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ORIGINAL PAPER

Effects of season and agro-ecological zone on the microbial quality of raw milk along the various levels of the value chain in Uganda

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Abstract Dairy production in Uganda is pasture-based and traditional Ankole cattle make up 80% of the cattle herd, reared in both pastoral and agro-pastoral ecological zones. Regardless of the zone, milk quality is lowest in production basin during the dry season when ambient temperatures are highest and water is scarce. Poor hygiene and quality management contributed to the deterioration of raw milk quality during its storage and delivery to the final consumer, and concealed the seasonal effect when milk reached urban consumption areas. Poor milk quality is a challenge for the Ugandan Dairy Development Authorities who wish to make the milk value chain safe. This study provides baseline information for the implementation of an HACCP-based system to ensure the hygienic quality of milk from the farm to the market place.

Keywords Microbial contamination · Quality insurance · Raw milk · Uganda

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Introduction

The Ugandan economy is largely based on agriculture, and livestock farming is the second major agricultural activity after cereal production. An estimated 900 million liters of milk is produced per year, of which 85% is produced from indigenous Ankole cattle (Mpairwe 2005). They produce on average 2 liters of milk per day and graze over a wide expanse of land (Okello and Sabiiti 2006; Grimaud et al. 2007b). Holstein-Friesian cows and their cross breeds with the indigenous Ankole cattle produce the remaining proportion of milk.

A large proportion of the national milk supply is provided by Mbarara region in south-western Uganda. This region has a mean altitude of 1,200 m, rainfall of 700–1,000 mm mostly during the two rainy seasons of March-May and September-December, and minimum and maximum temperatures averaging 14.6 and 26.3°C. Dairy production in this region occurs in two major agro-ecological zones, namely, the pastoral and agro-pastoral zones. In the pastoral zone, indigenous cattle are predominantly kept whereas in the agro-pastoral zones exotic breeds and their crosses with the

indigenous breeds are predominantly reared under relatively modern farming systems (Alary et al. 2007; Grimaud et al. 2007b).

Over 80% of the milk is produced under the largely subsistence pastoral system with the attendant inefficiencies and quality problems commonly associated with such production systems. Several marketing levels occur along the distribution channels which characterize the milk value chain in Uganda. These include the farm, bicycle, roadside pick-up points, milk collecting centres in the rural areas, urban milk cooling points, cyclist vendors (Grillet et al. 2005).

There is a need to evaluate the relative importance of the various factors that are likely to influence the quality of raw milk along the value chain, a major concern in Uganda where the consumption of raw milk is still practiced in the rural areas (Grimaud et al. 2007a). This survey was aimed at studying the relative influence of seasons and agro-ecological zones on the loss of quality of raw milk along the distributions chain. This study will also provide baseline information for development of a HACCP (Hazard Analysis Critical Control Point) system in order to identify the critical points for quality insurance, in accordance to the FAO (1998) guidelines, and in an effort to improve the quality and safety of milk in Uganda.

Materials and methods

Sampling procedure

One hundred and seventy six (176) samples were collected from Mbarara region and Kampala City, during both dry and rainy seasons. Samples were collected at six different levels where raw milk was either consumed or sold, namely, (i) fifteen farms, distributed in both the pastoral and the agro-pastoral zones of Mbarara region; (ii) nine bicycle-collectors; (iii) seven pick-up collecting centres; (iv) five milk cooling centres, at the rate of two samples per centre (before and after cooling); (v) twelve urban coolers in Kampala, at the same rate of two samples per cooler (in and out); and (vi) fourteen vendors, distributed around Kampala, and collected as they transported the milk to the selling points. Typically the milk spends 1 hour in transit from farm to milk collecting center, then a further 1 hour to the urban market in Mbarara or 4 hours to Kampala where it is transported by trucks with insulated tanks or in cans loaded on open vehicles. All samples were collected in sterilized containers and put in cold boxes for delivery to the laboratory. For comparison in quality of milk from pastoral and agro-pastoral areas, data collected at both farm and bicycle-collector levels were pooled. Furthermore, nine samples were collected from three heat-treatment units, locally known as pasteurization units in Kampala, where the milk is heated at 80°C for 2 h. At each unit, three samples were collected as follows: the first when milk arrived in the unit, the second and the third 1 h and 4 h after heat treatment, respectively.

