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Evaluation of the Microbiological Quality of Raw Cow's Milk Collected at Hahotoé and Kpémé (Southern Togo)

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A B S T R A C T

In most African countries south of the Sahara, raw cow's milk is appreciated and consumed without any treatment. In order to assess the microbiological risks associated with consumption of this foodstuff, this study was carried out in Hahotoé and Kpémé, two localities in Southern Togo. The hygienic quality of raw cow's milk was assessed on 20 samples, including 5 samples taken at sale and 5 samples taken at milking in each of the localities considered. Microbiological analyses showed the presence of total coliforms, faecal coliforms, moulds, yeasts and total aerobic mesophilic flora. However, the absence of sulfite-reducing anaerobes was noted in all samples. These results indicate unsatisfactory hygienic quality, due to non-compliance with good hygiene practices. Raw cow's milk consumed in the surveyed localities would therefore constitute a health risk for the population.

Introduction

Food production areas, including milk and its by-products, are a breeding ground for numerous diseases, most of which are preventable. Not only is this proliferation of food-borne diseases due to a lack of hygiene and quality control of foodstuffs a cause for concern, but in the event of food poisoning, it has a considerable impact on the health system and the economy. This is a real public health problem, given the ever-increasing number of consumers of this foodstuff; hence the need to set up a quality control system for these various foods (Alais, 1948). The dairy sector, which produces milk and its by-products, is highly dynamic in our country, linked to the general conditions of demand in sub-Saharan and West Africa (Faye and Alary, 2001).

To meet this demand, Togolese milk production has been steadily increasing over the last ten years. Artificial bovine insemination campaigns have been initiated to increase the cattle herd through the introduction of milk-producing breeds. In 2012, Togo's national production of fresh whole cow's milk was 13,878,644 Liters (Essodina

et al., 2017). Despite this enormous production effort, Togo still resorts to importing dairy products. Indeed, dairy products, whether traditional or semi-modern, are essentially represented by raw milk, curdled milk, pasteurized milk, Peulh or Wagashi cheese and butter. Imported dairy products, on the other hand, cover a wide variety, including pasteurized milk, yoghurt, creams of various types and cheeses of various types (Essodina *et al.,* 2017).

The local marketing and production circuit for milk and its by-products is essentially informal (Essodina *et al.,* 2017). Deguè bars and restaurants nationwide, are the demanders of raw milk at the informal channel level for its organoleptic and nutritional qualities most sought after by the consumer (Lovett *et al.,* 1989; Mani-lópez, 2014).

However, the dairy sector and its by-products suffer from quality problems essentially linked to the hygienic conditions under which farmers milk and store their products, and is heavily affected by major zoonoses such as tuberculosis and brucellosis, the transmission of which through the consumption of raw milk represents a major risk factor (Faye *et al.,* 2005).

In 2020, the World Health Organization (WHO) stated that unfit food containing bacteria, viruses, parasites or harmful chemicals would cause over 200 diseases ranging from diarrhoea to cancer (WHO, 2020). The main dangers associated with the consumption of raw milk are microbiological and physico-chemical.

Raw milk is a favourable environment for the development of germs and the storage of lipophilic xenobiotics. It is extracted in an unhealthy environment, depending on the state of health of the animals and the milking conditions of the dairy cows.

Furthermore, milk composition is variable and depends on the genotype of the dairy cow, age, season, stage of lactation and, above all, diet (Pougheon *et al.,* 2001). This last factor plays an important role in the physico-chemical composition of milk. Indeed, where cows graze determines the presence of certain chemical elements in milk (Yahaya *et al.,* 2010).

This is the background to the present study, which aims to contribute to the health safety of the population in the localities of Kpémé and Hahotoé through microbiological monitoring.

Materials and Methods

A survey sheet was drawn up and submitted to the participants. This survey sheet comprises 3 parts, namely: Socio-demographic characteristics of cow breeders, stakeholders' perception of pollution at phosphate mining and processing sites, and General infection prevention measures and Milk marketing. The biological material was raw milk collected in the Hahotoé and Kpémé areas during milking and the sale of milk packaged in plastic bottles. These milk samples were immediately transported to the microbiological laboratory at a temperature ranging from 4 to 8°C, in a cooler equipped with frozen dry ice. A total of 20 raw milk samples were taken in Hahotoé and Kpémé, including 10 samples taken in each locality. Of the 10 samples taken in each locality, 5 were taken at milking under normal conditions and the other 5 at sale. The samples were packaged in 125 mL sterile vials before being sent to the laboratory for testing.

Survey

The survey was carried out in the phosphate processing and extraction area of Kpémé and Hahotoé from June 14 to August 14, 2022 by submitting the survey form to seventy-one (71) participants recruited at random and according to their availability to participate in the study.

Microbiological analyses

Microbiological analyses were carried out using methods standardized by AFNOR.

Germs tested included Total Aerobic Mesophilic Flora (TAMF), total coliforms (TC), thermo-tolerant or fecal coliforms (TC), sulfite-reducing anaerobes (SRa), yeasts and molds. The results of these analyses were interpreted in accordance with European Commission regulation EC N°2073/2005 of November 15, 2005 on microbiological criteria for foodstuffs, and discussed in relation to similar previous studies. This regulation sets out the microbiological criteria for defining the acceptability of foodstuffs. All results were expressed in colony-forming units per gram of sample (CFU/g).

