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Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania

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ABSTRACT

Objective: To evaluate microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. **Methods:** A microbial quality assessment of marketed raw milk was undertaken by evaluating 59 samples of milk from selling points (collecting centres =15), bicycle boys (12) and kiosks/restaurants (32) in Tanga city during April-May 2005. Quality and milk-borne hazards were assessed using a combination of tests in order to quantify the occurrence of *Brucellosis* (milk ring test), *Escherichia coli* (*E. coli*) O157:H7 (culture), the coliform bacteria as well as standard plate count (SPC). Specific gravity (SG) determination was used as an indicator of adulteration. **Results:** The mean coliform plate count (c.f.u/mL) of milk handled by bicycle boys (4.2×10^6) was significantly higher than that handled by collecting centres (3.0×10^6) and kiosk/ restaurants (1.4×10^6), respectively ($P < 0.05$). Of the 59 milk samples collected, 33 (56%) were *Brucella* milk ring test (MRT)-positive and 78% and 17% of the samples graded satisfactorily based on SG and coliform plate counts as prescribed by East African Community standards for raw milk. There was no verocytotoxigenic *E. coli* (VTEC) O157: H7 in any of the milk samples collected and analysed during the present study. **Conclusions:** It can be concluded that raw market milk in the study area is of poor bacteriological quality and hazardous for human consumption. This highlights the need to implement good hygiene practices and effective monitoring from production through the delivery chain to the consumer. Further studies are needed for detection of toxins that are produced by *E. coli*, other pathogenic spore forming bacteria (*Bacillus* spp. and *Clostridium* spp.) and other harmful microorganisms.

1. Introduction

In common with other countries in the East and Central Africa region, the informal dairy industry in Tanzania plays a dominant role in milk marketing, handling over 80%-90% of all milk sold[1]. The informal milk markets pathways persist because they provide social and economic benefits to smallholder producers, small market agents and consumers in terms of higher farm gate prices, creation of employment and competitive consumer prices[2-4].

Being a nutritious food, milk serves as an ideal medium for the growth of various microorganisms[5-7]. It is a highly perishable commodity and poor handling can exert both a public health and economic toll, thus requiring hygienic vigilance throughout the production to consumer chain[8,9]. Although freshly drawn milk from animals may possess temporary 'germicidal' or 'bacteriostatic' properties, growth of microorganisms is inevitable unless it is processed by freezing, heat treatment or irradiation[10,11]. Microorganism in raw milk can originate from different sources such as air, milking equipment, feed, soil, faeces and grass[12,13]. The microorganism load and types found in milk shortly after milking are influenced by factors such as animal and equipment cleanness, season, ambient temperature, storage, personnel health, cleanness and animal health[14,15]. On this basis the daily production and eventual marketing and sale of milk requires special consideration to ensure its delivery to the market in hygienic and acceptable condition.

In developing countries such as Tanzania, outlets for the purchase of milk are numerous but most operate under unsanitary conditions and are not adequately monitored or regulated[16,17]. Under such conditions the food-borne zoonotic risk posed by milk and dairy products is of great public concern[18]. However, the need for milk hygiene

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standards as a public health requirement for providing wholesome milk and milk products, consequently protecting the human population against milk borne zoonoses, cannot be overstated. However, there is limited information available on the microbial load contained in raw market milk in Tanzania[19,20]. This paper reports on an assessment of the microbial quality of raw market milk from milk marketing agents in Tanga city, Tanzania.

2. Materials and methods

2.1. Study area

This study was carried out between April and May, 2005 in Tanga city, northern coastal area of Tanzania. The area is located between 4° 21′ - 6° 24′ S, 36° 11′ -38° 26′ E, and characterized by hot and humid tropical climate with two rainy seasons: heavy rains during the months of March, April and May, and light rains occurring in November and December. The mean annual rainfall varies from 500 to 1400 mm/year. The relative humidity ranges from 60% to 90% for most of the year. Monthly mean ambient temperatures range from 15 ℃ between June and August to 35 ℃ between December and March.