Milk quality evaluation

Density of raw milk was determined using a lactometer and values were corrected according to the temperature at analysis. The pH and resazurine values were determined immediately after the samples were delivered to the laboratory within four hours after sampling. The pH value was determined using a pH meter calibrated by standard buffer solutions of pH 4, 7 and 10. The 10-minute resazurine test was interpreted after 1 ml of freshly prepared solution of resazurine was mixed with 10 ml of milk sample, and then incubated at 37°C for 10 min. Depending on the degree of coloration, milk was graded according to a 1 to 6 scale (1: poor; 2: bad; 3: fair; 4: acceptable; 5: good; 6: excellent). Expected standard quality values were >1.030 for milk density, >6.4 for pH and >4, for resazurine coloration, respectively.

Samples were then frozen until further analyses. Samples were subsequently subjected to microbial analysis, which involved the following tests:

(i) Total plate count (TPC), using Plate Count Agar according to IDF standards (1987). Formed colonies were counted and expressed in colony forming units (cfu) per ml.
(ii) Total coliform count: coliforms were presumptively identified in Lauryl Tryptose (LT) Broth incubated at 35°C for 48 h. Positive tubes were confirmed by inoculation in Brilliant Green Bile Broth and incubated at 35°C for 48 h. Results were expressed as Most Probable Number of bacterial count per ml (MPN / ml) based on results of LT tubes.

(iii) Fecal coliform count: fecal coliforms were extracted from tubes positive in LT Broth, and confirmed by sub-culturing in EC Broth incubated at 45°C for 48 h. Results were expressed as MPN / ml based on results of EC tubes.

(iv) *Escherichia coli* count: inoculums were made from EC broth positive tubes onto L-EMB agar and incubated 24 h at 35°C.

Acceptable microbial standards for raw milk according to the Uganda National Bureau of Standards are $<2\times10^6$ cfu / ml for TPC and <100 MPN / ml for total and fecal coliforms and for *E. coli* count.

Zoonotic diseases

Analyses for specific zoonotic diseases were only done on samples from farms and urban coolers. Analysis for presence of *Brucella* spp. and *Salmonella* spp. in raw milk was done at MUST milk hygiene laboratory, while tuberculosis and *Listeria* spp. were analyzed at the Uganda National Bureau of Standards laboratory in Kampala.

The Rose Bengal (RB) buffered-antigen test (Porquier Institute, France) was used for brucellosis screening. Antigen and serum were maintained at ambient temperature for 30 min after clotting and before testing for false-negative reactions. Reading was taken after mixing antigen and serum on the plate for 4 min. Distinct agglutination was considered positive test.

Salmonella spp. were detected in 25 ml of milk which were inoculated into 9 ml of Selenite Cystine (SC) broth and 225 ml of lactose broth (LB), respectively, and incubated overnight at 42°C. One milliliter of pre-enriched milk in LB was inoculated into 10 ml of SC and Tetrathionate broth and incubated overnight at 37°C. Typical pink colonies with black centres on Xylose Lysine Desoxycholate were subjected to biochemical tests using standard methods (Macfaddin 1974).

For detection of tuberculosis, samples were decontaminated using Petroff's method and aliquots of the sediment inoculated on Lowenstein-Jensen (LJ) glycerol and LJ pyruvate slopes and incubated aerobically at 37°C for up 2 weeks. Cultures were checked twice a week for appearance of colonies.

