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HYGIENIC EVALUATION OF COW MILK PRODUCTION IN A RURAL AREA OF NIGER

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This study aimed to evaluate the hygienic status of cow's milk delivered to a dairy unit located in Say (Niger), representing a typical production area of Sahelian region. Milk from 6 groups of farmers was analyzed throughout a 1-year period (11 sampling sessions). Milk samples were withdrawn both before (at ICP = Intermediate Collection Points) and after the transport (at dairy unit), and analyzed for Total Viable Count, Enterobacteriaceae, Escherichia coli and Coagulase-Positive Staphylococci. High mean values were detected, both at ICP and at dairy unit, for Total Viable Count (6·0±0·7 and 6·9±0·8 log CFU·mL^{-1}, respectively) and Enterobacteriaceae (4.6±1.5 and 4.1±1.3 log CFU·mL^{-1}). A marked variability was observed for *E. coli* and Coagulase-Positive Staphylococci, with a high prevalence of low numbers, but also the presence of highly contaminated samples (>5 log CFU·mL^{-1}). Higher microbial counts were detected during the rainy season. Milk transport influenced negatively the microbial loads, especially when the distance was >10 km. Our results show the need for an improvement of local collection/delivery organization to ensure a hygienic milk supply. With this aim, the frequent training of the farmers and the use of equipped dairy units should be regarded as useful tools.

Keywords: Milk hygiene, Sahelian region, Microbiological contamination, Sampling season, Transport

INTRODUCTION

Milk and dairy products play an important role in the diet of developing countries population, in order to achieve a sufficient energy and protein supply. Their consumption has a growing diffusion in nutritional habits of several African people, and is strongly encouraged by FAO and WHO food security programs. Milk production in Sub-Saharan Africa has to face several obstacles due to climatic conditions (high environmental temperatures and marked rain seasonality) and an insufficient organization of the whole chain (Diop and Abellah, 1996; and Cheng *et al.*, 2017). The marked seasonality of Sahelian climate affects significantly quality and hygiene of milk production, in particular when traditional milking is applied. During the rainy season, from June to September, the presence of mud in the outdoor milking areas could result in high microbial initial contamination of milk, especially due to the use of open recipients, such as calabashes (empty gourds), plastic or metal buckets. During the dry seasons (cool dry season, from October to February, and hot dry season, from March to May), the high quantity of dust can also contaminate significantly the milk; in hot dry season, this factor is

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associated to very high environmental temperatures that allow the rapid growth of spoilage and pathogenic microorganisms. It's known that storage/transport temperatures >20 °C promote the development of lactic acid bacteria causing the milk acidification (pH≤6.5) (Pistocchini *et al.*, 2009) and the proliferation of pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* and *Clostridium* spp. (Mellenberger and Kirk, 2017). In such conditions, the duration of transport represents a critical factor, and international guidelines indicate that milking-refrigeration interval should be limited to 2-3 hours (IDF, 1990; and EU, 1992).

The development of a proper local milk chain is also hindered by wide dispersion of the production, traditional farming and milking, and lack of infrastructures allowing a proper management of the product (efficient roads, hygienic production units, energy supply for refrigerated storage). In Sub-Saharan Africa, during the last decades, several milk collection units have been realized; these structures pretend to give a better organization to milk chain by assuring a reliable collection point to farmers, the possibility of milk refrigeration and quality/hygiene controls (Nkya *et al.*, 2007; Pistocchini *et al.*, 2009; and Belli *et al.*, 2013).

Our study was a part of a research and cooperation project aimed to organize farmers of the Department of Say (Niger) to improve local cow's milk hygiene. A total of 129 local farmers from 19 villages were grouped in the cooperative *Association des producteurs laitiers de Say* (Say cow's milk producers association) - *Hawrinde Biradam* (*APL/Say*), and a small dairy processing unit, with a laboratory for basic microbiological analyses, was built in 2009. This experimental study aimed to evaluate the hygienic status of cow's milk delivered to the dairy unit of Say; in particular, the attention was focused on the trend of bacteriological contamination level during the different seasons and on the influence of transport phase on milk quality.

