Boiled milk, food safety and the risk of exposure to milk borne pathogens in informal dairy markets in Tanzania*

Authors: Kaiza Kilango[1], Kohei Makita[2, 3], Lusato Kurwijila[4], Delia Grace[2]

[1]*Tanzania Food and Drug Authority P.O.BOX 77150, Dar es Salaam, Tanzania.*

[2]*International Livestock Research Institute (ILRI), Nairobi, Kenya, PO Box 30709, 00100 Nairobi, Kenya*

[3]*Rakuno Gakuen University, Ebetsu, 069-8501, Japan.*

[4]*Sokoine University of Agriculture P.O.BOX 3000, Chuo Kikuu, Morogoro.*

Abstract

In Tanzania, more than 90% of the milk consumed is informally marketed as loose, raw milk. On the other hand, the practice of boiling milk before consumption is very common. The study was carried out to establish food safety status of informally marketed milk including boiled milk. Milk samples were collected in four wards of Temeke Municipality of Dar es salaam. A total of 69, 44 and 7 milk samples were collected from randomly selected farmers, milk kiosks and all milk vendors. The bacteriological quality of the milk with respect to Total Bacterial Counts (TBC) and *Escherichia coli* was lower at milk vendors level than farm and milk kiosk. The TBC of raw milk was found to be an average of $2.8 \pm 0.98 \times 10^6$ cfu/ml at producer level, $3.4 \pm 2.6 \times 10^7$ cfu/ml at vendor's level and $4.8 \pm 3.3 \times 10^7$ cfu/ml at kiosk level. TBC values for kiosk milk boiled and served hot was also determined and found to be an average of $3.7 \pm 2.3 \times 10^5$ cfu/ml. The samples were analysed for presence of toxin producing S*taphylococcus aureus*. Exposure assessment showed that the probability of purchasing boiled milk contaminated with *S. aureus,* served hot at kiosks was 0.227 (90%CI: 0.062-0.436). It was estimated that every day, 953 (90%CI: 718-1,249) people purchase milk from kiosks in peri-urban Temeke, and among them, 217 (90%CI: 62-427) people were likely to purchase contaminated milk. The present study found that while boiling made milk generally safer by killing most pathogens, it still carries the risk of consumer exposure to pathogenic bacteria due to possible recontamination.

Keywords: risk assessment*; S. aureus*; Dar es Salaam

---

* Paper presented at the World dairy Summit Conference, 4-8th November 2012, Cape Town , South Africa

Introduction

Informal milk marketing is the dominant channel through which milk produced by smallholders reaches consumers in urban centres in Tanzania. Due to the relatively undeveloped milk collection infrastructure, and organizational and institutional arrangements, less than 10% of milk sold off-farm is processed. Since there is little or no quality control for milk handling practices in the informal channels, there is potential presence of zoonotic pathogens, adulterants and antimicrobial drug residues in informal markets and these are public health risks to consumers (Blowey and Edmondson, 2000, Kurwijila et al, 2006??).

The present study attempted to assess public health risks from the informally marketed milk using participatory risk assessment approaches. Risk-based approaches for assessing and managing food safety offer a powerful new method for reducing the enormous health burden imposed by food borne disease, while taking into account other societal goals such as pro-poor economic growth. Initial studies by Grace *et al*. (2009), have shown the effectiveness and impact of risk-based approaches applied to the informal markets, in particular, the importance of consumer perceptions of risk and the mitigation measures they undertake in order to manage risk posed by unprocessed milk. The study reported here focused on the practices for managing of microbiological food safety hazard along the informal marketing value chain of smallholder milk producers. Due to high prevalence of mastitis in small-holder dairy herds in Tanzania (Mdegela *et al*., 2005), *S. aureus* was studied as an indicator of the potential pathogen likely to be transmitted through milk. Analyses were conducted to quantify Total Bacterial Count (TBC), *E. coli* and the prevalence of the pathogen of concern (*Staphylococcus aureus*); and, conduct a quantitative risk assessment of milk-borne public health hazards (presence of *S. aureus*) in (un-boiled) raw milk and kiosk-boiled milk served hot.

MATERIAL AND METHODS

Study design

The study was conducted in the Temeke Municpality in the City of Dar es Salaam (Figure 1). The study used a cross-sectional survey applying the combination of Hazard Analysis and Critical Control Point (HACCP) framework and *Codex Alimentarius* Commission microbiological risk assessment. The study identified potential critical points where microbial hazard contamination may occur in the dairy value chain from farmers to milk shops.

