



Short Communication

Microbiological analysis of raw milk in Rwanda

Megan M. Doyle¹, Sara Garcia², Emmanuel Bahati³, Dennis Karamuzi³, James S. Cullor^{1,2},
Somen Nandi^{1*}

¹Global HealthShare® initiative, Dept. of Molecular and Cellular Biology, University of California, Davis

²Dairy Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis

³Land O'Lakes International Development, USAID Rwanda Dairy Competitiveness Project II, Kigali, Rwanda

*Corresponding Author Email: snandi@ucdavis.edu

ABSTRACT

Milk spoilage and low quality milk are of special concern to developing countries such as Rwanda. Our objective was to measure bacterial loads in milk via total bacterial count (TBC) in three major segments of the dairy value chain from farm to consumer, including milk transporters, milk collection centers, and milk kiosks. In all cases, the increase in bacterial load was statistically significant. This indicates a general trend of decreasing quality from farm to consumer, which clearly raises food safety concerns about contamination by pathogenic organisms and suitability of raw milk consumption in Rwanda.

Key words: Dairy Value Chain; Unpasteurized Milk; Total Bacterial Count; Food Microbiology, Milk Quality

INTRODUCTION

In developing countries, such as Rwanda, milk spoilage and low quality milk are of concern. These countries rely on local small holder farms to supply milk to local consumers. Often, these products are transported with unreliable refrigeration. Breaks in the cold chain allow bacteria to quickly multiply, eventually reaching levels unsafe for consumption. The resulting decrease in milk quality and supply negatively impacts processors and consumers along the dairy value chain. The current market structure in Rwanda is fragmented, with 85% of milk sold in an informal, unregulated markets and 15% sold in a formal, urban market which requires regulatory oversight. Livestock ownership is an important source of sustenance and income for people in Rwanda, and is a potential pathway out of poverty and malnutrition for small holder farmers (Phiria *et al.*, 2010). However, many small holder dairy farmers lack basic knowledge of sanitation, hygiene, and do not have access to clean water, adequate feed, and training. The resulting low quality milk potentially exposes consumers, especially infants, children, pregnant, and nursing women to health risks (Rawlings *et al.*, 2014). In order to become competitive in regional milk markets in the coming years,

Rwanda will need to meet the Common Market for Eastern and Southern Africa (COMESA) grading standards for milk (Foreman and De Leeuw, 2013). Our aim was to evaluate the current bacterial loads in Rwandan milk against the regional COMESA standard. In Rwanda, scientific research regarding the dairy value chain and safety of dairy products is limited. To our knowledge, this is one of the first studies to evaluate total bacterial count (TBC) at multiple links in the Rwandan dairy supply chain.

MATERIALS AND METHODS

The formal milk distribution system in Rwanda has three major segments: Transporters, milk collection centers (MCC), and milk kiosks. Transporters are individuals paid by dairy farmers who collect raw milk from multiple dairy farms and transport to an aggregation point such as MCC or milk kiosk. At the MCC, milk is tested via platform tests for organoleptic properties, added water, and wholesomeness. The MCC has chilled or room temperature storage tanks depending on available infrastructure. Milk is then delivered to local processing

