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ORIGINAL ARTICLE

Microbiological Quality of Raw Milk Produced and Marketed in the Town of Moundou , Chad

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Abstract

Failure to comply with hygiene rules during the production and marketing of raw milk can induce contamination by potential pathogenic microorganisms. In this light, a study was carried-out with the objective to evaluate the microbiological quality of raw milk produced and marketed in Moundou, Chad. Standard microbiological analysis techniques were used for the thirty (30) samples collected. The identification and enumeration of the total aerobic mesophilic flora gave a total of 76.70% for an average of $5.9.10^{5}$ CFU/mL. The total coliform load was abnormally high in a proportion of 96.70%. *Escherichia coli, Staphylococcus aureus,* yeasts and molds respectively presented an unsatisfactory rate of 53.30%, 23.30% and 3.30%. *Bacillus cereus* and *Salmonella spp* were absent. From these results, it is clear that the raw milk produced in Moundou presents a generally not satisfactory hygienic quality and represents a real risk for the health of the population. The public authorities, through the Ministry of Livestock, must initiate efforts to create awareness among stakeholders in the sector regarding hygiene measures in order to restore this commodity to its place of choice in the diet.

Practical application

Sensitize milk producers on the danger of poor hygiene on milk quality and the potential health risk that it represents for humans.

Keywords: *Microbiological quality, raw milk, contamination, Moundou, Chad.*

1. Introduction

Chad is a livestock country per excellence, its livestock population has been estimated to about 93.8 million (FAO, 2018). This large-scale breeding is due to the availability of suitable pastures and favorable climate. Milk production, which is the result of breeding, keeps increasing with the fast population growth and varies according to seasons and the availability of pasture (Duteurtre *et al.,* 2000; Koussou *et al.,* 2007). Milk is naturally rich in high biological value proteins, in calcium, vitamins and trace elements. Its nutritional composition makes it to be a good food to meet energy needs, a good food for body building and maintenance. It is also recognized for its therapeutic virtue as it contributes to the protection against some diseases, and maintains bones and help in the prevention of heart diseases, hypertension,

obesity etc, it also contains bioactive compounds with beneficial effects on health (Renard, 2014; CELAGRI, 2020). In Moundou, Chad, the consumption of traditional dairy products such as fresh milk, curdled milk, fermented milk and butter is very popular. They are produced by Fulani herders living in camps on the outskirts of the city with their families and livestock. The production of dairy products is done by women, who carry out the necessary operations from milking to marketing. Each woman seller carries an average of twelve liters of dairy products and it has been estimated that during the high production season which is the rainy season, 5000 liters of milk enter the market and the various points of sale in the city (Duteurtre *et al.,* 2000). Despite its nutritional importance and its attractive character, milk is a highly perishable commodity due to its high water content, its pH close to neutral and its richness in lactose which make it to easily spoil through microbial or enzymatic alteration (Sboui *et al.*, 2009; Kalandi *et al.*, 2015). If the milk is collected under poor hygiene conditions, it can be contaminated and become a threat to consumers (Maïworé *et al.*, 2018; Gondimo *et al.*, 2023). Raw milk marketed in Moundou is milked by uneducated breeders, practicing mechanical milking, in open air at the animal farm without taking measures to avoid contaminations. This study was carried-out to evaluate the microbiological quality of raw milk produced and commercialized in Moundo, Chad.

2. Materials and Methods

2.1. Materials

About 30 raw fresh cow milk samples were collected from six different producers. Plate count agar, violet red bile agar with lactose, tryptone baird parked medium, mannitol egg yolk polymyxin agar and xylose-lysine-deoxycholate agar were used.

2.2. Methods

2.2.1. Study area and sampling

The raw milk samples analyzed in this study were collected from the town of Moundou in Southern Chad. Moundou has a Sudanese climate resulting in high availability of pasture in the rainy season. This is the reason why herders are attracted to this area, and most especially the Fulani and the Arab herders. They set up camps in the city and sale the milk and its products. Thirty (30) raw milk samples, collected from six different producers, were analyzed in this study. Sampling was done in a random manner. These raw milks were collected few minutes after milking in sterile 300 mL volume bottles and labeled. The collected samples were placed in a box with dry ice and then transported to the CECOQDA microbiology laboratory for analysis. During their transportation, they were kept at a temperature of 4°C. The analyses took place 24 hours after sampling, taking into consideration the distance separating the two cities.

2.2.2. Microbiological analyzes

2.2.2.1. Preparation and innoculation

The following methods were used to identify the bacterial strains: NF ISO 4833-1 (2013) for the total aerobic mesophilic flora, NF ISO 4832 (2006) for total coliforms, NF ISO 16649-2 (2001) for *Escherichia coli* ß-glucoronidease posifive, NF ISO 6888-1 (99) / Amd.2 (2018) for coaglulase-positive staphylococci, NF ISO 7932 (2005) for *Bacillus cereus*, NF ISO 21527-1 and 2 (2008) for yeasts and molds and NF ISO 6579-1 (2017) for *Salmonella spp.*