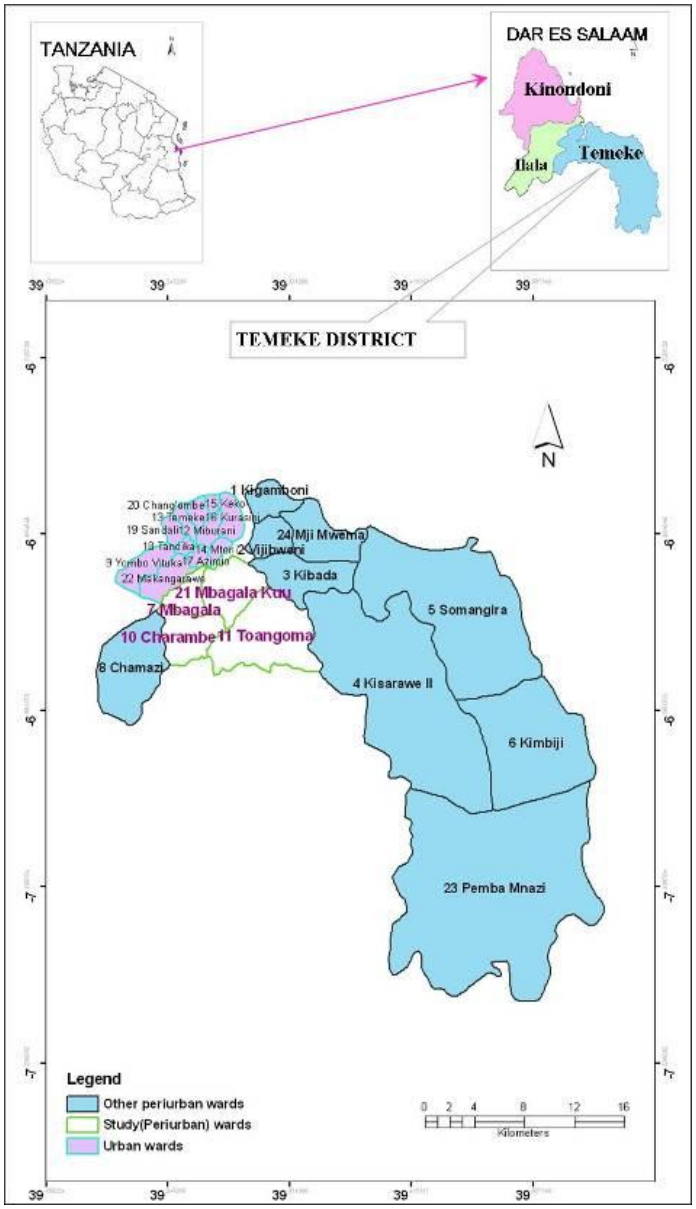


Figure 1: Map showing the study area

Using information from key informants, a scenario tree was constructed to describe basic steps involved in informal milk marketing channel of smallholder farmers' milk (Figure 2).