MATERIALS AND METHODS

Milking, Transport and Collection Procedures

Milking was done twice a day, following traditional procedures in outdoor conditions; each farmer collected the milk in a calabash, and shortly afterwards put it into a recipient (e.g., a bottle). In each village, all the milk was gathered into a single food-contact plastic churn at an Intermediate Collection Point (ICP), then transported by bicycle to the dairy unit by 10 a.m. The churns, supplied by the project, were used to avoid the traditional use of recipients intended for different materials (e.g., fuel), and were washed by a NaOH solution and disinfected by NaClO at the dairy unit after each milk transport.

Milk quantity from each village was very variable, depending on the season and consequently on the availability of feed and water. During the Rainy Season (RS) and the Cool Dry Season (CDS) the good quality of pastures allowed to obtain relatively high production levels (15-20 L/village/day), while during Hot Dry Season (HDS) they fell to about 10 L/village/day.

At the arrival at the dairy unit, milk was evaluated for pH and density. If defined ranges were respected (pH≥6.45, density≥1,028 $g \cdot L^{-1}$), the milk was accepted, filtered and stocked into a cooler tank at 0-1 °C for subsequent sale.

Milk Sampling

For the evaluation of raw milk quality, 6 groups of farmers were selected, involving a total of 21 familiar groups. The groups were chosen considering the distance from the ICPs to the dairy unit (≤10 km for groups 1, 2 and 3; >10 km for groups 4, 5 and 6), to be representative of the whole productive area of Say. A total of 11 sampling sessions were performed during the period August 2011-July 2012, aiming to obtain samples during all the seasons (5 sessions during both RS and CDS, and 1 session during HDS).

Each time, milk samples were withdrawn both at ICP (from the plastic churn before bike transport) and at the dairy unit (at the reception of the churns). Milk samplings were performed by sterile syringe; at the same time, the environmental temperature and the hour of sampling were registered, and milk temperature and pH were measured, using digital pH meter/thermometer (HI 8424, Hanna Instruments, Baranzate, I). The samples withdrawn at ICP were kept refrigerated and transferred to the laboratory within 1 hour, while the samples picked at the dairy unit were analysed immediately.

Microbiological Analyses

Milk samples were put into sterile tubes, and decimal dilution were prepared by NaCl/tryptone saline (0.85%); then, microbiological analyses were performed using a spread plate technique, considering the main bacteriological indicators of contamination. Total aerobic Viable Count (TVC) was performed on Milk Count Agar (MCA,

Biogenetics, Ponte S. Nicolò, I). Plates were incubated at 37 °C for 48 hours. The temperature of 37 °C for the incubation of MCA, instead of the usual value (30 °C) was chosen as it was more representative of the environmental temperatures of the area considered. The number of *Enterobacteriaceae* was determined according to the ISO: 21528-2:2004 method (ISO, 2004); *E. coli* counts were determined according to the ISO: 16649-2:2001 method (ISO, 2001), and Coagulase-Positive Staphylococci (CPS) were enumerated following the ISO: 6888-1:1999 method (ISO, 1999).

Statistical Analysis

All microbiological data were submitted to analysis of variance (ANOVA) using the NLMIXED procedure by SAS/stat package version 8.0 (SAS Inst. Inc., Cary, NC). The sampling point (ICP or dairy unit), the distance of the ICP from the dairy unit ($\leq$10 km or >10 km) and the sampling season were considered as fixed effects. A value of $P<0.05$ was considered statistically significant.

Data concerning milk transport duration, environmental temperature at ICP, and milk temperature and pH both at ICP and at the dairy unit were also submitted to ANOVA, considering the sampling season and the distance as fixed effects. Correlation coefficient (r) of all bacterial counts with values of the parameters registered during sampling was also calculated.

RESULTS AND DISCUSSION

Milk Temperature and pH

Milk temperatures and pH values, and environmental temperatures are reported in Table 1. Milk temperature decreased gradually during the transport, due to the lower environmental temperature (as the delivery was performed within 10 a.m.) and to the application of wet cloths on the churns, as suggested to farmers during the training courses. No statistically significant differences among the data collected in the three seasons were detected. Nevertheless, during the hot dry season, higher milk temperatures both at ICP and at the dairy unit were recorded, with a very low decrease between the two points. A statistically significant correlation was observed between environmental and milk temperatures ($P<0.01$). Milk pH values were within the normal range for similar contexts (Millogo *et al.*, 2010), and didn't show any difference among the seasons.