2.2. Study design

Limited information concerning milk quality coupled with logistic problems affected the ability of this study in estimating the required sample size. Furthermore, given the fact that most raw milk marketing is undertaken in urban and peri-urban areas where market opportunities are high, the study sampling frame (n=107) was limited to milk market agents (MMAs) confined to a radius of 40 km around Tanga City. Sampling frame consisted of all milk collection centres(CC), both cooperative and private owned, and kiosks and restaurants (KR) selling milk in town. In addition to these, bicycle boys (BB), who act as traders or middlemen and are important for marketing milk from peri-urban and rural areas around Tanga, were also included. Overall, 59 milk market agents were randomly selected and sampled.

2.3. Data collection and milk sampling

Data on milk handling practices by MMAs were collected during the sampling. Important data collected included categorization of MMAs (cooperative/private centre, restaraunts/hotel, kiosk, bicycle boys), number of litres collected/handled per day, number of suppliers, source of milk (from traditional herds or smallholder crossbred cow), type of containers (plastic or metals) and whether there were any quality checks conducted (based on specific gravity, acidity test and visual cleanness), or pre-treatment of milk prior to selling (cooling, boiling, *etc*). Milk samples (30 mL in duplicate) were aseptically collected from each milk marketing agent by a sterile syringe into sterile bottles for laboratory analyses. The samples were kept in a cool box on melting ice and transported within 5 h of collection to the laboratory. The collected milk samples were tested for *Coliforms* and *Brucella* sp., as well as for adulteration.

2.4. Determination of coliform plate counts

Milk samples for evaluation of quality as defined by specific gravity (SG), exposure to *Brucella* pathogen (MRT) and coliform plate count (CPC) were examined at the Veterinary Laboratory, Tanga, using standard procedures[21]. Briefly, ten-fold serial dilutions of each sample from 10^{-3} to 10^{-6} were prepared in phosphate buffered saline solution (PBS), using disposable pipettes. The wide range in dilutions was selected due to the expected wide variation in bacterial counts. From each dilution, 1 mL was placed on a sterile Petri dish followed by the addition of 15-20 mL sterilized (autoclaved at 121 ℃ for 15 min) of Levine eosin methylene blue agar (Levine EMB) (Oxoid) and then cooled to 45 ℃ onto the dish. The sample and agar were then mixed and left to solidify after which the plates were incubated in inverted positions at 37 ℃ for 24-48 h. Plates showing green colonies with metallic sheen in the countable range of 15-250 colony forming unit per plate (c.f.u/plate) were chosen and counted.

2.5. Determination of specific gravity (density)

Adulteration with water was tested for by specific gravity (SG) using a lactometer at a standardized milk temperature. The lactometer was allowed to float freely in a cylinder, containing sufficient milk sample, until it reached equilibrium and readings taken below the meniscus. A SG below 1.026 kg/L[22] was considered suspicious of adulteration by adding water.

2.6. Brucella milk ring test (MRT)

The MRT was performed by adding 30 μL of stained *Brucella abortus* (*Br. abortus*) antigen (VLA, UK), both to a volume of 1 mL and 3 mL, of whole milk that has been stored at 4 ℃ for at least 24 h. The height of the milk column in the tubes was at least 25 mm. The tubes were thereafter incubated at 37 ℃ for 1 h. The test is read using a uniform light source. If the blue colour in the cream layer at the top of the fluid column is deeper than the remaining milk column (*i.e.* presence of a blue coloured ring) the test is considered positive. If the intensity of colour in the cream layer is equal to or less than that in the milk portion, the test is considered negative. The MRT, when compared to indirect enzyme linked immunosorbent assay (iELISA), has shown a sensitivity of 68% and a specificity of 98.9% on bulk milk and has been described by other researchers[23,24]. Confirmation of positive samples with tests of higher sensitivities and

specificities such as iELISA or culture was not done due to the lack of resources (funds) to buy the required kits / reagents.