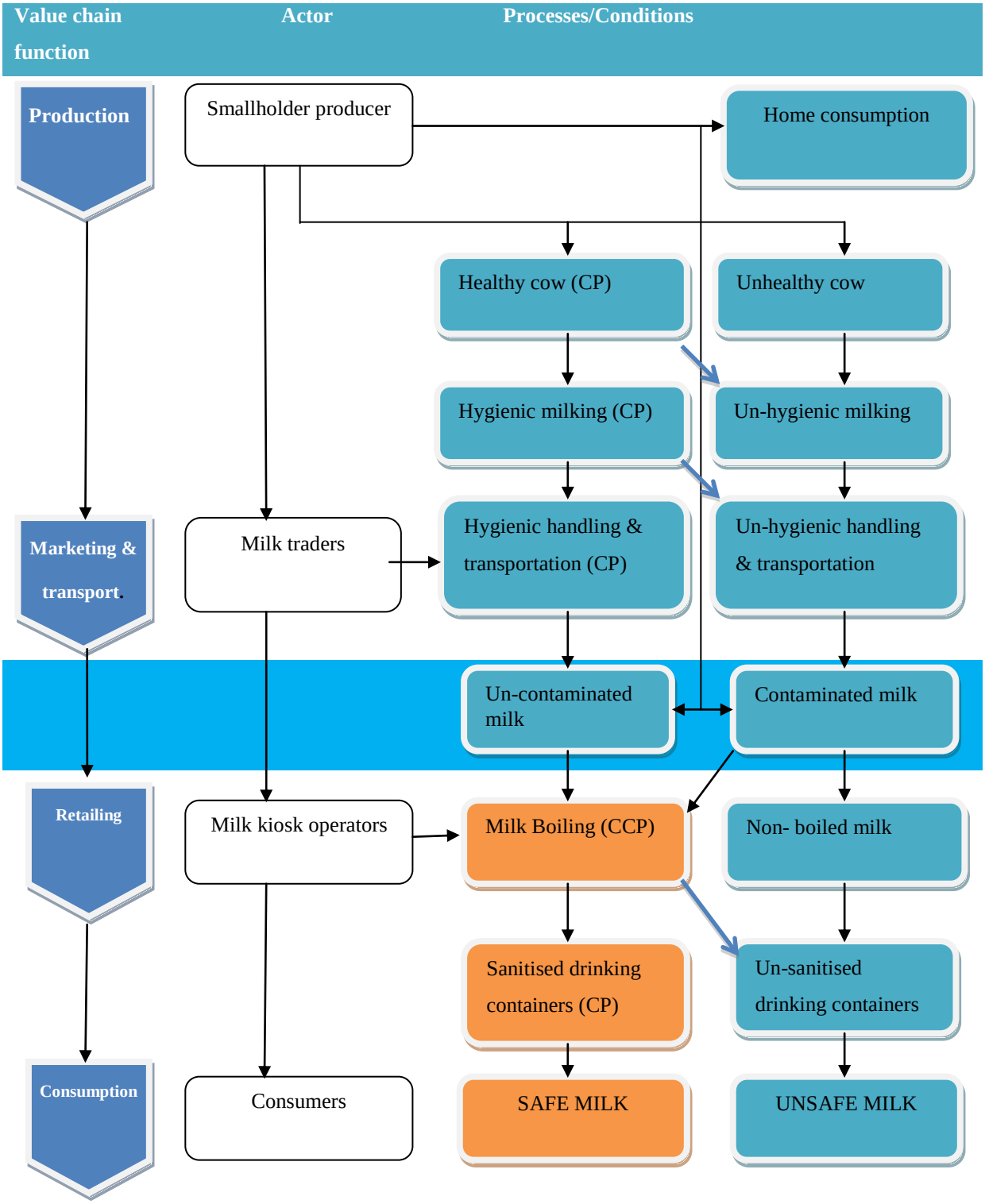


Figure 2: Milk marketing channels identified in Temeke peri-urban wards and potential critical control points for sampling frame (CP = control points; CCP = critical control points)

Data collection and sampling procedure

The study was conducted by following the pathway of the dairy value chain from consumption point (milk kiosks/restaurants) backward to milk producers (farmers). The study was conducted by using two approaches.

Firstly, information from farmers with at least one milking cow and from marketing agents was collected through face-to-face interviews using a structured questionnaire. Marketing agents were restaurants and other milk shops/kiosks that sell loose (unpacked) fresh milk. In addition, Focus Group Discussions (FGD) were conducted with consumers at milk kiosks.

Secondly, for assessment of milk quality, a Quantitative Microbiological Risk Assessment (QMRA) was carried out through sampling and testing presence of *S. aureus* in the milk samples along the dairy value chain.

Sampling procedure

Out of 13 peri-urban wards of Temeke municipality in Dar es salaam, four wards namely Mbagala, Mbagala Kuu, Toangoma and Charambe were purposely selected for the study due to high concentration of dairy activity. The sample size was estimated according to Fisher *et al*. (1991)

$N= Z^2 \times P (1-P)/d^2$

Where

Z = confidence level/ confidence internal (CI)

P= Estimated prevalence

1-P = the probability of having no pathogenic hazards b;

d= precision level

N= sample size

Level of confidence = 95%

Precision = 0.05 (5%)

The estimated prevalence was 14% based on prevalence of *S. aureus* from smallholder dairy and pastoral cattle herds in some urban and peri-urban areas of Tanzania reported by Mdegela *et al*. (2005). Employing the above formula suggested in 185 samples but for practical

reasons (such as relatively low numbers of vendors) we collected slightly fewer samples. The farmers included in the study were among those identified by milk kiosk as their milk suppliers. A total of 120 samples including 69, 44 and 7 were aseptically collected from randomly selected 29 farmers, 44 from milk shops and all the seven milk vendors active in the study area respectively.

At each consumption point (milk kiosk), a milk sample (20 ml) was aseptically collected. The collected sample were placed in a clean sterile vacutainer tube labeled accordingly and immediately stored in a cool box with ice cubes ready for shipment to the laboratory for use in the microbiological analysis.

As a control four milk samples were collected at farms in the four wards and brought to the laboratory for testing of presence of *S. aureus* before and after boiling 20 ml samples at 95°C for 5 minutes as in a thermostatically controlled water bath, cooled and handled aseptically thereafter until cultured.

Laboratory microbial tests

The laboratory tests were carried out at Central Veterinary laboratory (VIC) Temeke in Dar es Salaam.

Total plate count

Total plate count was done according to standard procedures (FAO (1987); Lampert (1975).

Isolation of *S. aureus*

Milk samples submitted to the laboratory were cultured using standard microbiological methods. Briefly, 0.01 ml of milk was streaked on a portion of a Blood Agar plate, MacConkey Agar and Mannitol Salt Agar (Becton-Dickson Microbiology). The plates were incubated at 37°C overnight in anaerobic jars with CO_2.

Plates were examined for growth at 24 and 48 hours. Bacteria were identified by colony morphology and Gram stain. For gram-positive cocci, catalase tests were performed to distinguish catalase-negative *Streptococcus* spp. from catalase-positive *Staphylococcus* spp. Catalase-positive gram-positive cocci were further identified using a coagulase test as summarized in Figure 3.

Data analysis

Laboratory results were entered into MS-ACCESS and MS-EXCEL and then analysed using SPSS.

