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Research Application Summary

Raw cattle milk quality from smallholder dairy farms with respect to *E. coli* and Salmonella quality indicators

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Abstract

The study examined the quality of raw cattle milk produced by smallholder farmers from selected districts in central Uganda. Two major "quality indicators", i.e., presence of *Salmonella* spp. and *Escherichia coli* were considered. The colony forming units for *Salmonella* spp. and *E. coli* were established following the standard plate count method. Absolute certainty was achieved by subjecting sample isolates to Gram staining. Milk samples were purposively collected at the respective milking times and tested in a laboratory using serial dilutions of 10,000 and 100,000 mls for *E. coli* and *Salmonella* spp., respectively. Analysis was done using the pour plate method on McConkey and dulcitol selenite agar media for *E. coli* and *Salmonella* spp., respectively. Plates were incubated at 35^{o}C and data collected after 48 hours. There was a strong positive correlation (r = 0.9) between experimental data and national standards for both *Salmonella* and *E. coli.* The t-test results (P=0.05) for *Salmonella* (P=0.301) and *E. coli* (P=0.1206) were not significantly different for different raw milk handling levels. Therefore, *Salmonella* and *E. coli* exist in smallholder farmers' raw milk in variable quantities, though not significant to affect the quality of milk. Routine milker training and assigning responsibilities for all areas of prevention of contamination on smallholder farms is a recommended practice. Periodic milk examination to provide microbiological count records on the prevalence of *E. coli* and *Salmonella* spp. at different milk handling chains is a practice that is recommended to all milk handlers.

Key words: *Escherichia coli*, quality, quality indicators, raw milk, Salmonella

Résumé

La présente étude a examiné la qualité du lait brute des bovins produit par les petits exploitants de quelques districts sélectionnés du centre de l'Ouganda. Deux 'indicateurs de qualité' majeurs, à savoir la présence de *Salmonella* spp. et *Escherichia* coli ont été considérés. Les unités de colonie de *Salmonella* spp. et de *E. coli* ont été établies en suivant la méthode de comptage sur plaque standard. La certitude absolue a été obtenue en soumettant les isolats de l'échantillon à la coloration de Gram. Les échantillons de lait ont été collectés pendant les périodes de traite et testés dans un laboratoire à l'aide des dilutions en série de 10000 et 100000 ml respectivement pour *E. coli* et *Salmonella* spp. L'analyse a été effectuée

en utilisant la méthode de la plaque de coulée sur des milieux de gélose McConkey et de sélénite au dulcitol respectivement pour *E. coli* et *Salmonella* spp. Les plaques ont été incubées à 35°C et les données collectées après 48 heures. Il y avait une forte corrélation positive (r = 0,9) entre les données expérimentales et les normes nationales pour *Salmonella* et *E. coli*. Les résultats du test t (P = 0,05) pour *Salmonella* (P = 0,301) et *E. coli* (P = 0,1206) ne montraient pas de différence significative pour les différents niveaux de manipulation du lait brute. Par conséquent, *Salmonella* et *E. coli* sont présents dans le lait brut des petits exploitants en quantités variables, bien que non significatives pour influencer la qualité du lait. La formation régulière des trayeurs et l'attribution de responsabilités dans tous les domaines de la prévention de la contamination des petites exploitations agricoles sont des pratiques recommandées. L'examen périodique du lait pour documenter les données microbiologiques sur la prévalence de *E. coli* et de *Salmonella* spp. à différents niveaux de manipulation du lait est recommandé à tous les entreprises laitières.

Mots clés: *Escherichia coli*, qualité, indicateurs de qualité, lait brut, Salmonella

Background

The quality of raw milk is affected by microbial contamination (Ernest *et al*., 2001), from microorganisms and macro elements of several sources (Kirk, 2001). Contamination of milk begins when raw milk leaves the alveoli of the udder (Feng *et al.*, 2002). As milk leaves the udder of healthy cows it normally contains very low numbers of microorganisms (Kurweil, 1973; Asaminew and Eyassu, 2010) usually less than 1,000 total bacteria per ml. (Gonzalez *et al.,* 1986; Lues *et al.*, 2010). While healthy animals should contribute less bacterial organisms to the raw milk (North, 2001), poor animal health and hygiene, dirty equipment, and marginal cooling introduce *Salmonella* and *Escherichia coli* into the milk (Bramley and McKinnon, 1990; FAO, 2004; Franz *et al.*, 2007). *Salmonella* and *E.coli* have been found to contaminate raw milk (Harold, 2002) and largely affect the superiority of that milk although smallholder producers are largely unaware of this phenomenon (Mubarak *et al*., 2010). In raw milk, *Salmonella* and *E.coli* population growth is favored by temperature and length of storage (Gwendolyn, 1988).