Microbiological Quality of Milk

The results obtained by microbiological analyses showed high bacterial numbers in milk samples, considering international dairy quality standards, but they were in line with data obtained by several studies conducted in similar climatic and management contexts.

Table 1: Milk Temperatures and pH Values in Relation to the Sampling Season

Parameter	RS	CDS	HDS
Environmental temperature (°C) at ICP	26.8±2.5	28.0±2.0	32.5±2.1
Milk temperature (°C) at ICP	31.7±2.5	33.0±2.9	33.5±2.8
Milk temperature (°C) at dairy unit	30.2±3.1	30.1±4.0	33.1±2.2
pH at dairy unit	6.54±0.06	6.55±0.07	6.55±0.02

Note: ICP = Intermediate Collection Point, RS = Rainy Season, CDS = Cold Dry Season, HDS = Hot Dry Season.

TVC ranged frequently between 6 and 7 log CFU·mL^{-1}; the mean values measured in samples collected at the ICP and at the dairy unit were 6.0±0.7 log CFU·mL^{-1} and 6.9±0.8 log CFU·mL^{-1}, respectively; the difference observed was statistically significant ($P<0.05$). A high frequency of relatively low counts was detected in ICP samples, as more than 50% values were <6 log CFU·mL^{-1} (12% of the dairy unit samples were below this limit), while very high counts (>8 log CFU·mL^{-1}) were observed only in samples withdrawn at the dairy unit (12%). *Enterobacteriaceae* numbers ranged mostly between 4 and 6 log CFU·mL^{-1}, with high variability. Mean values recorded for dairy unit samples (4.6±1.5 log CFU·mL^{-1}) were higher, also if not significantly different ($P = 0.37$) from those detected in ICP samples (4.1±1.3 log CFU·mL^{-1}). About 88% of the samples withdrawn at ICP had counts below 5 log CFU·mL^{-1}, while this rate decreased to 53% in samples from the dairy unit.

Escherichia coli counts were very variable, with a high prevalence of low numbers (<2 log CFU·mL^{-1}) especially in samples withdrawn at ICP (50%), but with high contamination levels (>5 log CFU·mL^{-1}) in some cases; no significant differences were observed between ICP and dairy unit samples ($P = 0.96$). A marked variability was observed also for CPS, with potentially dangerous contamination levels (>4 log CFU·mL^{-1}) in a significant percentage of samples (11 and 36%, at ICP and at the dairy unit, respectively); the comparison of values obtained from ICP and dairy unit samples didn't show any statistical difference ($P = 0.59$).

High contamination values can be linked to traditional milking procedures, such as manual milking in non-dedicated, uncovered areas. The studies conducted in Mali and Burkina Faso, with similar contexts to the area considered by our evaluation, gave comparable counts (Bonfoh *et al.*, 2003; and Millogo *et al.*, 2010), but these values agree also with the results of previous studies carried out in Uganda, Tanzania and Morocco (Lues *et al.*, 2003; Kivaria *et al.*, 2006; Sraïri *et al.*, 2006; Donkor *et al.*, 2007; and Grimaud *et al.*, 2009). The importance of the application of hygienic procedures along the whole production-sale milk chain has been already highlighted by Kouamé-Sina *et al.* (2012), showing the potential reduction of pathogens prevalence and of the number of human foodborne illness cases due to the application of proper practices.