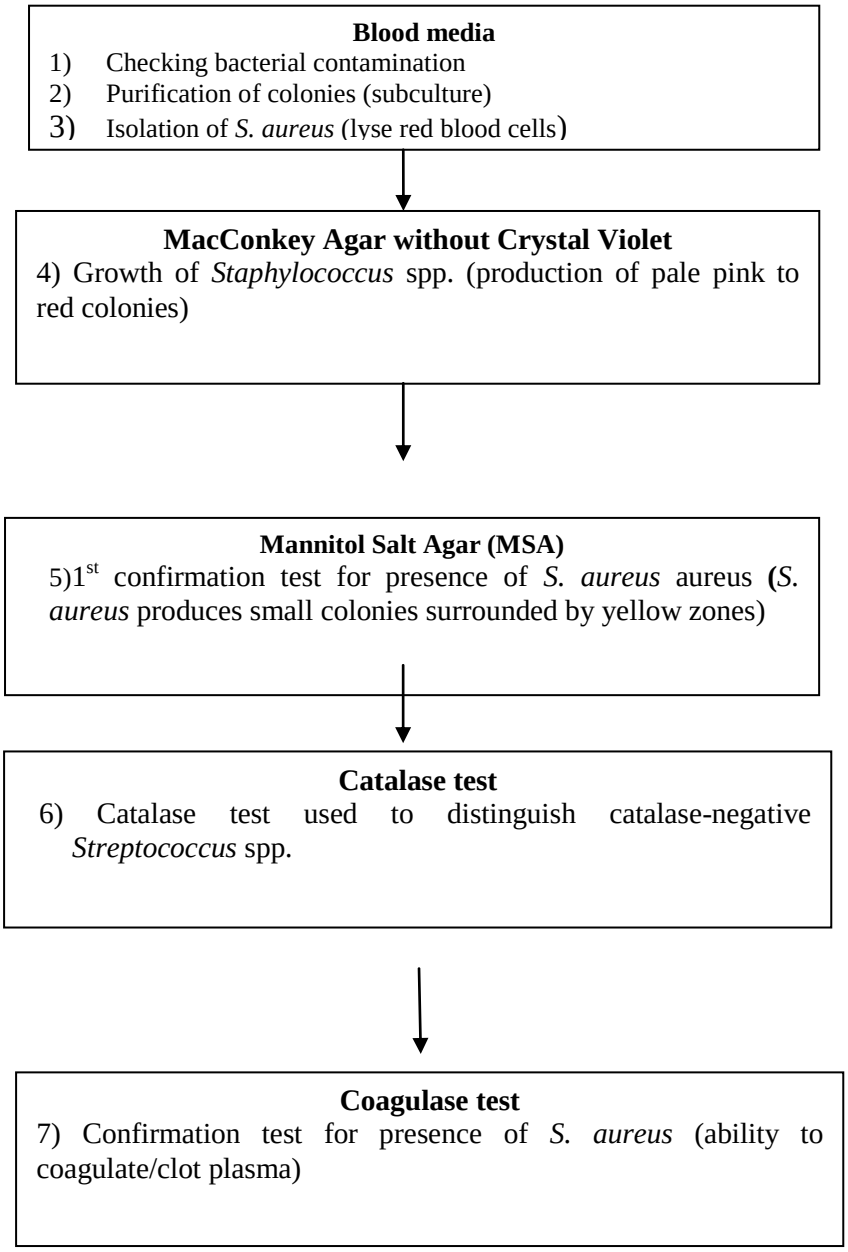


Figure 3: Flow chart for isolation of *S. aureus*

Exposure assessment of milk contaminated with *Staphylococcus aureus*

In the present study, length of storage time and temperature profiles during harvesting, storage and transportation of milk were not measured. Therefore only exposure to milk contaminated with the pathogen was assessed. Also, based on data on number of kiosks and

average number of consumers served per kiosk, the number of consumers consuming milk sold at kiosks in peri-urban areas of Temeke municipality was estimated.

The exposure to milk contaminated with *S. aureus* purchased in kiosks in peri-urban Temeke Municipality was stochastically modelled following the methodology below.

To calculate the total quantity of milk sold in kiosks in the areas per day, the total quantity of milk sold by 22 interviewed kiosks was estimated stochastically by summing randomly sampled quantity data in each kiosk under the same probability (from uniform distribution) using the bootstrap technique. Then, average quantity of milk sold in each kiosk was estimated by taking the summation of litres sold in all kiosks and divide it by 22 (number of kiosks). Secondly, the number of kiosks in peri-urban areas of Temeke Municipality was estimated by estimating the numbers of kiosks in Mbagala, Mbagala Kuu, Charambe and Toangoma Wards (surveyed peri-urban Wards in the Municipality) by sampling integer values between 50 to 70 (these numbers were estimated by Ward officials from the four surveyed wards as expert opinions) in uniform probability distribution and summing up these numbers. Finally, the quantity of milk sold per day in kiosks in peri-urban Temeke (Q) was estimated using formula below.

$Q = Y/22 * \text{Number of milk kiosks/ward} * N$

Where

- Q = Quantity of milk sold per day in kiosks in per-urban wards of Temeke Municipality
- Y= Estimated total quantity of milk sold by 22 interviewed kiosks
- N = Total number of peri-urban wards in Temeke municipality

The quantity of contaminated milk sold in these kiosks in peri-urban Temeke was estimated as follows. Firstly, the model showing a single purchase of milk contaminated or not was constructed using binomial distribution. The contamination rate fed into the model was estimated from the microbiological tests (5/22 = 22.7%- boiled and sold milk samples in kiosks were contaminated). Secondly, quantity data from each of 22 kiosks were sampled randomly in uniform probability distribution and each sample was multiplied with a sample taken from above mentioned binomial distribution (contaminated or not, 1 or 0) to obtain total amount of milk contaminated among milk sold in 22 kiosks.