To detect *Listeria* spp. in 25 ml of milk, the sample was enriched twice in 225 ml then 9 ml, respectively, of nutrient broth (Difco, USA) at 4°C for 6 weeks. Subcultures were made onto selective *Listeria* media with Oxford supplement (Oxoid, UK) fortnightly. Inoculated plates were incubated at 37°C in 5% CO_2 controlled incubator (Forma Scientific, USA) for 24–48 h. Typical black colonies were Gram-stained and tested for catalase and oxidase activities. Colonies that were Gram-positive short rods were inoculated into Triple Sugar Iron (TSI) agar slants, Urea slants and motility medium. Isolates with acid/acid, reaction without gas in TSI, urea-positive and typical umbrella-shaped motility were subjected to further biochemical tests (Macfaddin 1974).

Statistical analyses

Statistical analysis was performed by analysis of variance using the GLM procedures of SAS (1985), according to the model $Y_{ij}=\mu + s_i + t_j + s_it_j + \varepsilon_{ijk}$, where Y was the dependent variable, μ was the overall mean, s_i was the effect of season (i=1 or 2), t_j the effect of type of selling point, and ε_{ijk} was the error. Values of j varied according to the calculations: (i) 1 or 2 for the effect of agro-ecological zone, (ii) 1 to 5 for the effect of the type of selling point in Mbarara region, and (iii) 1 to 4 for the effect of the type of selling point for raw milk leaving Mbarara region and reaching Kampala, in order to study the effect of transportation from production location to the capital city. When the effect of the type of selling point was significant, further analyses with the test of Student-Newman-Keuls (SNK) were performed. Significance was declared at $P<0.05$.

Results

Raw milk quality in the primary production area (Mbarara region)

Agro-ecological zone affected neither milk density, pH and resazurine values, which were close to the expected standard, nor microbial quality (Table 1). Microbial analysis reflected a seasonal effect on total and fecal coliforms, higher in the dry season ($P<0.05$; Table 1). However, there was no significant seasonal effect on TPC and *E. coli* count (Table 1).

Observation of the different quality parameters at each stage of the raw milk chain in Mbarara region

Table 1 Platform tests (pH, density, and resazurine) and microbiological analyses - Total Plate Count (10^6 cfu / ml) and coliforms count (MPN / ml) - of raw milk in Mbarara region according to the agro-ecological zones

Parameter	Season [a]	AP [b] zone	P [b] zone	SEM [c]	SS [d]
pH	DS	6.46	6.40	0.12	
	RS	6.42	6.44		
Density	DS	1.028	1.030	0.0004	
	RS	1.029	1.031		
Resazurine test	DS	5.8	5.9	0.04	
	RS	5.9	5.8		
Total Plate Count	DS	32	15	0.3	
	RS	2	15		
Total coliforms	DS	594	317	24.6	S*
	RS	379	211		
Fecal coliforms	DS	193	371	12.3	S*
	RS	50	27		
Escherichia coli	DS	45	71	3.9	
	RS	22	6		

[a] DS: dry season; RS: rainy season.

[b] AP: agro-pastoral; P: pastoral.

[c] Standard error of treatment means, with N=4.

[d] Statistical Significance: S = season. * P<0.05.

revealed a significant effect of the season on pH value, resazurine test and fecal coliforms (P<0.05, Table 2). Significant interactions between season and type of selling point were also observed to affect pH value (P<0.01; Table 2) and fecal coliforms (P< 0.05), which presented better values during the rainy season at both farm and bicycle-collector levels, and during the dry season at the other three levels. There was also a significant influence of the level of selling point on TPC and fecal coliforms (P<0.05) as well as on total coliforms and resazurine test (P<0.01), and on density (P<0.001): SNK tests (results not shown) isolated the outlet of milk collecting center as the worst point in terms of microbial quality; however, they highlighted milk collecting centers – in and out – to present the best values of milk density.

