levels of coliform bacterial contaminations in raw cow milk collected from different dairy farms in Morogoro Municipality. A total of 20 farms with lactating dairy cattle were visited and 20 raw cow milk samples were collected for coliform bacteria analysis using standard procedures. East African Standards for coliform count were used to ascertain for raw milk coliform contaminations. Before sampling, some basic information on milking, milk handling, storage and processing were gathered through a questionnaire. It was found that the mean cattle herd size from which the sample was taken was 48 ±8 with mean lactating cows in the herd being 15 ± 28. The general status of coliform bacteria in milk indicated that the contamination rate was 95%. The average total coliform count was 8.1 ± 8.2 (log TCC cfu/ml). The assessed farm activities including type of milking, containers used in milking and storage, occurrences of mastitis and milk storage conditions significantly contributed to coliform bacterial contamination in raw cow milk ($p<0.05$). It was concluded that almost all the raw cow milk assessed were heavily contaminated with coliform bacteria which implies that either the cows had bacterial infections or there was unhygienic practices in the process of milking, milk handling and storage. This calls for the need of education to the farmers on hygienic way of handling milk along the value chain so as to minimize unnecessary contaminations which can be of public health significance.

Key words: Raw cow milk, coliforms, Morogoro

INTRODUCTION

Coliforms refer to the group of bacteria in the family Enterobacteriaceae that are found in gastrointestinal tract of humans, livestock and other mammals which forms part of the intestinal micro flora (Facklam *et al.,* 2002). Presence of coliform bacteria normally indicates recent fecal contamination and therefore they are used as indicator bacteria. Unhygienic production of animal and plant source foods predisposes them to microbial contaminations predetermined by coliform bacteria. They are also common in environment that is contaminated with human and animal faeces which forms potential sources of surface water contaminations with coliform bacteria (Hayes *et al.,* 2001). The common coliforms include *Escherichia coli, Klebsiella, Citrobacter* and *Enterobacter*. Coliforms are frequently isolated from contaminated food products such as meat, milk and lettuce. Apart from contaminations, coliform bacteria can

cause food spoilage and some are known to be pathogenic to humans and animals. Some strains of *E. coli* like enteropathogenic, *e*nteroinvasive, *e*nterotoxigenic *e*nterohaemorrhagic *e*nteroaggregative *E. coli* causes different disease conditions in humans and some associated with severe diarrhoea. An enterohaemorrhagic *E. coli* O157:H7 is known to cause haemorrhagic colitis (Leclerc *et al.*, 2002) and its occurrence have been reported in raw cow milk from traditional cattle farms in Tanzania (Schoder *et al.*, 2013). A similar study by Lupindu (2014) isolated a highly pathogenic *E. coli* O157:H7 in cattle manure in Morogoro urban and peri-urban.

Coliform bacteria are normally shed in the faeces of healthy livestock, including dairy cattle. Thus, poor herd hygiene, contaminated water, unsanitary milking practices, wet milking and improperly washed and maintained equipment can all lead to elevated coliform counts in raw milk at the dairy farm. Milking of cows with wet and manure-soiled udders and inadequately cleaned milking equipment are the most common ways for coliform bacteria to enter milk on farm.

Morogoro is among the Municipality with several small- and medium-scale dairy farmers who produce and sell raw cow milk to the community in the city nevertheless, there are limited information on microbiological quality of the produced. Therefore, the current study aimed to establish the status of coliform contamination in raw cow milk produced by selected small- and medium-scale dairy farmers in Morogoro Municipality. Factors for contaminations like milking environment, personnel and equipment were also assessed to ascertain their contribution to coliforms contamination.

MATERIALS AND METHODS

Study area and animals

This study was conducted in Morogoro Municipality in Morogoro region Tanzania. Morogoro is a city with a population of 315,866 (PHCT, 2013) in the eastern part of Tanzania, 196 kilometers west of Dar es Salaam, the country's largest city and commercial Centre, and 260 kilometers east of Dodoma, the country's capital city. Morogoro is the capital of the Morogoro Region. The study was conducted in small- and medium-scale dairy farms in Morogoro Municipality. Most of the animals kept are crosses of Friesian, Ayrshire and Jersey. In most cases, the dairy cattle are managed under semi-intensive management system whereby the animals are grazed on natural pasture and are supplemented with cut grasses and concentrates when animals are back home. A few farmers practice total confinement (zero grazing) where the animals are fed indoorly. The average milk production per cow per day ranged between 6 and 12 litres.