Thirdly, the total quantity of milk contaminated among milk sold in kiosks in peri-urban Temeke, was calculated using the estimated number of kiosks in the areas as the same manner used for total quantity of milk sold in kiosks in the areas. Finally, the probability of purchasing contaminated milk from a kiosk in the peri-urban areas of Temeke Municipality was estimated by dividing the quantity of milk contaminated with *S. aureus* by the total quantity of milk sold in kiosks the peri-urban areas of Temeke Municipality.

The number of consumers purchasing contaminated milk per day through kiosks in peri-urban Temeke was estimated taking steps explained as follows. At first the average quantity of milk consumption per person was estimated by using answers from 60 consumers in the interviews, taking average of daily milk consumption (a point estimate). Then the total quantity of milk sold in kiosks in peri-urban Temeke was divided by this average quantity of milk consumption per person to calculate the number of people purchasing milk from kiosks in peri-urban Temeke. Finally, this number of people purchasing milk was multiplied with the probability of purchasing contaminated milk obtained above to calculate the number of people purchasing contaminated milk per day. The Monte Carlo simulation was performed for these all stochastic outputs by running 5000 iterations using @Risk (Palisade).

Ethical consideration

Permission to conduct the study was sought from the District and Municipal authorities before starting the study. The aim and purpose of the study was explained to all study participants. Non disclosure or identification of source of information in any publications of the research results was guaranteed.

RESULT AND DISCUSSION

Bacteriological quality of milk

Total Bacterial counts

Raw milk intended for further processing is considered very good when it contains less than 200,000 colony plate count per millilitre (EAS, 2007). The proportions of raw milk samples with Total Bacterial Count above East Africa Standard specification for grade 3 milk farmers', vendors' and kiosk raw milk (received un-chilled) were 29%, 100% and 100 % respectively. The TBC values of farmers' milk samples ($2.8 \pm 0.98 \times 10^6$) in the present study is higher than those reported for smallholder milk elsewhere in Tanzania (Karimuribo *et al*.

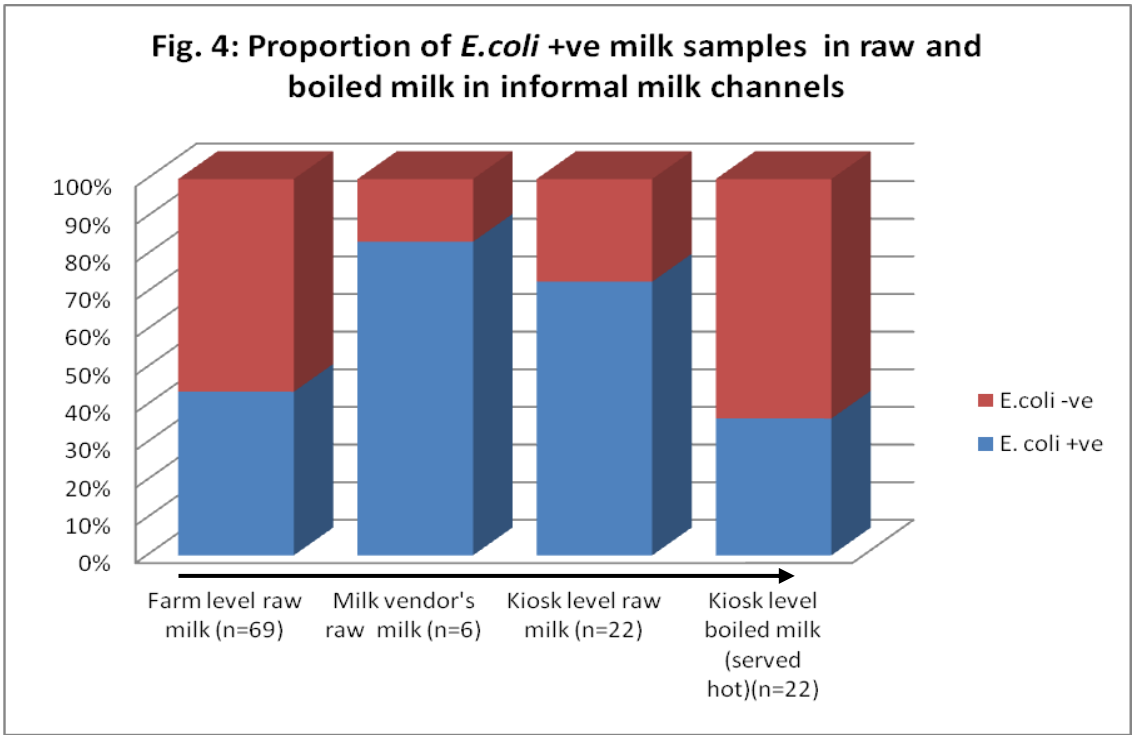
(2005). TBC values for un-chilled vendors' raw milk samples ($3.4 \pm 2.6 \times 10^7$) and un-chilled kiosk raw milk samples ($4.8 \pm 3.3 \times 10^7$) were higher than values for farmers' milk. This is in line with findings by Omore *et al*. (2005) who reported that bacterial counts increase (and subsequently, milk quality decreases) as milk passes through increasing numbers of intermediaries. The failure of milk handled by informal traders to meet basic quality standard is not surprising given the fact that most of the milk is handled in plastic containers that are not easy to clean and sanitise.