Results and Discussion

Survey results

The results of the survey are shown in Table 2. Of the participants who completed the questionnaires, 57.14%

were male compared with 42.86% female in Hahotoe, and 52.77% male compared with 47.23% female in Kpémé. Most participants were aged between 19 and 59 in both study areas, 88.58% in Hahotoé and 88.89% in Kpémé. The majority of study participants were illiterate: 82.85% in Hahotoé and 80% in Kpémé.

The rest of the milk is used to produce Peulh cheese and curdled milk. The majority of participants stated that they had not experienced any discomfort related to milk consumption in the two zones.

Direct consumption of milk is the most important destination of milk, 86.11% in Kpémé and 80.00% in Hahotoé zones, but around 6% and 21% stated that they had experienced discomfort in Kpémé and Hahotoé respectively. However, all participants reported that the cows' udders were covered with flies.

Microbiological quality of raw milk samples

The results of microbiological analyses of raw milk samples taken from the two study areas are shown in Table 3. According to this table, all samples showed a total aerobic mesophilic flora (TAMF) value greater than 10^5 CFU /g. Total coliforms (TC) were also very high, reaching an average value of 4,580,000 CFU/g.

The results of the same table show that faecal coliform (FC) values are above 130 CFU/g, except in the case of samples taken for sale in the Goumoukopé area (V2). Yeast values were low in the Goumoukopé and Haotoé sales samples, but above 1000 CFU/g in the Haotoé milking sample (T2). Molds, on the other hand, ranged from 360 CFU/g to 7900 CFU/g in both zones.

The survey revealed that the majority of participants were in the 19-59 age bracket, which groups together people with the physical strength to carry out cow-breeding activities, especially milking, which requires a certain amount of physical stamina. This age group is in line with the current trend in developing countries, where a higher proportion of young people have the physical strength required for work such as farming and animal husbandry. In the survey carried out, milk is milked exclusively by men, some of whom sell it at the milking site. Itinerant sales of milk, handled at room temperature for 6 to 11 hours with little control over container hygiene, are carried out mainly by the herdsmen's wives and their children. These results are higher than those reported by Essalhi (2002) in the 06 outlets selling fresh milk and dairy products in Togo's maritime region, where women account for 86% of raw milk vendors and men for only 14%.

The illiteracy rate is lower than those obtained by Leyral and Vierling (2007) during their study of 6 fresh milk sales and collection outlets in Lomé, where illiterates accounted for 85.7%. Few of them, therefore, had a school education: 17.15% in Hahotoé and 11.11% in Gounoukopé. In Gounoukopé, 5.25% have completed secondary school. The level of education of breeders and cow's milk retailers would be essential for a good understanding and practice of hygiene in the course of their activities.

The survey also shows that most milk is consumed directly, i.e. 86.11% in Kpémé and 80.00% in Hahotoé. This trend can be explained by the conservation of its nutritive elements. Indeed, for the Fulani population, milk is a very precious raw material that is consumed at any time, sometimes mixed with rice or millet (Guilhem, 2006). The discomfort felt after drinking milk, ranging from stomach ache to diarrhoea, could be explained by the fact that some people's bodies are unable to tolerate the lactose present in raw cow's milk, leading to lactose intolerance after consumption. In addition, the presence of flies in the udder could lead to microbiological contamination of milked milk. According to Florence (2010), this poor lactose tolerance could be explained by glucose-galactose transporter deficiency, manifesting itself in severe diarrhoea that rapidly leads to dehydration, under nutrition and death.

With regard to microbiological quality, the raw milk samples presented an unsatisfactory hygienic quality according to EC regulation No. 2073/2005, which set a FMAT level of less than 10^5 CFU/g of sample. This could be associated with inadequate hygiene measures during milking and milk sales in both areas.

In Algeria, Total Aerobic Mesophilic Flora ranging from $0.4.10^5$ to $0.7.10^5$ CFU/ml in raw milk, with an average of $0.56.10^5 \pm 0.114.10^5$ CFU/ml. These values were lower than those found in the present study. Raw milk produced by a healthy animal milked under good hygienic conditions normally yields milk with low levels of contamination, containing an overall flora of 10^3 to 10^5 CFU/ml (Faye and Loiseau, 2002). These results reveal a lack of respect for good production practices and storage conditions for milk from milking to sale (Amhouri *et al.,* 1998).