Study design and sample size

This study employed a cross-sectional study design to establish the prevalence of coliform contamination in cows' milk at farm level. The samples from the study population were taken by using convenient sampling. A total of 20 raw milk samples from 20 different farms found in Morogoro Municipality were selected for sampling. The sample size was calculated by using the formula:

$$N = \frac{Z^2Pq}{L^2}$$

Where by n = number of samples, Z = standard normal deviation (1.96), p = proportional of the targeted population (50% for unknown population), q = 1-p and

L= degree of accuracy (5%).The calculated sample size was 200, but due to time and resources limitations, only 10% was used hence about 20 milk samples were collected from different 20 dairy farms.

Data Collection

General data collection

Data was collected by field visit and assessment of hygienic milking practices at all selected dairy farms and taking milk samples for laboratory analysis per each farm. The assessment of milking procedures hygiene used the following criteria: (i) Hygiene of the udder, presence of wounds on the udder, (ii) Personnel hygiene, during milking (wash their hands and maintain the cleanliness throughout the milking period), (iii) Presence of wounds and bruises on milkers hands (iv) Hygiene of milking equipment, surrounding environment, and handling of milk post milking. Other information recorded at the farm were herd size, number of lactating animals, method used for milking, use of teat lubricants and incidences of mastitis. A checklist with the afore stated information was used in data collection from each herd.

Milk sampling and sample handling

From each household visited, 50 ml of milk was aseptically collected and put into a sterile screw capped falcon tubes and subsiquently stored in the cool box with ice packs before transportation to the laboratory for analysis. All samples were drawn from pooled containers containing milk that were milked on that particular day.

Laboratory analysis of milk samples

Raw cow milk samples were assessed for the total coliform count (TCC) using direct culture methods. The raw milk samples were thoroughly shaken and then diluted tenfold serial dilution of milk sample from 10^{-1} to 10^{-10} in sterile normal saline solution, using disposable pipettes. From each dilution, 1 ml of diluted sample was inoculated on MacConkey's agar (Oxoid Ltd., Basingstoke, UK) using the spread plate method. The plates were then incubated at 37°C for 36 hours (Harley, 2013). The number of colonies was recorded using a colony counter. Only the plates with 30 - 300 colonies were considered in calculating the colony forming units per ml of sample. TCC exceeding 4.5 log CFU/ml in raw cow milk samples means the count is above recommended levels (EAC, 2006).

Data analysis

Data were entered into Microsoft Excel spreadsheet and analyzed using Epi Info version 7 (Centre for Disease Control, Atlanta, USA). The Chi-square (χ^2) test was used to compare proportions of categorical variables like microbial load and the possible factors for contaminations using Chi-square test at 95% confidence intervals (CI) at the critical probability of $p < 0.05$.

RESULTS

General results

A total of 20 farms were visited for milk sample collection and assessment of risk factors for milk contamination. The farms were from five different wards in Morogoro Municipality which includes Kingorwila 5% (1), Magadu 20 % (4), Mazimbu 25% (5), Mbuyuni 1 (5%), and Mlimani 45% (9). Most of the dairy cattle reared were crosses of Friesian, Ayrshire and Jersey. The mean cattle herd size from which the sample was taken is 48 ± 8

(ranged from 2 to 310). The mean lactating cows in the herd was 15 ± 28 (ranged from 1 to 110).

Bacteriological results

The general status of coliform bacteria in milk indicated that out of 20 raw milk samples analyzed; only one sample had the recommended levels of total coliform according to East Africa Community standard of total Coliform count of 4.7log TCC cfu/ml. This means that the TCC contamination rates in raw cow milk was 95%. The average total coliform count was Log 8.0378 ± 8.2006 cfu/ml (minimum Log 4.602 and maximum Log 8.806).

Results on risk factors of milk contamination

Different farm activities were recorded during the farm visits as detailed in Table 1. The farm activities were also assessed as factors for coliform contamination in milk. The assessed activities include type of milking, udder washing, water status used for udder washing, towel drying of udder after washing, type of uses of towel in udder drying, type of milking container, use of milking salve, incidence of mastitis in farms, milking place, milk sieving, milking area hygiene, refrigeration of milk after milking. Since the contamination rates were very high (95%), each factor appeared to significantly contribute to coliform bacterial contamination in raw milk. Most of the milking environment, utensils and the personnel were dirty. Plastic containers were the common utensils used in milking and milk storage.