Raw milk source	Raw milk samples with TBC higher than grade III standard[1]	Average TBC/ml ($x10^6$)	Minimum	Maximum
Farmer (n=69)	29%	2.75±0.98	1.03	5.04
Vendor (n=7)	100%	34.1±25.5	17.2	71.9
Kiosk raw (n=22)	100%	4.81±3.2	2.87	8.59

Note: EAC Standard for raw milk Grade I= < 200,000 TBC/ml; Grade II= 1000,000-2,000,000 and Grade III = > 2,000,000

According to East Africa Standards (EAS), the standard plate count per milliliter for pasteurized milk shall not exceed 30,000 (EAS, 2007). Out of 22 kiosk heated milk (served hot) samples only 72.7% of the contained TBC less than 30,000 cfu/ml. The failure of 27.3% of ready-to-eat milk samples that failed to meet the EAS specification for pasteurized milk could be associated with spore forming, heat resistant bacteria or from re-contamination, during handling subsequent to boiling (Kitagwa *et al*., 2006).

To assess the effect of boiling and subsequent handling in milk served hot at milk kiosks an analysis of the presence of *E. coli* was performed. The results (Fig 4) show that the proportion of milk samples testing positive for the presence of *E. coli* was higher for vendors (83.3%) compared to farmers (43.5%) and milk received at kiosks directly from farmers (73%), while boiling reduced the proportion of positive samples to 36.4%.