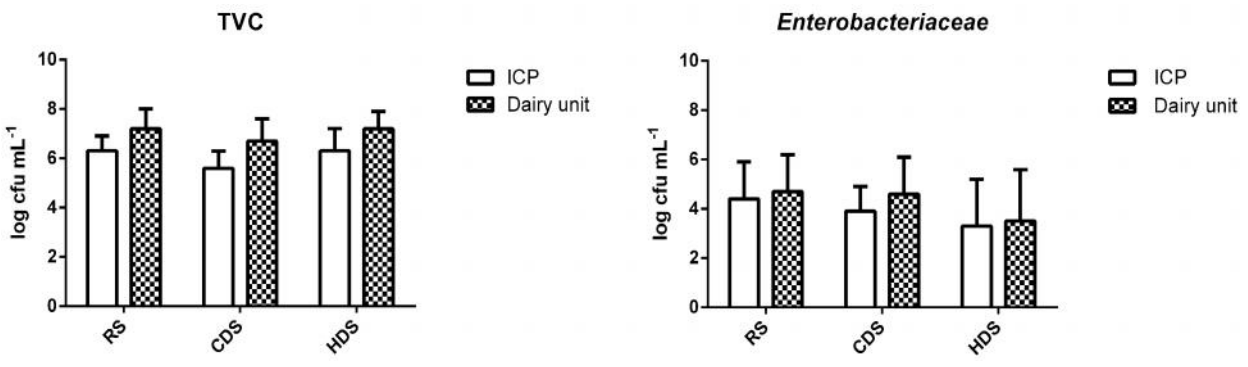
Comparison Among the Sampling Seasons

Data from microbial counts were clustered, based on sampling season, in order to evaluate the influence of climatic factors (temperature, rainfall) on milking hygiene and milk transport. Figure 1 shows the mean values observed for TVC and *Enterobacteriaceae*, while in Figure 2 is reported the frequency distribution analysis of all the data obtained during RS and CDS sampling; due to a too low number of data, HDS values weren't submitted to this analysis.

TVC values were not significantly influenced by the sampling season (P = 0.97), but lower counts were observed in CDS milk samples, and more than 90% of the samples withdrawn at ICP showed values <6 log CFU·mL^{-1}. During RS, higher counts were registered, with 20% of the samples overcoming 7 log CFU·mL^{-1}; this could be due to milk contamination during the rainfall (presence of water and mud on the skin and udder surface). Our data agree with those reported by Sraïri *et al.* (2009), who underlined the clear effect of seasonal climatic variations on the cow's dirtiness, resulting in higher counts during the rainy periods; during HDS, high TVC values could be favoured by the presence of heavy dust diffusion associated with high environmental temperature. A similar situation was observed also in milk samples from the dairy unit; a higher percentage of TVC values >8 log CFU·mL^{-1} was in fact detected in RS and HDS samples.