Table 2. Different factors that may predispose the raw milk to coliform contaminations

Parameter assessed	Category	Frequency	Percent
Cattle herd size	Big farms (>50)	5	25
	Small farms (<50)	15	75
Type of milking	Hand milking	20	100
Udder washing	Yes	20	100
Water status used for udder washing	Warm	11	55
	Cold	9	35
Towel drying of udder after washing	Yes	13	65
	No	7	35
Type of uses of towel in udder drying	Different towel each cow	1	7.7
	Same towel to all milking cows		92.3
Type of milking container	Metallic	4	20
	Plastic	16	80
Use of milking salve	Yes	16	80
	No	4	20
Incidence of mastitis in farms	Yes	7	35
	No	13	65
Milking place	In a crush	1	5
	Milking parlor	4	20
	Cattle resting place	15	75
Milk sieving	Yes	17	85
	No	3	15
Milking area hygiene	Clean	4	20
	Dirty	16	80
Refrigeration of milk after milking	Yes	14	70
	No	6	30

DISCUSSION

Coliforms are normal flora of the warm blooded gastrointestinal tract which are normally shed through animal faeces resulting into contamination of the environment. The current study was conducted to establish the status of coliform contamination in raw cow milk produced by selected small- and medium-scale dairy farmers in Morogoro Municipality and assess the risk factors for contaminations. The results indicated that there was high count of coliforms in milk that indicated poor hygiene in the whole process of harvesting milk and post-harvest handling. The possible sources of coliforms included unhygienic milk environment where most of farmers were milking in the dirty environment contaminated animal faeces and flies. The unhygienic milking personnel, unwashed udder, dirty storage facilities and uncovered milk containers were predisposed the milk to contaminations. Milk is sterile only when it is in the udder of a healthy animal but becomes contaminated with bacteria mainly during and/or after milking (Karimuribo *et al.,* 2005). Milk from subclinical mastitis cows usually contains etiological agents but milk from non-mastitis cows is often contaminated from extraneous dirty or poor quality water (Kivaria *et al.,* 2006). Other studies in Tanzania reported that unhygienic practices along the milk value chain predisposed milk to high bacterial load (Kanyeka 2014; Bukuku *et al.,* 2015, Nonga *et al*., 2015).

The high coliform counts depends on the level of hygiene and is an indication of faecal contamination from animals, humans or environmental materials (Robinson 2002; Omore *et al.,* 2002). Although most of Coliforms are not pathogenic, they are closely associated with pathogenic bacteria which cause milk-borne diseases, and hence poor milking hygienic practices results in high bacteria count including coliforms and other pathogenic like *Staphylococcus*, *Campylobacter* and *Salmonella* (Chye *et al*., 2004; Mennane *et al*., 2007).

The health of a cow is important in the quality of milk since some pathogenic bacteria affecting the cow like *Brucella abortus* and *Mycobacterium bovis* shed in cow milk also that can result into infecting human. Milk quality depends on the milking hygienic procedures used and the environment of milking also in the farms. The hand milking method was used by all farmers which possibly contributed to high coliform count because many people were washing hand by cold tap water which ultimately lead to milk contamination. Nevertheless, apart from washing udder by tap water also some farmers were not drying the udder before milking and hence this contributed to increase of coliforms count.

Use of plastic containers has contributed to increase of coliforms because of difficulties in cleaning and hence much milk fats remain giving good condition for bacterial growth on the containers (Bukuku *et al.,* 2015). Some farmers were not using refrigeration in storage of milk and this contributed also to high coliform count because keeping milk under room temperature favours microbial activities resulting into microbial growth by multiplication (Gwandu *et al.,* 2017).

The herd size as the factor was not significantly risk factor in increased raw milk total coliform count in this study as the total coliform count was high in both small and large herd. The time from milking to sample collection contributed to increased microbial count because the

mean time was 2.3 ± 1.1 hours which was high as most farmers were storing milk under room temperature after milking that favoured microbial multiplication.

