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Microbiological Characterization of Raw Cow's Milk Sold in Zongo in the Commune of Cotonou

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Abstract: Milk is a favorable environment for the growth of microorganisms. Thus, the microorganisms likely to be found in the milk can be at the base of several food poisonings. This work aims to assess the microbiological quality of raw cow's milk sold in Zongo in the commune of Cotonou (Benin). After taking samples of raw cow's milk, microbiological analyzes were carried out in the laboratory in accordance with Good Hygienic Practices (BPH). The results of these microbiological analyzes revealed the presence at high loads and therefore, outside the norm of certain microorganisms such as total mesophilic aerobic flora, enterobacteria, thermotolerant coliforms, coagulase positive staphylococci, *E. coli* and yeasts and molds. Thus, for proper monitoring of hygiene rules, a quality control system must be put in place to allow the population to enjoy good health by consuming good quality raw cow's milk.

INTRODUCTION

Food is the most important need among human daily needs. Milk, intended for human consumption, is the integral product of the total and uninterrupted milking of a healthy, well-nourished and non-overworked dairy female[1]. Milk is a food of choice due to its richness in mineral elements. The name "milk" is reserved exclusively for products of normal breast secretion, obtained by one or more milking (s), without any addition or subtraction and not having been subjected to any heat treatment[2]. Milk is a food of high nutritional value very rich in proteins, lipids, carbohydrates and above all by a supply of trace elements such as calcium[3]. It is roughly the only food that can meet most of human nutritional needs in a balanced way[4]. As a result, it occupies a prominent place in the human food intake in most countries in the world. This is why international

organizations such as FAO and WHO have taken an interest in this product and its derivatives. Milk has always been used to feed infants and children in general. It is also used in the diet of pregnant women and the sick because it is universally recognized as a complete and easily digestible food[4]. Benin, like many countries in the sub-region, pays particular attention to milk production as part of the livestock sector development policy. Indeed, milk is produced over almost the entire national territory and its quality can vary from one region to another, from one breed of cattle to another. Likewise, its consumption is now experiencing a considerable boom[5]. Milk contributes >50% of the annual income of Peulh households. Despite its importance in human nutrition, it is common to observe ignorance and non-mastery of the rules of good hygienic practice during the milking and sale of milk which does not guarantee the hygienic quality of the product. In fact, the players in the sector (producers and sellers) work in unsanitary conditions, thus, constituting enormous sources of contamination of milk by microorganisms. However, by virtue of its highly perishable nature and very favorable to the development of microorganisms, cow's milk can constitute a potential source of contamination and intoxication for human consumption. The risk of possible deterioration of cow's milk by various useful or pathogenic microorganisms requires rigorous microbiological monitoring from milking to consumption. Assessing the sanitary and hygienic quality of raw milk intended for consumption is therefore essential for consumer protection. It is in this context that this study takes place in order to assess the risks to which consumers are exposed. The objective of the study is to assess the microbiological quality of raw cow's milk sold in Zongo in the commune of Cotonou.

MATERIALS AND METHODS

The material used consisted mainly of samples of raw cow's milk (Fig. 1) collected at random (at random) from three milk sellers on three different occasions (regular period of 5 days) in Zongo in the commune of Cotonou. For each sample, three samples were taken from each salesperson. On each occasion, the 9 coded samples were sent to the laboratory in their original packaging (Fig. 1) in a cooler containing cold accumulators for microbiological analyzes. Thus, the 09 samples made up of the 27 samples were characterized from a microbiological point of view. Samples from point of sale N°1 were coded A1, those from point of sale N°2 were coded A2 while samples from point of sale N°3were coded A3.