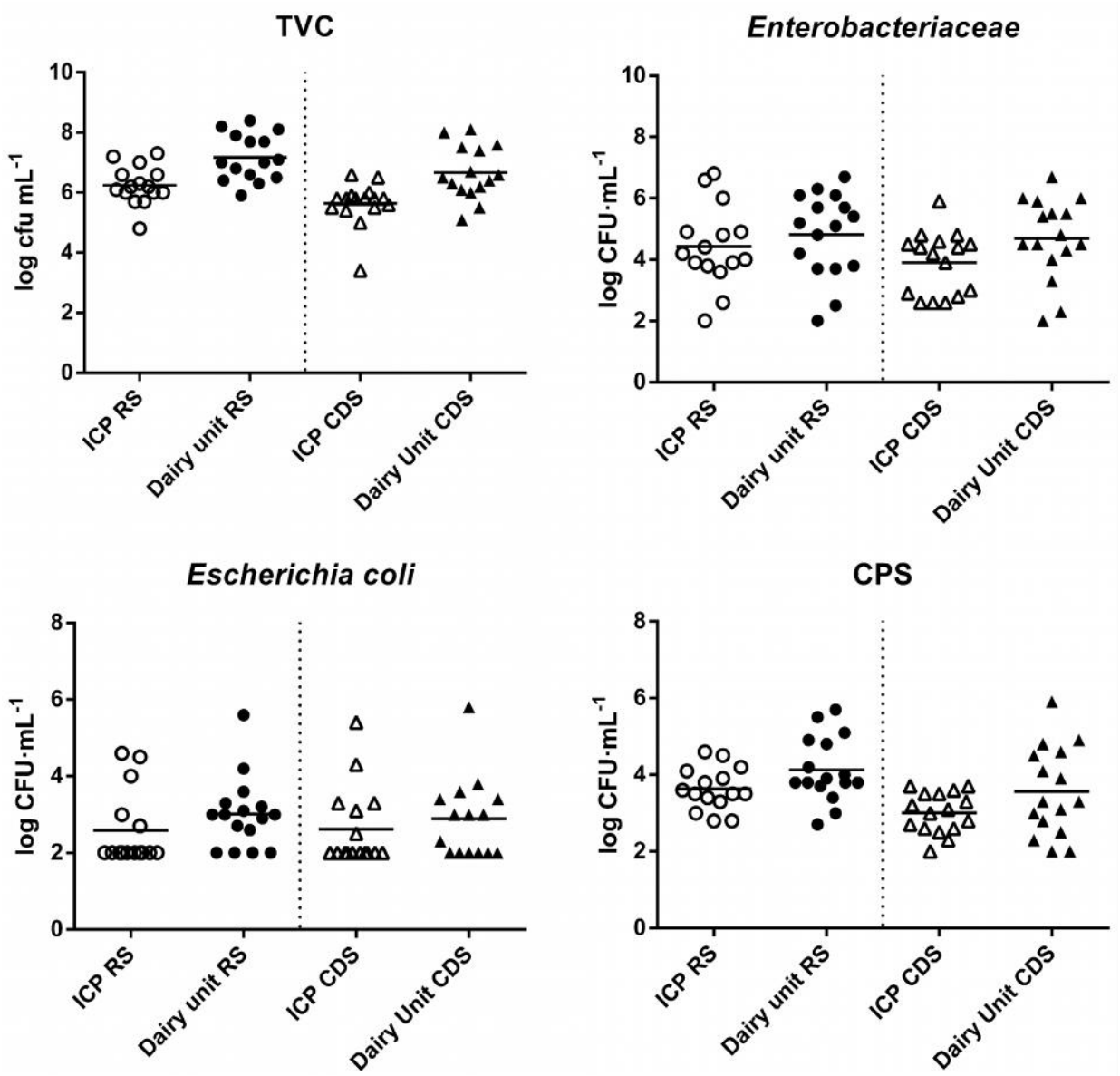
Also for *Enterobacteriaceae*, no statistically significant influences by the sampling season were observed (P = 0.25), also if slightly higher mean counts were detected during RS if compared to CDS. 20% of samples taken at ICP during RS had counts >6 log CFU·mL^{-1}, while all the counts at CDS were below this limit; for samples withdrawn at the dairy unit, this percentage raised to 25% and 12.5% for RS and CDS, respectively. These results can be explained by the negative influence of rainfall, as for TVC, but the environmental temperature, in this case, didn't seem to have an evident influence on microbial counts. In fact, data concerning HDS samples indicated the presence of lower counts both at ICP and at the dairy unit; such results could be due to the lower possibility of faecal contamination in very dry milking areas, but the small number of data didn't allow a complete analysis. *E. coli* counts weren't significantly different among the seasons (P = 0.13), also if slightly higher values were detected during RS, and frequency distribution of data was similar between RS and CDS. The small number of HDS data confirmed the trend

Figure 1: Total Viable Counts (TVC) and Enterobacteriaceae Counts in Milk Samples Collected in Different Seasons



Note: ICP = Intermediate Collection Point; RS = Rainy Season; CDS = Cold Dry Season; HDS = Hot Dry Season.

Figure 2: Distribution of Microbial Counts Considering Season and Sampling Point



Note: Median - Value. TVC = Total Viable Count; CPS = Coagulase-Positive Staphylococci; ICP = Intermediate Collection Point; RS = Rainy Season; CDS = Cold Dry Season.

observed for *Enterobacteriaceae*; it was expected, as these bacteria share the same contamination source. Considering CPS, a statistically significant influence of sampling seasons was detected ($P<0.01$), with higher values observed in RS. The frequency of CPS counts above 4 log CFU·mL^{-1} was evidently higher in RS samples, if compared to CDS ones, both at ICP (25% vs 0%) and at the dairy unit (46% *vs* 31 %). During HDS, only low counts (<3 log CFU·mL^{-1}) were detected both at ICP and at the dairy unit, but the limited amount of available data should be considered.