Study description

The study was shaped by Freter's Nutrient-Niche theory, which suggests that the ecological niches of microorganisms depend on the nutrient availability within the host organism (Weagant and Feng, 2002), and was based on two variables; excellence, superiority or quality of raw milk and Numbers (composition) of salmonella and coliforms. The quality and class of milk from the farms was predicted by colony forming units counted from the investigated samples for *Salmonella,* and also the number of coliforms in the same sample. The numbers of the microorganisms could be modified (intervening variables) by temperature and hygiene of the surroundings as shown in the conceptual framework (Fig. 1).

A good grade of milk, and/or a high class of raw milk was predicted by the number of coliforms and colony forming units for salmonella in the milk. Temperature and poor hygiene

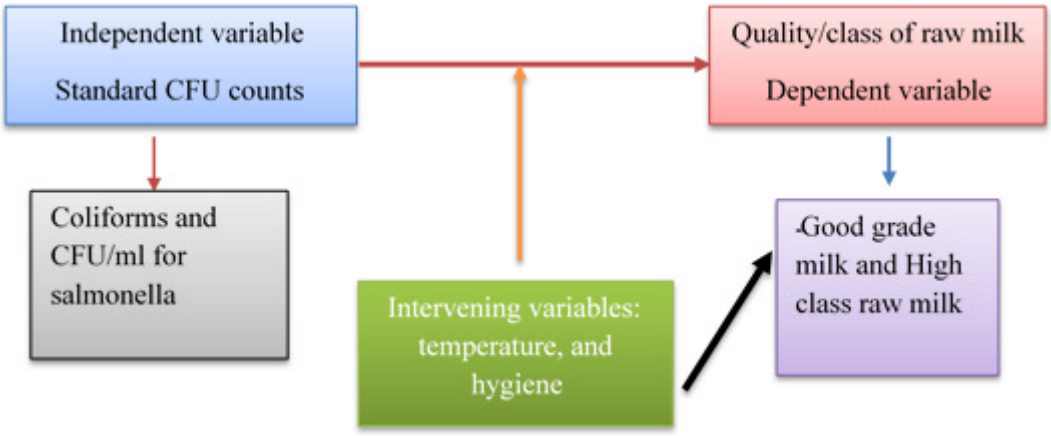

Figure 1. Conceptual framework

were controlled by keeping the untested samples in a cooler at 4-7^{0}C, and having all apparatus sterilized, and the working area disinfected with 70% alcohol.

Smallholder farms from selected central Uganda districts of Kalungu, Mpigi, Wakiso and Kampala were engaged in the study. These districts have an approximate stocking capacity of 14,000 and 350,000 herds of exotic crosses and indigenous breeds, respectively. The smallholder farmers considered in this study were not processing milk before sale, and the numbers of *Salmonella* and *E.coli* in the raw milk had not been quantified and assessed against the national standards hence the purpose for this study.

Milk samples from individual dams and the mixed milk were examined and quality indicators isolated using the Standard Plate Count (SPC) method (Gwendolyn, 1988: Harold, 2002). In each case, the raw milk sample was diluted with a series of peptone water in the water blanks to a final dilution of 1:1.000.000. Four-99 ml peptone water blanks were labeled, N_1, N_2, N_3, and N_4, and four Petri dishes were labeled (W) 1:100 (X) 1:10.000, (Y) 1:100.000, (Z) 1:1,000,000. A bottle of nutrient agar was liquefied under the autoclave at 15Kpa and 121^{o}C. The culture of the organisms (raw milk) was shaken and 1.0 ml of the organisms transferred to blank N_1, N_2, N_3 and N_4 using a sterile 1.1ml pipette. Blanks were shaken vigorously for about 25 times to ensure good distribution and breakdown of bacterial clumps. Using another sterile pipette, 0.1ml was transferred to plate W and to plate X, Y, and Z respectively, and 20 ml of the nutrient agar was poured in each of the four plates. The plates were rotated gently to get adequate mixing of the media and the organisms and incubated in an inverted position at 35^{o}C for 48 hours. Selective plating using Mac Konkey agar for *E. coli* and selenite agar (dulcitol selenite) for *Salmonella* spp. was used to isolate the organisms before gram staining was done to prove whether the maroon colonies were for *E. coli* and the white for *Salmomella*.