2.7. Isolation of Escherichia coli O157: H7

For each milk sample cultured on Levine EMB agar (Oxoid) for coliform counting, up to ten green colonies with metallic sheen were inoculated on cefixime-tellurite sorbitol MacConkey (CT-SMAC) (Oxoid) agar plates. Plates were incubated in inverted positions at 37 ℃ for 24 h. Non-sorbitol fermenting colonies were counted and re-inoculated on Levine EMB for reconfirmation. Green colonies with metallic sheen were stored on tryptose soy agar slants. Following standard procedures, colonies were further tested for indole reactions and sero-tested for the O157 somatic antigen using a latex agglutination test (Prolex™-Pro-Lab Diagnostic)[25,26].

2.8. Data handling and analysis

Data collected were entered and managed in an Epi Info database (CDC, version 6.04). Descriptive statistics were then computed for different variables. Continuous and the proportions of categorical variables were computed and compared for statistical significance by *Chi*-square test at a critical probability of $P<0.05$.

3. Results

3.1. Milk marketing agents characteristics

A total of 59 milk samples were collected, of which 15, 32 and 12 were from CC, KR and BB, respectively. On average, 799 liters of milk are handled daily by the 3 categories of agents and most (82%, 662/799) of the market raw milk is handled by milk collection centers. Average amount of milk handled by CC, KR and BB was 662, 86, and 51 L with an medium amount of 500, 30 and 50L. A quality check of the collected milk is mainly performed at the milk collection centers owned either privately or cooperatively. Most milk collecting centers (93%) do not process milk and mainly cool bulk milk for delivery (33%) to bigger processors in the region, although some centers do undertake some milk retail. Most of the restaurants and kiosks pre-treat milk, mainly by cooling and boiling (72%), before they sell it to their customers. Milk collecting centres and bicycle boys receive 40% and 42% respectively of the milk from traditional herds whereas smallholder dairy sector is the main (91%) supplier of milk for kiosks and restaurants.

3.2 Physical and microbial quality of whole milk

The quality of milk as defined by CPC and SG is summarized in Table 1. Milk samples (n=1, 6%) from CC, KR

Table 1
Adulteration of milk with water and milk bacteriological quality.

Parameter		MMAs			
		CC	RK	BB	Overall
Specific gravity (kg/L)	Mean	1.028 (1.022-1.031)	1.027 (1.019-1.032)	1.026 (1.020-1.030)	1.027 (1.019-1.032)
	Median	1.029	1.027	1.027	1.027
Samples with SG< 1.026 kg/L (%)		6	19	42	22
CPC	Mean	3.0×10^6 (8×10^6-14×10^6)	1.4×10^6 (7×10^6-11.2×10^6)	$4.2\times10^{6*}$ (1×10^6-21.0×10^6)	2.8×10^6 (1×10^6-21.0×10^6)
	Median	10 000	900	3 000	2 100
Samples with CPC >5.0×10^4c.f.u/mL(%)		87	81	83	83

*$P<0.05$, comparing with milk samples from CC and RK.

Table 2
Proportion of raw milk samples positive for brucellosis using the MRT and isolation results of *E. coli* O157:H7 [n (%)].

Parameter	MMAs			
	CC	RK	BB	Overall
Brucellosis				
Samples MRT positive (%)	67	59	33	56
E. coli O157:H7 (VTEC)				
Samples with metallic green sheen colonies on EMB	15 (100)	32 (100)	12 (100)	59 (100)
Samples with Sorbitol(-) colonies on CT-SMAC	10 (66)	12 (38)	4 (33)	26 (44)
Samples with metallic green sheen after re-inoculation on EMB (confirmed suspect *E.coli*)	1 (10)	4 (12)	1 (8)	6 (10)
Indol test (+)	0 (0)	0 (0)	0 (0)	0 (0)
Serologically confirmed O157(+)	0 (0)	0 (0)	0 (0)	0 (0)