Effect of Transport Distance Between the ICP and the Dairy Unit

Considering the influence of milk transport on microbial counts, an increase in mean values, associated to a higher rate of highly contaminated samples, was observed for all

Table 2: Comparison of Data Considering the Distance from the ICP (Intermediate Collection Point) to the Dairy Unit

Parameter	? 10 km	> 10 km
Transport duration (min.)	40* ±31	97* ±30
Milk temperature at dairy unit (°C)	31.6* ±3.4	28.5* ±7.0
Milk pH at dairy unit	6.53±0.06	6.56±1.68
TVC at dairy unit (log CFU·mL^{-1})	6.9±0.8	7.0±1.8
Δlog TVC	1.0±0.6	1.0±0.7
Enterobacteriaceae at dairy unit (log CFU·mL^{-1})	4.5±1.8	4.8±1.1
Δlog *Enterobacteriaceae*	0.4±1.0	0.8±1.4
E. coli at dairy unit (log CFU·mL^{-1})	2.2±1.2	3.2±1.3
CPS at dairy unit (log CFU·mL^{-1})	3.4±0.7	4.0±0.8

Note: TVC = Total Viable Count; CPS = Coagulase-Positive Staphylococci. * P<0.01.

the parameters (Figures 1 and 2). For TVC and *Enterobacteriaceae*, after milk transport, a mean increase of 0.99 and 0.61 log CFU·mL^{-1} was detected, respectively. Comparing values obtained from samples withdrawn in the different seasons, no significant influences were observed on transport count rises (P = 0.83 for TVC and 0.30 for *Enterobacteriaceae*); nevertheless, a lower increase during CDS was observed, in particular for *Enterobacteriaceae* and CPS.

In order to evaluate the influence of transport distance on the quality and microbial load of milk delivered to the dairy, microbiological data, milk temperature and pH values were clustered in two groups, based on the distance from the ICP to the dairy unit (Table 2).

The transport duration was significantly higher (P<0.01) for milk delivered from ICP located at a distance >10 km from the dairy unit, and lasted almost 1 hour more than that coming from shorter distances. Such high variability, also for small kilometric differences, was due to the poor quality of routes and to the use of the bicycle as the main transport medium. Milk temperature at the dairy unit was significantly lower (P<0.01) for longer transport distances; this was due to the gradual refreshment of milk kept in plastic churns surrounded by wet cloths. The statistical analysis of microbiological data didn't show any significant difference (TVC: P = 0.08; *Enterobacteriaceae*: P = 0.80; *E. coli*: P = 0.45; CPS: P = 0.52); however, the combination of transport time and temperature led to higher microbial counts for ICP located at a distance >10 km from the dairy unit. The importance of the distance between production sites (villages) and refrigeration points has already been evidenced by Gran *et al.* (2002), who correlated this parameter to TVC values. The increase in delivery time is a critical factor in favoring milk deterioration; to keep a low microbial level in raw milk, it should reach the dairy and be refrigerated as long as bacterial growth is still in the lag phase, that is known to last around three hours.

CONCLUSION

The results of this study highlight the importance of the application of a set of interventions along the whole chain to improve local milk production in Sahelian areas. In addition to the application of good milking practices to the traditional procedures (dedicated and proper milking areas, teats and milkers' hands cleaning, elimination of foremilk), a deep attention should be taken also to ensure a minimization of milk transport phase. In particular, considering the lack of infrastructures and the high costs of fast transport means, the main intervention should be an improvement of the collection/delivery organization. With these aims, a frequent training activity for farmers, giving a higher awareness on the importance of microbiological contamination of milk, and the local presence of dairy units equipped with simple laboratories, that can ensure refrigeration, quality evaluation and eventual treatment of milk, should be regarded as important means to be applied in future interventions in Sahelian areas.

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CONFLICT OF INTEREST

Anna F A Cantafora, Simone Stella, Filippo De Monte, Abdoul Kader, Ivan Corti and Casimiro Crimella state the absence of conflicts of interest related to the publication of the manuscript.

ETHICAL STANDARDS

This article does not contain any studies with human or animal subjects performed by any of the authors.

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