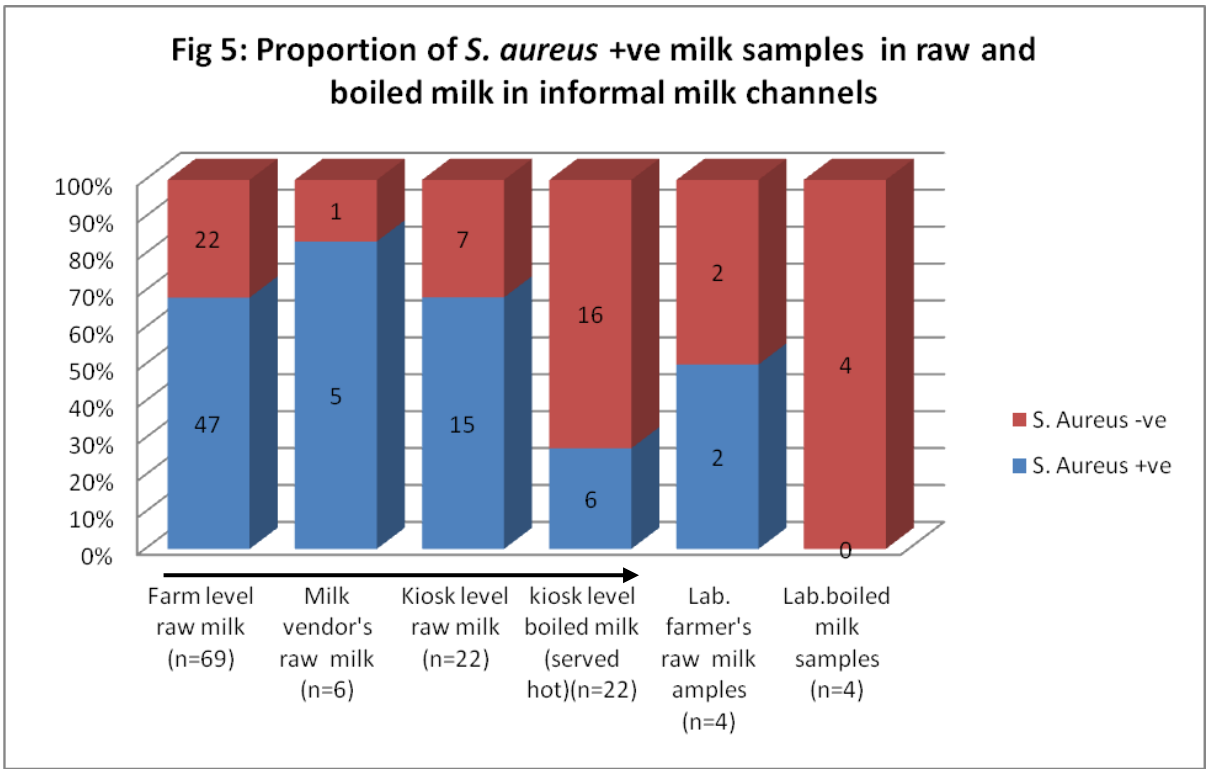


***Staphylococcus aureus* in un-boiled and boiled milk kiosk samples**

Results in Table 1 (Fig 5) indicate that 22.7% of boiled milk served hot, obtained at milk kiosk contained *S. aureus.* The bacterium is heat labile and does not compete well with other microorganisms and therefore, contamination usually occurs after the food has been processed when there is little competition from other microorganisms. The organism usually gains access to foods from food handlers or other surfaces like the processing equipment (Leenalitha and Peter, 2007). Kitagwa *et al.* (2006) reported that presence of *Staphylococcus aureus* in boiled milk could be due to insufficient boiling, people with poor personal hygiene handling the food or serving the food using dirty utensils. This is collaborated by the results of the laboratory boiled samples **.**There was no bacterial isolates found in laboratory boiled milk (Fig. 5). This is in agreement with reports by other researchers. Omore *et al*., (2005) reported that boiling of milk effectively destroys all milk-borne pathogens in raw milk. This suggest that sufficient cooking, storage and serving of food using clean utensils/equipment can reduce food safety risks associated with microbial contamination.

Table 1: *Staphylococcus aureus* in raw and boiled kiosk milk

Type of milk	N	Staphylococcus aureus	
		Positive	Negative
Raw milk	22	6(27.27%)	16 (72.73%)
Boiled milk served hot	22	5(22.72%)	17(77.27%)
Total	44		
$X^2 = 0.12$		df =1	P= 0.728



Exposure assessment of milk contaminated with *Staphylococcus aureus*

The total amount of milk sold in kiosks in peri-urban areas of Temeke Municipality a day was 1792L (90%CI: 1337-2358). Among this amount, 407L (90%CI: 119-799) was contaminated with *S. aureus* and the probability of purchasing contaminated milk was 0.227 (90%CI: 0.062-0.436) as shown in Figure 6. Everyday, 953 (90%CI: 718-1,249) people purchase milk from kiosks in peri-urban Temeke, and among them, 217 (90%CI: 62-427) people were estimated to purchase contaminated milk (Figure 4).

The result of the assessment indicated that a large fraction of the milk solid in milk kiosks of Temeke peri-urban wards could be contaminated by *S. aureus* at the time of consumption. The amount of milk contaminated found in this study 407L (22.71%) is however low when compared to amount reported by Lindqvist *et al*. (2002) who indicated that about 30% of the samples contained *S. aureus* above theoretical detection limit of the analytical method, 100cfu/g when doing a similar study.

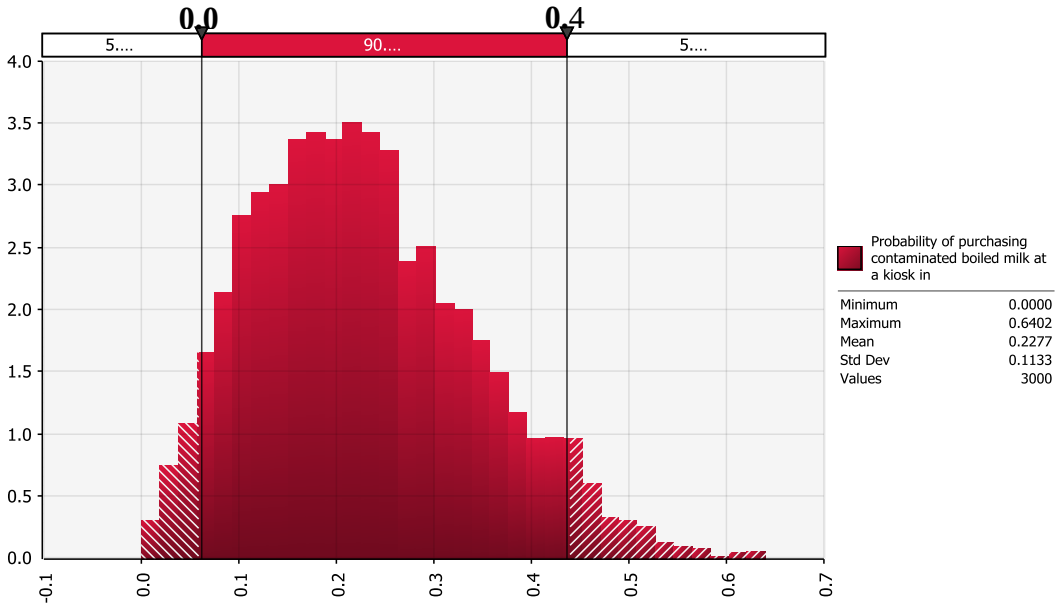


Figure 6: Monte Carlo simulation of Probability of purchasing contaminated milk with *S. aureus* from a kiosk in peri-urban Temeke