Table.1 Germs sought in samples taken and culture methods used

N°	Desired germs	Methods	Culture Media	Growing Conditions
1	**Total germs**	NF EN ISO 4833-1, September 2013	PCA	30°C, 24 – 72h
2	**Total coliforms**	NF EN ISO 4832, february 2006	VRBL	30°C, 24h
3	**Thermotolerant coliforms**	NF V08060, april 2009	VRBL	44°C, 24h
4	**Sulfito-reducing anaerobes**	NF EN ISO 15213, may 2003	TSN	44°C, 24 – 48h
5	**Yeasts and molds**	NF EN ISO 21517, July 2008	Sabouraud + Chloramphenicol	30°C, 48 – 72h

Table.2 Survey results

Parameters considered		Goumoukopé	Hahotoé
Sex	**M**	52.17 %	57.14 %
	F	47.23%	42.86%
Age range (years)	**12 to 18**	11.11%	11.42%
	19 to 35	22.22%	28.57%
	36 to 59	66.67%	60.01%
Grade level	**No level of school education**	83.33%	82.85%
	Primary	11.11%	17.15%
	Secondary	5.56%	0%
Consumption of Milk Expressed	**Yes**	86.11%	80.00%
	No	13.89%	20.00%
Raw milk processing forms	**Raw**	51.61%	67.86%
	Fulani cheese	35.48%	17.86%
	Crudled milk	12.91%	14.28%
Discomfort after drinking milk	**Yes**	6.45%	21.43%
	Non	54.84%	64.29%
	No answer	38.71%	14.28%
Presence of flies on the udders of dairy cows	**Yes**	100.00%	100.00%
	No	0%	0%
Utensils used for milking, storing and selling	**Washed canister**	36.11%	51.43%
	Plastic bottle	25.00%	42.86%
	Gourd	38.89%	5.71%
How often these utensils are cleaned	**During milking/selling**	50.00%	45.71%
	After milking/selling	30.55%	40.00%
	During and after milking/selling	19.45%	14.29%

Table.3 Microbiological analysis results

Indicator values (CE N° 2073/2005)		TAMF (10^5)	TC (10^5)	FC	Y	M
		< 10^5	< 100	< 100	< 100	< 100
Goumoukopé	T1	692.4 ± 277.4	23.77 ± 17.02	144.4 ± 100.3	44.6 ± 23.8	360.0 ± 153.72
	V1	1030.0 ± 469.72	24.1 ± 13.32	13.2 ± 4.83	3.2 ± 3.2	7880.0 ± 5615.0
Hahotoé	T2	2620.0 ± 453.21	45.8 ± 11.52	320.0 ± 134.02	1474.0 ±1382.0	1230.0 ± 960.0
	V2	1236.0 ± 232.15	38.4 ± 6.04	134.0 ± 89.98	4.0 ± 4.0	3590.0 ± 3357.0

TAMF: total aerobic mesophilic flora, TC: Total coliforms, FC: faecal coliform, Y: Yeast, M: Molds.Values are expressed as mean ± SD

Total coliforms, faecal coliforms, yeasts and moulds were found in smaller quantities in the milk samples analyzed from both zones. The presence of total coliforms is not necessarily a direct indication of faecal contamination. Some coliforms are present in wet residues found in dairy equipment (Larpent, 1997).

Heavily soiled bedding also contains coliforms, and the prevalence of mastitis in some cases increases, leading to greater contamination of the milk. Other sources of contamination are also to be considered, such as poor transport conditions and lack of hygiene during milking (Magnusson *et al.,* 2007).

However, fecal coliform contamination of milk would indicate definite fecal contamination (Gouvernement du Québec, 2019). Their presence in raw milk also reflects a lack of the required sanitary provisions during milking and milk harvesting, contamination during transport or defective storage. The main vectors are teat skin soiled with faeces and poorly designed milking equipment.

The main vectors are feces-soiled teat skins and poorly designed and cleaned milking equipment. Fecal coliforms are *Escherichia coli* in 95 to 99% of cases and are the most common in the feces of dairy cows (Mocquot and Guittonneau, 1939; Bouchibi and Boulame, 1997).

The raw milk samples showed a satisfactory hygienic quality compared to the yeasts, as their number was below the recommended limit (EC Regulation No. 2073, 2005) except for those taken at milking in Hahotoé, which could be explained by poor cleaning of the utensils used The presence of mould indicates that the milk samples were of unsatisfactory quality, as their number was higher than the threshold value according to Regulation (EC) No. 2073/2005. These results can also be explained by poor hygiene both during milking and storage. The utensils used, improperly washed because of their configuration, can also be a factor in the presence of mold in the milk. Study populations are therefore at risk by regularly consuming milks containing yeasts and moulds. A total absence of sulphito-reducing anaerobes in all samples was noted.

The microbiological quality of the milk samples analysed in Kpémé and Hahotoe does not generally comply with the established microbiological standards. Most contamination is mainly the result of poor hygiene in milking, storage and sale.

The presence of germs such as faecal coliforms, *Escherichia coli* in 95 to 99% of cases responsible for food poisoning, can become a public health problem if appropriate measures are not taken to avoid contamination. For its development in Togo, the dairy sector must organize itself with the State to create collection centers with cold chains in order to reduce contamination. The hygienic quality of raw milk at milking and sale can also be improved by the use of the dynamic model of the 6M (Material, Labour, Machine, Method, Medium and Measure).

Author Contributions

Sissowou Mihesso: Investigation, formal analysis, writing original draft. Diantom Agoura: Validation, methodology, writing, reviewing. Melila Mamatchi: Supervision and material contribution.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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