(*n*=6, 19%) and BB (*n*=5, 42%) had a SG below the standard of 1.026 kg/L. The mean CPC of the raw milk handled by BB (4.2×10^6) was significantly higher than that of raw milk from CC (3.0×10^6) and KR (1.4×10^6) respectively($P<0.05$). Milk samples with a SG < 1.026 kg/L had a significant higher coliform plate count (5.3×10^6 *vs.* 1.6×10^6, $P<0.05$). Overall, 83% of all milk handled by the 3 categories of agents showed a higher CPC than recommended values of < 50 000 c.f.u/mL. Factors like 'quality check by the agent' and 'milk cooling by the agent' did not have a significant effect on the coliform plate count. Milk from agents which reported receiving milk mainly from traditional herds had a significant higher coliform plate count compared to milk from agents which reported receiving milk mainly from smallholder dairy farms ($P<0.05$).

Brucella abortus antibodies were found in more than 50% of all milk samples, with a lower proportion of milk samples positive in the bicycle boys group.

All samples showed growth of *Escherichia coli* (*E. coli*) suspicious colonies on Levine EMB. 44% of these doubtful colonies were Sorbitol negative on cefixime-tellurite sorbitol MacConkey (CT-SMAC). However based on further Indole- and O157 antigen sero-testing, no *E. coli* O157: H7 was isolated in anyone of the milk samples (Table 2).

4. Discussion

This study has shown that milk handled by the 3 categories of milk marketing agents was of poor quality considering the high percentage of MRT positive and high CPC in milk samples and widespread levels of adulteration as reflected by lowered specific gravity values. High CPC values are indicative of contamination of milk with faecal (of animal or human origin) and environmental materials[27,28]. Coliform organism can rapidly build up in the moist residues on the milking equipment and become a source of contamination for the milk[27,29]. A high bacterial count reduces the shelf life and enhances the risk of milk-borne bacterial infections if milk is not properly heated or if thermally injured pathogens recover under suitable temperatures[30]. CPC were the lowest in the group of restaurants and kiosks, followed by collection centres and bicycle boys. If a CPC acceptable level of ($<5.0 \times 10^4$) is considered as quality index as defined by FAO[17] and East African Community Standard (EACS)[22], hence 83% of the samples of the present study should be considered to be of poor quality and hazardous for human consumption. Omore *et al* [29]. reported 39%-69% of the urban samples in the Kenya highlands did not meeting EACS. This might show the unsuitability of these standards under local circumstances, as the majority cannot meet them. The CPCs in the current study were 10-100 times higher than those reported in the Kenyan sample. This could be caused by poorer hygiene, but also by higher ambient temperatures in coastal Tanga compared to the Kenyan highlands. Consistent with studies in Kenya, the finding of this study reveals higher bacterial counts as the milk moves up the market chain, suggesting poor handling along the process[29]. The use of plastic containers, scooping of milk, high milk temperature, the long duration and lack of cold chain between milking and sale may be factors contributing to rapid bacterial multiplication[31]. Milk produced under hygienic conditions from healthy cows should not contain more than 5.0×10^4 bacteria per milliliter[5]. The total coliform bacteria counts from the three MMAs, which were higher than acceptable standards, could be primarily associated with a lack of pasteurization and improper hygienic management of the milking utensils and plastic containers used in keeping the milk.

Overall 20% of samples from the 3 MMAs had a SG below 1.026 kg/L, indicative of adulteration by adding water (either intentional or accidentally), which was also likely to be of poor bacteriological quality. It should be noted that the SG can also be lowered if the milk is aerated, for example by bumping during transport. Adulteration of milk by addition of water may introduce chemical or microbial health hazards as well as reducing the nutritional and processing quality, palatability and marketing value of the milk[32,33]. All the samples with a low SG came from agents where no quality check was done. The practice of adulteration of milk by adding water is more common during the dry season when milk is scarce and market demand is high[29]. Verification of this observation could not be ascertained in the present study because sampling was only once carried out during the wet season. Milk samples with SG's lower than 1.026 kg/L had significant higher CPC values. Also milk collected by agents from traditional herds using plastic utensils, had a higher CPC values. Compared to metal utensils, plastic materials are difficult to clean. Cooling and quality check by the milk agents, however, had no influence on the CPC. The high CPCs values found and lack of association with cooling, shows that most of bacterial growth occurred before reception by the milk agent. High CPCs are also not detected by the quality check practiced at the collecting centres, unless the acidity of the milk has changed, which will show in the alcohol test.