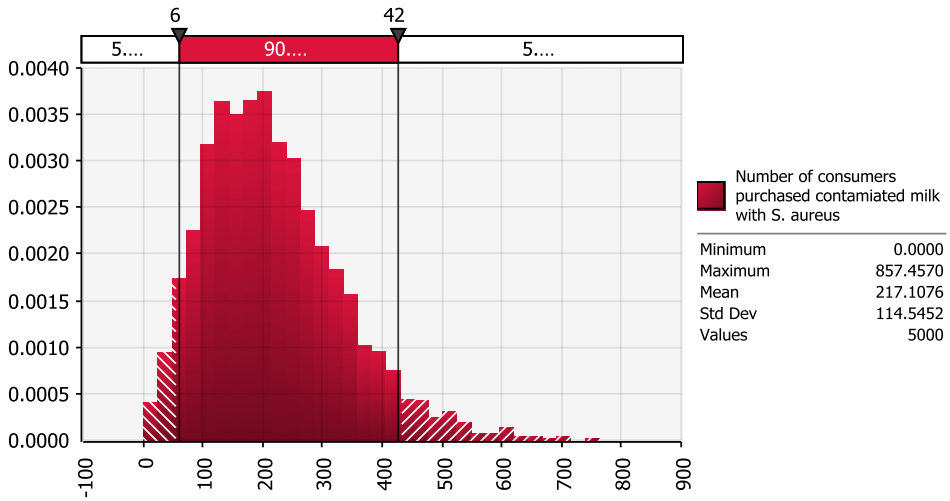


Figure 7: Probability of number of consumers purchased milk contaminated with *S.aureus*

CONCLUSION

The high values of TBC and *E. coli* in milk samples in this study showed that quality of milk in study area was poor and deteriorates further as it is moved from farm to retail kiosk outlets. The results of the study show that while boiling made milk generally safer by killing most pathogens, it still carries the risk of consumer exposure to pathogenic bacteria due to possible re-contamination.

Acknowledgement

The authors wish to acknowledge the ASARECA Dairy and Meat Project and the ILRI Safe Food Fair Food Project for joint technical and financial support of the field and laboratory research by the senior author as part of his MSc study at Sokoine University of Agriculture.

References

Blowey, R. and Edmondson, P. (2000). *Mastitis control in dairy herds: an illustrated practical guide*. Farming Press, Tonbridge. pp. 1-138.

European Academies Science Advisory Council (2008). Combating the threat of zoonotic Infections. [http://www.leopoldina.org/fileadmin/user_upload/Politik/Empfehlungen/EASAC/EASAC Statement_Zoonoses_2008.pdf]. Site visited on 25/11/2010

East African Standards (2007). Raw cow milk-Specification [http://*www.dairyafrica*.com/documents/*EAS%2067%20raw%20milk%20cleaned.doc*]. site visited on 25/11/2010.

East African Standards (2007). Pasteurized milk — Specification [http://www.Dairyafrica .com/documents/EAS%2069%20pastueised%20%20milk% 20cleaned .pdf]. site visited on 25/11/2010

FAO (1987). Food analytical procedures. *FAO Bulletin for Regional Dairy Development and Training Team for English-Speaking Countries in Africa*. January 4, 1987. Pp 80.

Fischer, A.A., Liang, J.E. and Townsend, J.W. (1991). *Handbook for family operationsresearch and design*. 2nd edition. Population Council USA. 84pp.

Grace, D., Baker, D. and Randolph, T. (2009). *Innovative and Participatory Risk Based Approaches to Assess Milk safety in Developing Countries*: A case study in North east India. Paper presented at the International Association of Agricultural Economists (IAAE) conference in Beijing, China. pp 17-22

Karimuribo, E.D., Kusiluka, L.J., Mdegela, R.H., Kapaga, A.H., Sindato, C. and

Kambarage, D.M. (2005). Studies on mastitis, milk quality and health risks associated consumption of milk from pastoral herds in Dodoma and Morogoro regions Tanzania. *J.Vet Sc: 6(3):213-21*

Kitagwa, W.G.I., Bekker, J.L. and Onyango, R.O. (2006). The influence of knowledge, attitudes and practices of food handlers on food kiosk hygiene. Eldoret, Kenya. *Environment and Health International* 8(2): 19-29.

Leenalitha, P. and Peter, M. (2007). Signal Amplification and Detection of *Staphylococcus aureus* Enterotoxins in Foods. [http://www.ansi.okstate.edu/research/research-reports-1/2007/panneerseelan_008.pdf]. site visited on 18/08/2011

Lindquist, R., Sylvan, S. and Vagsholm, I. (2002). Quantitative microbial risk assessment exemplified by *Staphylococcus aureus* in unripened cheese made from raw milk. *International Journal of Food Microbiology 78(2):155-70*

Lampert, L.M. (1975). *Modern dairy products*. 3rd Ed. Chemical Publishing Company. Inc. New York, U.S.A pp130-144

Mdegela, R.M., Karimuribo, E., Kusiluka, L.J.M., Kabula, B., Manjurano, A., Kapaga, A.M. and Kambarage, D.M. (2005). *Mastitis in smallholder dairy and pastoral cattle herds in the urban and peri-urban areas of the Dodoma municipality in Central Tanzania*. Livestock Research for Rural Development Volume 17, Article # 123.

Omore, A., Lore, T., Staal, S., Kutwa, J., Ouma, R., Arimi, S., Kang'the, E. (2005). *Addressing the public health and quality concerns towards marketed milk in Kenya*. SDP Research and Development Report No.3 Nairobi (Kenya): Smallholder Dairy (R and D) Project.pp.1-45