Research application

The grand mean for *Salmonella* spp. obtained from evening and morning samples for the tests was $8.27x10^5$ colony forming units ($CFUml^{-1}$) (Fig. 2A) with the sample from the milk vendor recording the highest average bacterial counts for salmonella ($4.0x10^5$ $CFUml^{-1}$) and *E. coli* ($19.3 x10^4$ $CFUml^{-1}$) (Fig. 2B), respectively. Poor storage hygiene and sanitation were suspected at this level. The vendors supplied milk to their clients using plastic utensils which are not easy to clean, but are able to allow proliferation of the "quality indicators". This corrolates with the report by Hein *et al.* (2001), who established that multiplication of contamination in raw food is amplified by poor processing and inappropriate storage conditions.

The counts for the quality indicators were very low in all samples obtained directly from dams (coded here as "evf"), but progressively increased with the handling chain. The high

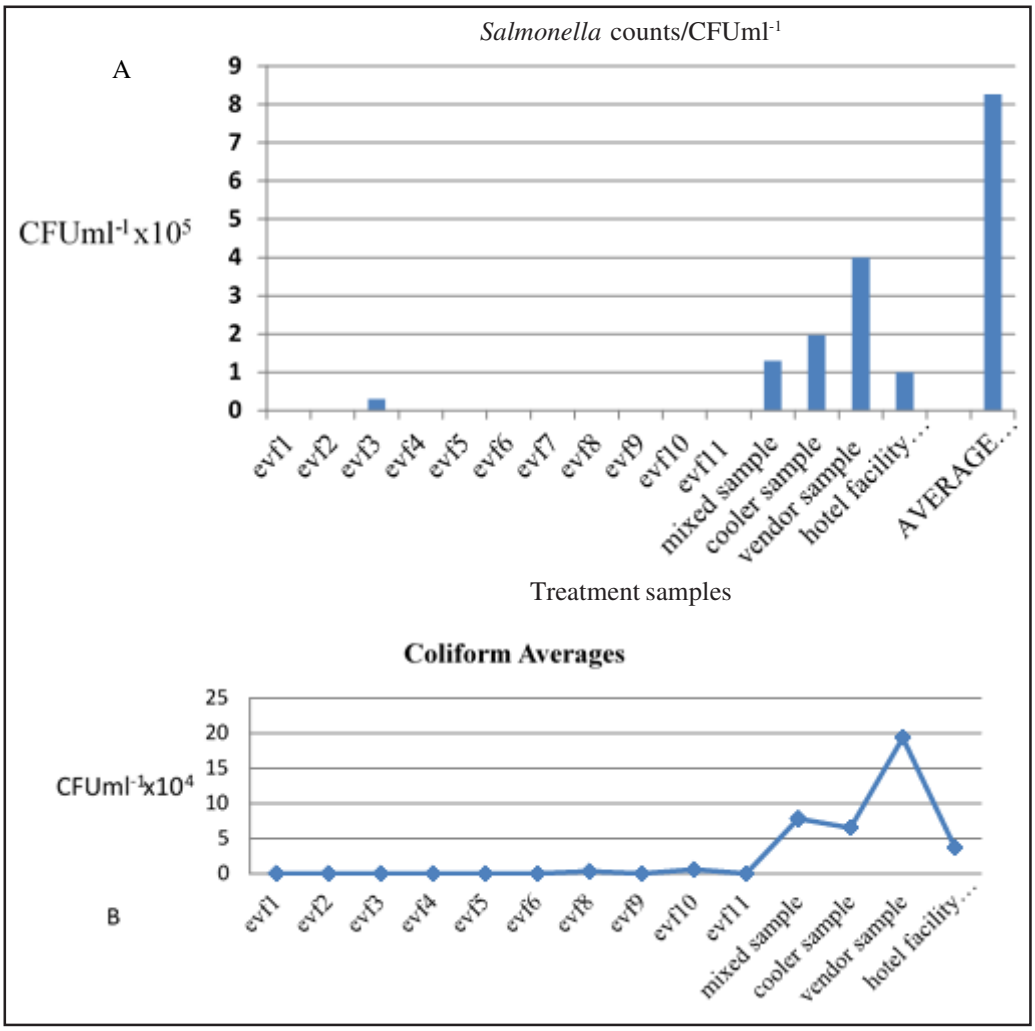

Figure 2. Colony forming units for salmonella in raw milk from central Uganda

range of microbial counts obtained from the individual dams' samples (0.32 x10^4 CFU ml^{-1} - 0.3x10^5 CFU ml^{-1}) was attributed to inconsistencies in the milking procedure. In some, cases, the multiplication shot a hundred fold when milk from individual animals was mixed together. However, samples from coolers and hotel facilities had arrested multiplication rates (1.97 x10^5 CFU ml^{-1},6.8 x10^4 CFU ml^{-1}) for *Salmonella* and *E. coli,* respectively. Cold temperatures limit bacterial multiplication, but the presence of the "quality indicators" in samples cooled within 4°C - 7°C cannot rule out psychrotrophic bacteria strains which survive and multiply under low temperatures. Even when raw milk was boiled at the hotel facility an average of 1.0 x10^5 CFU ml^{-1} for *Salnomella* and 3.7 x10^4 $^{-1}$ *E.coli* loads were still noted. Boiling killed most of the bacteria; the reason this count is relatively low. However, mesophilic and thermophilic strains which survive boiling and multiply when milk has cooled could not be ruled out in such samples. The widely distributed organisms in nature, and the interaction of the animal with its farmer, pasture, manure, wind, dust and water create avenues for contamination of raw milk. The colony forming units of these microorganisms were correlated against the national provisions. The correlation coefficients obtained are shown in Table 1.