Results of the present study further revealed that 56% of the bulk milk samples showed ring test positive for *Brucella* suggesting that more than half of the market milk is derived from herds or animals infected or previously exposed to *Brucella* spp. pathogen. It must, however, be realized that since pooled milk samples were studied, the finding do not directly reflect the status of individual cows or herds. The proportion of MRT brucellosis positive samples from milk collection centres was significantly higher than from bicycle boys. This could be due to the fact that collecting centres bulk milk from a larger number of animals and herds as compared to bicycle boys. The detection of *Brucella* antibodies in the milk samples might be due to excretion of the antibodies by infected carrier or vaccinated cows[23]. This result differs from the findings of Bertu *et al*[34-38]. in

Nigeria and Omore *et al*[39]. in Kenya, who reported 13.5% and 3.5% prevalence of *Brucella* antibodies in bulk milk, respectively.

All the 59 milk samples tested showed growth of colonies with a metallic green sheen on Levine EMB agar, which was highly suspicious for *E. coli*. These colonies were cultured on CT-SMAC, the recommended method for the isolation of *E. coli* O157. The addition of potassium tellurite further selects serogroup O157 from other *E. coli* serogroups and inhibited *Aeromonas* spp. Cefixime is inhibitory to *Proteus* spp. *E. coli* O157 did not ferment sorbitol and form pink colonies. March and Ratnam reported that the detection of *E. coli* O157 on this medium had a sensitivity of 100% and specificity of 85% and recommended it as a simple, inexpensive and reliable means of screening *E. coli* O157. Forty-four percent of all samples showed growth of non-sorbitol fermenting colonies. All isolated colonies, however, were indole negative and none of them agglutinated with the O157 agglutination test. Had a positive O157 sample been found, it would still have required a last step to test for the potential to produce verotoxins, before the isolation of verocytotoxigenic *E. coli* (VTEC) could be confirmed. Within the milk-borne pathogens, *E. coli* species, a specific verocytotoxigenic strain may cause haemorrhagic colitis, inclusive of the important enterohemorrhagic type *E. coli* O157:H7[39]. The high proportion of unclassified *E. coli* observed in this study is therefore a source of concern since in the presence of a verocytotoxigenic *E. coli* enough toxins may be produced to cause illness to consumers. Omore *et al*[29] isolated *E. coli* O157:H7 in 1% of the samples in a milk marketing survey in the Kenyan highlands. Despite the fact that most MMAs claim to boil the milk that may destroys verocytotoxins, the possibility of inadequate treatment cannot be ruled out. The fact that a majority of the Tanga city residents consume raw milk will increase the risk of milk-borne *E. coli* poisoning. Although no VTEC was isolated from any of the samples tested, 100% of the samples contained unclassified *E. coli*. The high proportion of *E. coli* positive samples found in the raw milk marketed in Tanga must be considered a significant health risk, particularly in the light of the confirmed presence, by other studies, of VTEC in East Africa.

In conclusion, the findings of this study highlight the poor microbiological quality of milk handled by the MMAs in Tanga Region, Tanzania. This is most likely due to poor handling, the use of unsterile milk transport equipment, and high ambient temperature prevailing in the study area. The presence of *Brucella* antibodies, the high counts of coliforms and the high levels of adulteration are indicative of a potentially hazardous product which is likely to be posing a serious public health risk to consumers, particularly if the milk is not pasteurized or adequately boiled. These findings highlight the need to implement improved hygiene practices and to apply effective monitoring throughout the production to delivery chain. Moreover, further studies are needed to positively eliminate the occurrence of toxins produced by *E. coli* and other pathogenic spore forming bacteria (*Bacillus* spp and *Clostridium* spp) and other harmful microorganisms.

Conflict of interest statement

We declare that we have no conflict of interest.

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