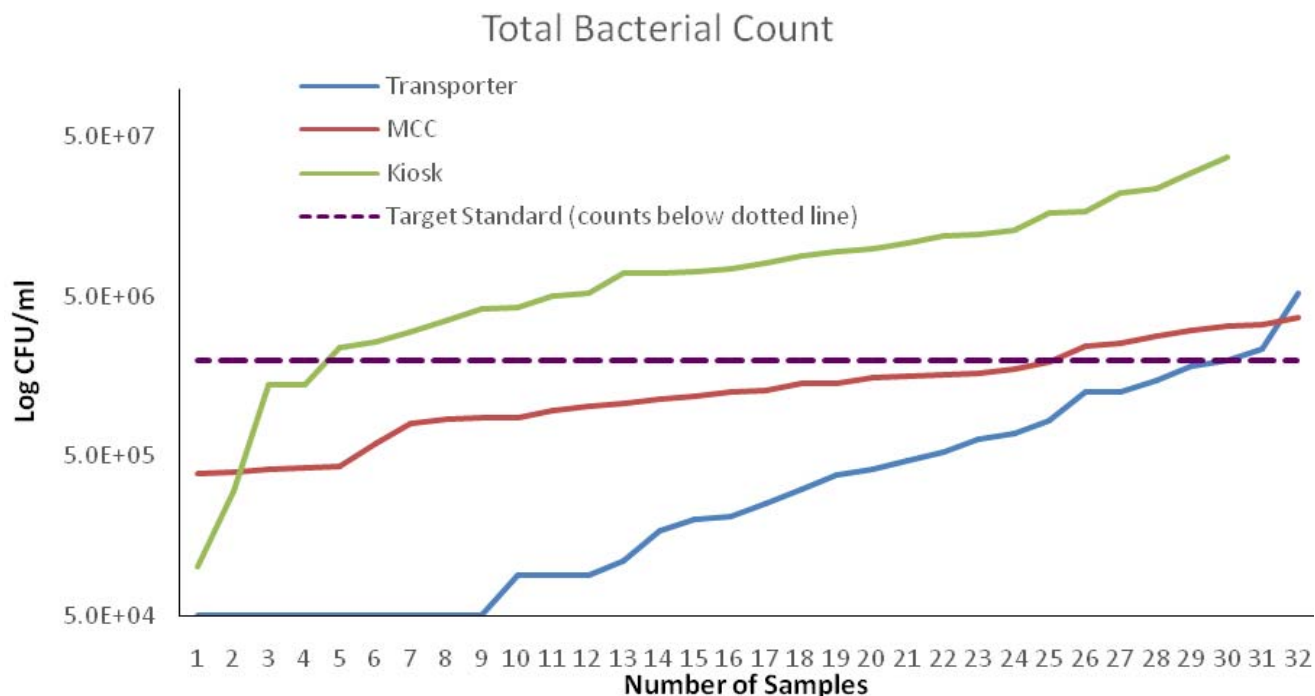


Figure 1. The samples here are presented in ascending order against the upper limit of the Common Market for Eastern and Southern Africa (COMESA) milk grading standard of 2,000,000 CFU/ml (Grade III or C, target standard). Samples were randomly collected from three major segments of milk value chain and total bacterial count (TBC) was performed.

units and /or kiosks. Milk kiosks sell raw milk, traditional fermented/cultured milk (e.g. *Ikivuguto*), and boiled milk.

Ninety four milk samples from transporters ($n=32$), MCCs ($n=32$), and kiosks ($n=30$) were randomly sampled from the Eastern, Southern and Kigali milk sheds between November 2013 and March 2014. Milk samples were collected from milk cans or tanks after bulking. All samples were collected in sterile tubes and immediately stored on ice. Total bacterial count was determined by standard plate count on 3MTM Petrifilm Aerobic Count (PAC) according to the manufacturer's standard operating procedures. Plates were incubated at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 18-20 hours. Colonies were counted using a 3MTM Plate Reader. Beside PAC, blood-agar plate counts were also performed as described by Gerhardt *et al.* (1994). Various enumeration experiments have shown very little or no variance between counts obtained through Petrifilm and standard blood agar counts (Nero *et al.*, 2006). All data was analyzed using the IBM Statistical Package for the Social Sciences (SPSS). Levene's test (Levene, 1960), Welch test (Welch, 1951), and Games-Howell post-hoc test (Games and Howell, 1976) were performed to determine homogeneity of variance and overall statistical significance.

RESULTS AND DISCUSSION

The mean TBC of transporters, MCCs, and kiosk samples were 670,000 CFU/ml ($\text{SD} \pm 1,100,000$), 1,500,000 CFU/ml ($\text{SD} \pm 930,000$), and 9,800,000 CFU/ml ($\text{SD} \pm 8,900,000$), respectively. The minimum TBC was 50,000 CFU/ml and there was a significant difference ($p < 0.001$) in the mean values among the three groups. The TBC was higher at the MCC ($p = 0.004$) and kiosk ($p < 0.001$) stages compared to the transporter stage. The mean TBC was highest ($p < 0.001$) at the kiosk compared to the MCC stage. Also, the TBC was lower ($p = 0.004$) at transporter stage compared to the MCC stage. About 94% of samples from the transporter segment had less than 2,000,000 CFU/ml and fell within COMESA's grade III or C, the lowest quality milk standard. About 78% of the MCC samples met grade III standard in comparison to transporters. However, less than 13% of samples from kiosks, where milk is sold directly to the consumer, meets the safe levels of TBC per target standard. The increases in TBC observed along the value chain may be due to several factors, including contamination on the farm, storage and transport in uncleaned milk cans, and lack of controlled temperature along transportation.