Methods of microbiological analysis: Microbiological analyses were done not only on the search for pathogenic microorganisms but also on germs indicative of good hygiene practices in food products[6, 7]. The culture media

Fig. 1: Photos of fresh cow's milk

were prepared according to the manufacturer's instructions and maintained in supercooling until the time of plating except for Baird Parker agar enriched with egg yolk and potassium tellurite which was pre-cast in Petri dishes. To prepare the stock solution, 10 g of each sample was taken with a sterile spatula from a sterile stomacher bag and 90 mL of tryptone salt broth was added. This mixture was homogenized in a sample blender. Successive decimal dilutions were made from the stock solution. Inoculation was done by the method of incorporating the inoculum into the agar (mass inoculation technique) except for the Baird Parker which was surface inoculated. Total Aerobic Mesophilic Flora (TAFM) was enumerated on PCA agar after incubation at 30°C for 72±2 h[8], Enterobacteria on VRBG agar after incubation at 30°C for 24±2 h[9], staphylococci on Baird Parker agar with egg yolk and potassium tellurite after incubation at 37°C for 48±2 h[10], thermotolerant coliforms on VRBL at 44°C for 24±2 h[11]. The search for Escherichia coli β-glucuronidase was done from coliform plates on VRBL by performing the Mac-Kenzie test (indole and oxidase) using Kovacs and oxidase reagents[12]. The enumeration of Anaerobic Sulfito-Reducing Bacteria (ASR) was carried out on TSN agar after incubation at 46°C for 20±2 h[13]. As for yeasts and molds, they were counted on OGA agar enriched with oxytetracycline after incubation at 25°C for 3-5 days[14]. Salmonella was tested on SS Agar according to NF 6579-2[15]. Microbiological analyses were performed in three repetitions on each sample.

Analysis of data: The data collected were analyzed using SPSS Version 16 software which allowed for Analysis of Variance (ANOVA) and Tukey's test for comparison of means. The level of significance retained was 5%. The means of microbial loads (CFU/mL) found in the samples were compared with the limit values "m" and "M" set for raw cow's milk for human consumption according to the microbiological criteria for foodstuffs[16]; "m" represents the number of CFU/mL below which the samples are considered satisfactory or of good bacteriological quality. If the number of CFU/mL obtained is between "m" and "M", the samples are judged as acceptable (poor) and the samples containing numbers of CFU/mL higher than "M" are unsatisfactory (non-compliant).

RESULTS AND DISCUSSION

The results of the microbiological analyses carried out on the samples of raw cow's milk collected were presented in Table 1.

The analysis of Table 1 shows that the microbial load in FAMT of the milk sampled in Zongo is higher than the limit values set by the Direction de Santé Luxembourg[16]. This high contamination can be explained by the lack of hygiene and sanitation during milking, equipment used for sale or to the infection of the cow's udders. The same observation was made by Villar *et al*.[17]. According to Aumaitre[18], the health of the dairy herd, milking and pre-storage conditions are also basic determinants of milk quality. Bacteria can enter the milk while it is still on the udder and most microorganisms found in raw milk are contaminants from the external surface of the udder, milking utensils and milkers as pointed out by Chye *et al*.[19]. Thermotolerant coliforms and *E. coli* were found in the milk samples analyzed at loads above the set criteria. The presence of thermotolerant coliforms in milk indicates contamination by cow feces or by the milker's soiled hands as pointed out by Farougou *et al*.[20]. The presence of *E. coli*, an indicator of fecal contamination, reveals the potential presence of a pathogenic enterobacterial host in the samples. This same observation was made by Chye *et al*.[19]. Thermotolerant coliforms are a subgroup of total coliform bacteria which includes *Escherichia coli* species. *E. coli* is the best indicator of fecal contamination as it is present in the digestive tract of animals and humans and is the only member of the coliform group that is exclusively fecal in origin. According to Canadian standards, the presence of *E. coli* in a ready-to-eat food indicates the potential presence of enteric pathogens in that food and, therefore, makes the food at risk for human consumption, since, no further processing will be applied to the food. It should not be detected in a ready-to-eat food even if a tolerance is allowed. The main recommendations associated with the presence of *E. coli* in food and water are to first identify potential sources of fecal contamination. Increased hygiene measures for handlers, equipment, instruments and premises should also be applied. As for coagulase positive staphylococci, their presence in milk is due to the environmental conditions and the milk handlers, who are healthy carriers of these germs. Staphylococcus aureus is a contagious agent that lives on the cow's udder and is transmitted from cow to cow[21]. This bacterium can enter the milk either by direct shedding from udders with clinical or sub-clinical staphylococcal mastitis or by environmental contamination during handling and processing of raw milk[22, 23]. When the udder is infected, S. aureus is shed into the milk in amounts that can vary widely, from 0-108 CFU/mL[24]. These results which are consistent with those of Kouame-Sina *et al*.[25], showed that this bacterium came mainly from the water used during the different milking steps (50.9%), from the milker's hands (39.6%) and from the udders (28.9%). The contamination of milk by yeasts and molds is explained by the long exposure to the ambient air during milking and packaging. This contamination can also be explained by prolonged storage of milk. The samples of cow's milk taken at Zongo in the commune of Cotonou are therefore of unsatisfactory hygienic quality. Indeed, yeasts and molds are widespread in the environment. Some of them are part of the normal flora of various food products. They are used in the fermentation process of several foodstuffs. But, when they proliferate in food and their population reaches excessive levels, yeasts and molds can cause product spoilage (taste, texture, appearance) and lead to significant economic losses. Under given conditions, some mold species can synthesize mycotoxins that are toxic metabolites, making them potentially pathogenic to humans. Mycotoxins are produced by molds that grow on plants and foods. Many types of mycotoxins exist but only a few are found in foods. The severity also depends on the amount and duration of exposure. Some yeast and mold spores are resistant to heat, freezing, antibiotics and irradiation. It is essential to control the quality of food products from their origin to the consumer (harvesting, storage, transportation, processing and preparation). Maintaining mold populations at acceptable levels reduces the risk of food poisoning.