A correlation coefficient between the presence of *E. coli* and the quality of raw milk from the smallholder farms was 0.9 indicating a positive correlation. This meant that coliforms exist in smallholder farmers' raw milk but their presence as defined by National Standards vary together positively. There was also a strong positive correlation between the standard plate count for *Salmonella* and the quality of milk set by the UNBS guidelines with a correlation coefficient of 0.9. This suggested that any increase in the total number of *Salmonella* in raw milk lowers the quality of raw milk.

The t-test was performed to establish whether two sample averages or proportions of *Salmonella* and *E.coli,* respectively, from smallholder farmers' raw milk and National Bureau of Standards were equal and statistically significant to give concrete inference on the

Table 1. Coliform averages and selected UNBS values for comparison

Treatment sample	Lab result sample (CFU m l^{-1})	UNBS selected values (CFU ml^{-1})
Mixed sample	7.8 x10^4	7.76 x10^4
Cooler sample	6.8 x10^4	6.84 x10^4
Milk vendor sample	19.3 x10^4	19.00 x10^4
Hotel facility sample	3.7 x10^4	3.6 x10^4
Plate counts for *Salmonella* and UNBS selected values		
Mixed sample	130,000	130170
Cooler' sample	197,000	195000
Milk vendor sample	400,000	400200
Hotel facility sample	100,000	100130

hypotheses. The one- tailed p value at P=0.05 of the T-test between *Salmonella* and National standards was 0.301 or 30.1% at 5% chance. This statistic result was higher than (P=0.05) and therefore was statistically insignificant to disprove the null hypothesis. Hence the numbers of *Salmonella* present in raw milk from smallholder farmers were not higher than the the acceptable by the National standards and therefore could not affect the quality of milk. In a similar manner a one tailed p value at P=0.05 was 0.1206 or 12.1% at 5% chance. This result was higher than (P=0.05). These results suggest that the numbers of these microorganisms in milk were not significant to affect the quality of raw milk. Routine milker training and assigning responsibilities for all areas of prevention of contamination on smallholder farms is a recommended practice. Periodic milk examination to provide microbiological count records on the prevalence of *E.coli* and *Salmonella* at different milk handling chains is also recommended.

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