Raw milk is commonly boiled before consumption in Rwanda. However, Kilango *et al.* (2012) observed that while boiling generally makes milk safer by eliminating most microorganisms, it still carries the risk of consumer exposure to pathogenic bacteria (Kamana *et al.*, 2014) due to possible re-contamination. For example, *Staphylococcus aureus* (*S. aureus*) is heat-labile and does not compete well with other microorganisms, thus, *S. aureus* contamination usually occurs after milk has been boiled when there is little competition from other microorganisms. Further studies are warranted to investigate consumer behavior with regard to milk consumption, including boiling and storage of milk.

At the farm level, farmers should maintain proper animal hygiene, including disinfecting teat ends before and after milking, maintaining clean housing and bedding, and disinfecting milk cans. Cows with chronic udder inflammation (e.g. mastitis) may serve as reservoirs of mastitis pathogens which can infect other cows in a herd. In some cases, antibiotic therapy is of benefit but supportive therapy (e.g. fluids, anti-inflammatory drugs, etc.) will be equally important. Although in few occasions chronically infected cows cannot be managed via therapy but culling these chronically infected cows (e.g. *S. aureus* infection) might be the most effective control measure. Recently about 50 milk kiosks in Kigali have begun marketing pasteurized milk, however, surveillance in future studies is needed to determine the efficacy of pasteurization practices and safety of milk marketed as pasteurized.

In conclusion, high bacterial loads found in this study show that there is a failure to prevent bacterial growth in milk during transportation from farm to consumer and results in raw milk that is beyond the limits for safe consumption. Currently by the time milk reaches the consumer or end of the dairy value chain it does not meet COMESA standards for milk grading as reflected in this study, thus there is an immediate need to address food safety in the dairy supply chain in Rwanda.

ACKNOWLEDGEMENT

This work was supported by the Rwanda Dairy Competitiveness Program II, funded by the U.S. Agency for International Development (USAID). The authors

thank Pranaya Venkatapuram (Global HealthShare®, UC Davis) for assistance in statistical analysis.

Authors Disclosures

The author's views expressed in this publication do not necessarily reflect the views of the University of California, Davis, United States Agency for International Development, the United States Government or Land O'Lakes International Development. No competing financial and conflict of interest exist for publication of this manuscript.

REFERENCES

- Games PA, Howell JF (1976). Pairwise multiple comparison procedures with unequal N's and/or variances: A Monte Carlo Study. *J. of Edu. Statistics*. 1(2): 113-125.
- Gerhardt P, Murray RGE, Wood WA, Krieg NR (1994). *Methods for General and Molecular Bacteriology*. American Society for Microbiology, Washington, DC.
- Foreman I, De Leeuw B (2013). Quality Based Milk Payment Study. http://www.snvworl.org/download/publications/quality_based_milk_payment_study_8_may_2013_final.pdf (Accessed 12 Jan 2015).
- Kamana O, Ceuppens S, Jacxsens L, Kimonyo A, Uyttendaele M (2014). Microbiological quality and safety assessment of the Rwandan milk and dairy chain. *J. of Food Protection*. 77(2): 299-307.
- Kilango K, Makita K, Kurwijila L, Grace D (2012). Boiled milk, food safety and the risk of exposure to milk borne pathogens in informal dairy markets in Tanzania. World Dairy Summit Conference, 4-8 November 2012, Cape Town, South Africa.
- Levene H (1960). In *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, I. Olkin. Stanford University Press. Page 278-292.
- Nero LA, Beloti V, Barros MDF, Ortolani MBT, Tamanini R, Franco BDGDM (2006). Comparison of Petrifilm Aerobic Count Plates and De Man-Rogosa-Sharpe Agar for Enumeration of Lactic Acid Bacteria. *J. of Rapid Methods & Automation in Microbiology*. 14: 249-257.
- Phiria, BJ, Benschopa J, Frencha NP (2010). Systematic review of causes and factors associated with morbidity and mortality on smallholder dairy farms in Eastern and Southern Africa. *Preventive Veterinary Med.* 94 (1-2):1-8.
- Rawlings R, Pimkina S, Barret CB, Pedersen S, Wydick B (2014). "Got milk? The impact of Heifer International's livestock programs in Rwanda on nutritional outcomes." *Food Policy*. Vol. 44: 202-213.
- Welch BL (1951). On the comparison of several mean values: An alternative approach. *Biometrika*. 38: 330-336.