Table 1: Microbial loads of germs in raw cow's milk samples collected in Zongo in the commune of Cotonou

Germs	A1 (CFU/mL)	A2 (CFU/mL)	A3 (CFU/mL)	Critères[16]
TAMF	$>3.10^{7a}$	$>3.10^{7a}$	$>3.10^{7a}$	5.10^4
Enterobacteria	$>10^{4a}$	$>10^{4a}$	$>10^{4a}$	$10\text{-}10^2$
Thermotolerant coliforms	$>3.10^{3a}$	$>3.10^{3a}$	$>3.10^{3a}$	Absent
Escherichia coli	$4.4.10^{2a}$	$6.2.10^{2b}$	$2.6.10^{2c}$	Absent
Staphylococcus aureus	$8.2.10^{2a}$	$4.6.10^{2b}$	$1.1.10^{4c}$	$10^2\text{-}5.10^2$
Yeasts	$>1.5.10^{4a}$	$>1.5.10^{4a}$	$>1.5.10^{4a}$	Absent
Molds	Absent[a]	Absent[a]	10^{2b}	Absent
Salmonella spp.	Absent[a]	Absent[a]	Absent[a]	Absent/25 g
Anaerobic Sulfito-Reducing Bacteria (ASR)	Absent[a]	Absent[a]	Absent[a]	Absent
Appreciation	HQNS	HQNS	HQNS	-

Mean values with the same letter in the same line are not significantly different at the 5% level; Data represents in table is mean of three replications; HQNS: Hygienic Quality Not Satisfactory; TAFM: Total Aerobic Mesophilic Flora; A1: Samples of the point of sale N°1; A2: Samples of the point of sale N°2; A3: Samples of the point of sale N°3

CONCLUSION

The results of this work showed that the samples analysed did not meet the microbiological quality standards. The degree of contamination detected reflects a failure in hygiene, whether at the level of milking, handling personnel or storage and sale condition. The problem has shown that consumers of this product are exposed to the risk of food poisoning. To remedy this state of affairs, it would be advisable to encourage all actors in the production chain to adopt good hygiene practices through awareness programs in order to allow the population to enjoy good health by consuming raw cow's milk of satisfactory quality.

Disclaimer: The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing companies rather it was funded by personal efforts of the authors.

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