Milk quality along the value chain from production area to major urban market (Kampala)

Milk density was higher in rainy season (P<0.01; Table 3). We also observed a seasonal effect on the total and fecal coliforms (P<0.05) with a significant interaction between season and type of selling point (P<0.05; Table 3). Decreased values of pH and

Table 2 Platform tests (pH, density, and resazurine) and microbiological analyses - Total Plate Count (10^6 cfu / ml) and coliforms count (MPN / ml) - of raw milk in Mbarara region according to the type of selling points

Parameter	Season [a]	Farm	Bicycle	MCC [b] in	MCC out	SEM [c]	SS [d]
pH	DS	6.43	6.39	6.47	6.46	0.17	S*, SxT**
	RS	6.44	6.49	6.41	6.41		
Density	DS	1.029	1.026	1.030	1.031	0.0007	T***
	RS	1.029	1.028	1.030	1.031		
Resazurine test	DS	5.9	5.2	5.4	5.9	0.12	S*, T**
	RS	5.8	5.4	4.8	4.6		
Total Plate Count	DS	2	47	79	234	8.0	T*
	RS	8	10	88	360		
Total coliforms	DS	294	558	886	686	35.9	T**
	RS	279	271	699	1008		
Fecal coliforms	DS	175	345	206	337	33.4	S*, T*
	RS	9	47	282	833		SxT*
Escherichia coli	DS	9	142	31	197	18.6	
	RS	5	19	70	184		

[a] DS: dry season; RS: rainy season.

[b] Milk Collecting Center.

[c] Standard error of treatment means, with N=10.

[d] Statistical Significance: S = season, T = type, SxT = interaction season x type.

* P<0.05; ** P<0.01; ** P<0.001.

Table 3 Platform tests (pH, density, and resazurine) and microbiological analyses - Total Plate Count (10^6 cfu / ml) and coliforms count (MPN / ml) - of raw milk leaving Mbarara region and sold in Kampala, according to the selling points

Parameter	Season [a]	MCC [b] out	UC [b] in	UC out	Vendor	SEM [c]	SS [d]
pH	DS	6.46	6.33	6.37	6.26	0.13	T*
	RS	6.41	6.34	6.34	6.23		
Density	DS	1.031	1.027	1.028	1.026	0.0008	S**, T*
	RS	1.031	1.032	1.030	1.027		
Resazurine test	DS	5.9	4.0	4.1	4.2	0.09	T**
	RS	4.6	3.5	4.3	3.3		
Total Plate Count	DS	234	1173	1383	1417	9.7	T*
	RS	360	857	1130	953		
Total coliforms	DS	686	934	1100	946	41.2	S*, SxT*
	RS	1008	1018	784	854		
Fecal coliforms	DS	337	758	909	750	35.6	S*, SxT*
	RS	833	815	429	648		
Escherichia coli	DS	197	317	529	515	29.0	
	RS	184	281	254	357		

[a] DS: dry season; RS: rainy season.

[b] MCC: Milk Collecting Center in Mbarara region; UC: Urban Cooler in Kampala.

[c] Standard error of treatment means, with N=8.

[d] Statistical Significance: S = season, T = type, SxT = interaction season x type.

* P<0.05; ** P<0.01.

resazurine were significant from Mbarara to Kampala (P<0.05; Table 3). TPC were higher in Kampala (P< 0.01; Table 3); however, the level of selling point did not affect total and fecal coliforms or *E. coli* counts. SNK tests on density, resazurine test and TPC (results not shown) focused on loss of quality from Mbarara to Kampala.

Specific bacterial zoonotic diseases

All samples tested negative for tuberculosis. However, a high prevalence of brucellosis and listeriosis was observed at the farm level. The prevalence of brucellosis, salmonellosis and listeriosis was higher in the dry season (Fig. 1), irrespective of the agro-ecological zone (results not shown). With the exception of salmonellosis-causing bacteria, the prevalence of all detected zoonotic bacteria at urban cooler level was between 5 and 10% in the dry season (Fig. 1).

