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

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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\textsuperscript{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\textsuperscript{2-4}.

Being a nutritious food, milk serves as an ideal medium for the growth of various microorganisms\textsuperscript{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\textsuperscript{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\textsuperscript{10,11}. Microorganism in raw milk can originate from different sources such as air, milking equipment, feed, soil, faeces and grass\textsuperscript{12,13}. The microorganism load and types found in milk shortly after milking are influenced by factors such as animal and equipment cleanliness, season, ambient temperature, storage, personnel health, cleanliness and animal health\textsuperscript{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\textsuperscript{16,17}. Under such conditions the food–borne zoonotic risk posed by milk and dairy products is of great public concern\textsuperscript{18}. However, the need for milk hygiene...
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°C between June and August to 35°C 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, restaurants/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 metal) and whether there were any quality checks conducted (based on specific gravity, acidity test and visual cleanliness), 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 10⁻⁷ to 10⁻¹ 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°C for 15 min) of Levine eosin methylene blue agar (Levine EMB) (Oxoid) and then cooled to 45°C 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°C 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°C 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°C 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 °C 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 $P<0.05$.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CC</th>
<th>RK</th>
<th>BB</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity (kg/L)</td>
<td>Mean 1.028 (1.022–1.031)</td>
<td>1.027 (1.019–1.032)</td>
<td>1.026 (1.020–1.030)</td>
<td>1.027 (1.019–1.032)</td>
</tr>
<tr>
<td></td>
<td>Median 1.029</td>
<td>1.027</td>
<td>1.027</td>
<td>1.027</td>
</tr>
<tr>
<td>Samples with SG &lt; 1.026 kg/L (%)</td>
<td>6</td>
<td>19</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>CPC</td>
<td>Mean 3.0×10^5 (8×10^5–14×10^5)</td>
<td>1.4×10^7 (7×10^6–11.2×10^6)</td>
<td>4.2×10^6 (1×10^5–21.0×10^6)</td>
<td>2.8×10^6 (1×10^5–21.0×10^6)</td>
</tr>
<tr>
<td></td>
<td>Median 10 000</td>
<td>900</td>
<td>3 000</td>
<td>2 100</td>
</tr>
<tr>
<td>Samples with CPC &gt; 5.0×10^6 c.f.u/mL (%)</td>
<td>87</td>
<td>81</td>
<td>83</td>
<td>83</td>
</tr>
</tbody>
</table>

*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 (%)].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MMAs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brucellosis</strong></td>
<td></td>
</tr>
<tr>
<td>Samples MRT positive (%)</td>
<td>67</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7 (VTEC)</td>
<td></td>
</tr>
<tr>
<td>Samples with metallic green sheen colonies on EMB</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Samples with Sorbitol(–) colonies on CT–SMAC</td>
<td>10 (66)</td>
</tr>
<tr>
<td>Samples with metallic green sheen after re–inoculation on EMB (confirmed suspect <em>E.coli</em>)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Indol test (+)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Serologically confirmed O157(+)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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 50 L. 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
temperatures in coastal Tanga co
samples and widespread levels of adulteration as reflected
This study has shown that milk handled by the 3 categories
4. Discussion
Brucella abortus antibodies were found in more than 50%
All samples showed growth of Escherichia coli (E. coli)
suspicious colonies on Levine EMB. 44% of these doubtful
MacConkey (CT–SMAC). However based on further Indole–
and O157 antigen sero–testing, no E. coli O157: H7 was
Brucella abortus antibodies were found in more than 50%
Overall 20% of samples from the 3 MMAs had a SG below
1.026 kg/L, indicative of adulteration by adding water (either
Brucella abortus antibodies were found in more than 50%
Brucella abortus antibodies were found in more than 50%
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.[30] 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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References