To ensure hygiene of raw cow milk at farm levels, farmers should be educated on hygienic milking procedure and the importance of milking environment hygiene during milking as well as good storage of raw milk. The raw milk contamination should be minimized to the acceptable level which will not cause harm to the consumers and also minimize chances for milk spoilage.

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REFFERENCES

Bukuku JN, Nonga, HE, Mtambo MAM. Assessment of raw milk quality and stakeholders awareness on milk-borne health risks in Arusha City and Meru District, Tanzania. *Trop Anim Health Prod*, 47(5): 927-32, 2015.

Chye FY, Abdullah A, Ayob M K. Bacteriological quality and safety of raw milk in Malaysia. *Food Microb,* 21, 535-541, 2004.

EAC (East African Community). East African standard, raw cow milk specification. pp. 1–2, 2006.

Facklam R, Carvallo MG, Teixeira L. History, taxonomy, biochemical characteristics, and antibiotic susceptibility testing of *Enterococci*. The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistances. Gilmore ,Washinghton, DC: ASM Press. M. pp. 1–54, 2002.

Gwandu S H, Nonga HE, Mdegela RH, Katakweba AS, Suleiman TS, Ryoba R. Assessment of Raw Cow Milk Quality in Smallholder Dairy Farms in Pemba Island Zanzibar, Tanzania. *Vet Med Intern*, 2018: 1- 9, 2018.

Harley JP. *Laboratory Exercises in Microbiology*, McGraw-Hill, New York, NY, USA, 2013.

Hayes MC. Ralyea RD. Murphy SC. Carey NR. Scarlett JM, Boor KJ. Identification and characterization of elevated microbial counts in bulk tank raw milk. *J Dairy Sci*, 84: 292–298, 2001.

Kanyeka HB. Assessment of Microbial Quality of Raw Cow's Milk and Antimicrobial Susceptibility of Selected Milk-Borne Bacteria in Kilosa and Mvomero Districts, Tanzania. MSc Dissertation, 2014.

Karimuribo ED, Fitzpatrick JL, Bell CE, Swai ES, Kambarage DM, Ogden NH, Bryant MJ, French NP. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: risk, intervention and knowledge transfer. *Prev Vet Med Intern, 74: 84-98,* 2006.

Kivaria FM, Noordhuizen JPTM, Kapaga AM. Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Trop Anim Health Prod* 38: 185 - 94, 2006.

Leclerc V, Dufour B, Lombard B, Gauchard F, Garin-Bastuji B, Salvat G, Brisabois A, Poumeyrol M, De Buyser ML, Gnanou-Besse N, Lahellec C. Pathogens in meat and milk products: surveillance and impact on human health in France. *Liv Prod Sci*, 76: 195 – 202, 2002.

Lupindu AM. Risk of Enteropathogen infections in humans and animals associated with manure management in Urban and Peri-Urban livestock farming in Morogoro, Tanzania. Thesis for award of degree of Doctor of Philosophy (PhD) at Sokoine University of Agriculture Morogoro, Tanzania. pp. 1 - 149, 2014.

Mennane Z, Ouhssine M, Khedid K, ELyachioui M. Hygienic quality of raw cow's milk feeding from domestic waste in tow regions in Morocco. International *J Agric Biol*, 9(1), 46-48, 2007.

Nonga HE, Ngowi HA, Mdegela RH, Mutakyawa E, Nyahinga GB, William R, Mwadini MM. Survey of physicochemical characteristics and microbial contamination in selected food

locally vended in Morogoro Municipality, Tanzania. *BMC Res Notes*, 1–10, 2015.

Omore AO, Arimi SM, Kang'ethe EK, McDermott JJ, Staal SJ. Analysis of milk-borne public health risks in milk markets inKenya," in *Proceeding of the Annual Symposium of the Animal Production Society of Kenya*, vol. 9, pp. 21–30, Society of Kenya, Naivasha, Kenya, May, 2002.

PHCT, The 2012 Population and Housing Census of Tanzania General Report," Tech. Rep., National Bureau of Statistics, Dar es Salaam, Tanzania, 2013.

Robinson RK. Dairy Microbiology Handbook: The Microbiology of Milk and Milk Products, Third Edition. John Wiley & Sons, Inc; 2002, 51-305, 2002.

Schoder D, Maichin A, Lema B, Laffa J. Microbiological quality of milk in Tanzania: from Maasai stable to African consumer table. *J Food Prot,* 76(11): 1908 - 1915, 2013.