Effect of heat treatment

Heat-treatment decontaminated the milk to the extent that TPC and specific bacterial counts reached negligible levels after treatment (Fig. 2). There was a corresponding increase in pH (from 6.15 to 6.39 and 6.35 in the dry season, and from 6.05 to 6.33 and 6.22 in the rainy season) and resazurine values (from 3.7 to 6.0 and 6.0 in the dry season, and from 3.0 to 5.3 and 4.0 in the rainy season). Four hours after treatment, the bacterial count dramatically increased in rainy season (Fig. 2).

Discussion

Milk production on the farm

Higher microbial contamination was observed in dry season when temperatures are warmer. This is in agreement with the conclusions of a study carried out under Mediterranean climate (Soler et al. 1995), where all microbial groups reached their maximum levels in summer. The higher temperature favors bacterial growth, increases somatic cell counts due to heat stress in lactating cows, and leads to the use of dirty water from wells to wash equipments. It is also common that milk price increases during drought (Okello and Sabiiti 2006), and some farmers tend to mix evening milk and that drawn from sick animals

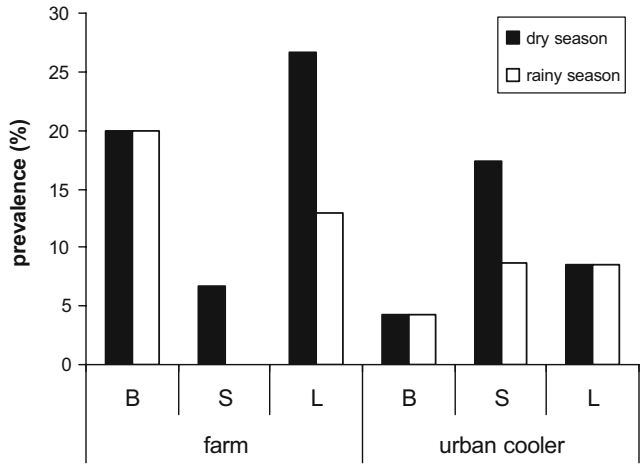

Fig. 1 Prevalence (%) of brucellosis (B), salmonellosis (S) and listeriosis (L) in farms and urban coolers, according to the season

with good quality milk so as to increase volume to get more money. During the rainy season, cows move away from soiled sleeping areas at night, and farmers also milk from clean areas outside the kraal to avoid mud.

Farming system is closely related to agro-ecological zone (Mpairwe 2005): settlers in the pastoral zone are livestock-specialized with indigenous Ankole cattle, and crop-livestock integrated farmers in the agro-pastoral zone do both crop cultivation and rear animals, typically exotic dairy cattle or cross breeds for market production. Nevertheless, due to a pasture-based dairy industry in every farming system, individual dairy productivity remains low (Grimaud et al. 2007b). Milk is produced in small quantities by most farmers, and such circumstances make farm-level milk quality assurance very difficult. Our study did not reveal any significant influence of ecological zone of production or farming system on milk quality at the farm level.

The presence of zoonotic microorganisms in raw milk is a cause of concern to consumers. The high prevalence of brucellosis did not seem to be dependant on season or agro-ecological zone. However, a more ambitious survey recently conducted in Mbarara region on more than 1,500 cattle indicated that pastoral production system was strongly associated with *Brucella* seropositivity (Grimaud et al. 2007a). A survey conducted by Faye et al. (2005) on the prevalence of tuberculosis in this area using the tuberculin test showed very high tuberculosis herd prevalence. Surprisingly our study did not confirm this result, most probably due to the lack of sensitivity of the *Mycobacterium* test in milk and the low number of samples which were used. The highest *Salmonella* spp. contamination occurred during the dry season. This could be attributed to use of dirty water from wells and valley tanks during dry spell and the unhygienic production and handling condi-

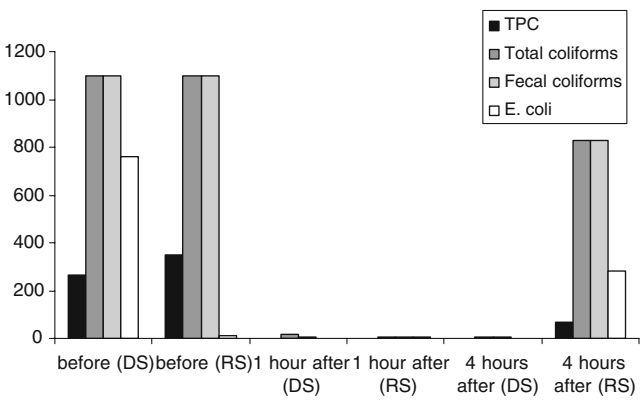

Fig. 2 Effects of heat-treatment on total plate count (TPC, 10^6 cfu / ml) and coliforms (MPN / ml): samples before, 1 hour and 4 hours after heat-treatment, in dry season (DS) and rainy season (RS)

tions (Faye and Loiseau 2002), and to the amount of dust in the environment.

Raw milk transportation to urban markets

From the farm, milk is first dispatched to milk collecting centers then further transported by road to the major urban markets, mainly in Kampala. The ideal temperature for milk transport is between 0 and 2°C to avoid microbial growth (Faye and Loiseau 2002). The dramatic decrease in hygienic quality of raw milk from the primary production region to the urban market which concealed the seasonal effect observed at the farm level could be attributed to the milk handling infrastructures along the chain, prolonged time and increased temperature during transportation to the final market. The time taken to cool the milk after milking, the rate at which it is cooled and the temperature to which it is cooled will influence the rate at which microbial multiplication will occur and hence the shelf life of the milk (Harding 1985).

Small-scale batch heat-treatment seems to be effective in reducing the microbial load of raw milk to safe levels for consumption. However, the observed increase in pH value implies that excessive heating of milk is applied, which causes physico-chemical changes in the milk constituents. According to Asperger (1993) and Montel et al. (2003), this could result from the denaturing of proteins as well as the potential Maillard reactions. Heat-treated milk that is not aseptically packaged poses a danger of post-processing contamination as it became an almost sterile substratum and could be colonized by any contaminating microorganisms which will rapidly multiply in the absence of competition, especially opportunistic bacteria like *Listeria monocytogenes* (Asperger 1993). Our study demonstrated that the so-heat processed milk was more vulnerable in rainy season.

Implications

Milk is a highly perishable product that deteriorates quickly at ambient temperatures that prevails in most tropical countries. To improve delivery of better quality and safe milk, Ugandan authorities need to encourage farmers to practice better milk production and handling practices as well as support the development of requisite infrastructures. For instance, farmers and traders could be encouraged to transport milk in metal cans rather than plastic jerry cans, and cooperatives to transport loose milk in insulated trucks rather than open vehicles. Farmers continue to be paid for milk by volume rather than by quality, which does not create incentive for production of high quality milk. In addition, an efficient cold chain should be promoted as this remains the best method of milk preservation and would present the advantage to implement an HACCP-like system to ensure the hygienic quality of milk from the farm to the consumer (FAO 1998).

Conclusion

Milk quality and hygiene ensure both consumers' safety and optimum economic value. This study revealed a decrease in milk safety and quality on arrival in the urban market. This is attributed to the original contamination at the farm and subsequent microbial multiplication during storage and handling including transportation to the market. Raw milk value chain is complex and any improvement should involve every stakeholder at every stage, and this study provides baseline information for development and implementation of HACCP-like system to ensure production and supply of improved quality and safe milk from the farm to the market place. The maintenance of a cold chain is a major constraint in tropical environment, and the promotion of small scale milk processing plants could be considered as one of the steps of the global strategy to improve milk quality at grass root level and to stabilize the raw milk production beside the processed milk value chain.

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