The raw milk samples were homogenized by inversion and 25 g was aseptically weighed, mixed with 225 mL of buffered peptone water. After mixing for two minutes using a blender (Stomacher interscience), the decimal dilutions

were made from 10^{-1} to 10^{-4}. These dilutions were inoculated on agar culture media and incubated (Binder KB53 incubator) at temperatures specific to each germ. The parameters considered were: total aerobic mesophilic flora (TMAT), total coliforms, *Escherichia coli,* Staphylococci, *Bacillus cereus,* yeasts and molds and finally *Salmonella spp.*

The search and enumeration of Total aerobic mesophilic flora (TAMF) were carried out on milk PCA medium (Plate Count Agar) by deep inoculation of 1000 µL of dilutions 10^{-1} to 10^{-4}. The incubation period was 3 days at 30°C. The results of all parameters were red using a colony counter coupled to a magnifying glass (Interscience Scan100). For the enumeration of coliforms, dilutions 10^{-1} to 10^{-3} were inoculated on VRBL (Violet Red Bile Lactose) agar at 37°C for 24 hours. The identification and enumeration of *E. coli* were carried-out on TBX (Bile X-Glucuronide Bile Tryptone) by inoculating dilutions 10^{-1} and 10^{-2} after 24 hours of incubation at 44 ° C. The colonies appearing in blue or blue/green after absorption of the chromogenic agent were considered.

The search for Staphylococci was carried out on Baird Parker medium. The 10^{-1} and 10^{-2} dilutions were inoculated for 24 hours at 37°C. Confirmation of *Staphylococcus aureus* was made by the hydrogen peroxide catalase test (LABELL), Gram staining (bioMérieux reagents) and the coagulase test on human serum. The slides for Gram staining were red using a light microscope (Olympus) at objective 100 with immersion oil. Catalase+ and Gram+ Cocci arranged in clusters or diplococcus were considered.

The search for *Bacillus cereus* in this study was carried out by surface seeding on MYP medium (Mannitol egg Yolk Polymyxin) after 24 hours of incubation at 30 °C.

For yeasts and molds, a 100 µL stock suspension was inoculated on Sabouraud agar for 72 hours at 25°C.

Salmonella constituting the last parameter of this study were searched for in three stages spread over 4 days as follows:

- ✓ The first step, which was pre-enrichment, consisted of mixing 26 g of sample with 125 ml of buffered peptone water. This stock suspension was then incubated at 37°C for 24 hours.
- ✓ The enrichment which was the second step consisted at inoculating the pre-enrichment suspension on two broths: RVS broth (Rappaport-Vassiliadis Soja) and MKTTn broth (Muller - Kauffmann with Tetra Thionate - novobiocin). Thus, 200 µL of the pre-enriched inoculum was inoculated on RVS and incubated at 41.5°C for 24 hours on one side and 1000 µL on MKTTn, at 37°C for 24 hours on the other side.
- ✓ The isolation which was the last step was carried-out simultaneously on XLD (Xylose Lysine Deoxycholate) and Hektoen medium. Using a single-use loop, a drop of culture on RVS was inoculated on XLD and another drop on Hektoen. The same operation was carried-out for the MKTTn inoculum. After 24 hours of incubation at 37°C, colony counting and characterization on an API20E Strep gallery followed.

After the enumeration, the expression of results in colony forming units (CFU) was made using the following formula:

$$N = \frac{\sum c}{\mathrm{VmLx(n1+0,1n2)xd1}}$$

Where,

- N represents the number of CFU per gram or per mL of initial product;
- ∑c: which is the sum of interpretable box colonies;
- V_{mL}: volume of solution (1mL);
- n_1: number of boxes considered at the first dilution;
- n_2: number of boxes considered at the second dilution used;
- d_1: first dilution factor retained

2.2.2.2. Microbiological standards used

The microbiological reference standards used in the interpretation of the results were those of the Luxemburg Food Safety Directorate (DSA/Luxemburg) and are summarized in table 1.

3. Results and discussion

3.1. Results

The microbiological analyzes carried-out during this study focused on the following parameters: TAMF, total coliforms, *E. coli*, Staphylococci, *B. cereus*, yeasts and molds and finally on Salmonella. Table 2 presents the results expressed in CFU/mL.

The analysis of these results shows variable load in TAMF; between $1.6.10^4$ CFU/ mL and 3.10^6 CFU/mL with an average of $5.9.10^5$ CFU/mL. The total coliforms presented a load varying from 10 CFU/mL to $8.8.10^3$. The enumeration of *E. coli* gave a result ranging from 05 CFU/mL to $4.9.10^2$ CFU/mL. The bacterial load of *S. aureus* varied from 22 CFU/mL to $1.5.10^4$ CFU/mL giving an average of $1.1.10^3$ CFU/mL. Yeasts and molds were ranged between 25 CFU/mL and $4.5.10^5$ CFU/mL. The yeast and mold loads representing the maximum were the only one above the normal value. Out of all the samples, *B. cereus* count was lesser than 10^2. The search for *Salmonella spp* was negative in all the analyzed samples, since a total absence of this germ was observed.

3.1.1. The TAMF (Total aerobic mesophilic count)

The TAMF count expressed according to the Luxemburg quality standard gave the distribution presented in figure 1.

According to the standard considered, 76.70% or 23/30 samples presented an unsatisfactory TAMF load compared to a satisfactory proportion of 23.30% or 7/30.

Table 1: microbiological quality criteria for raw milk (Luxemburg, 2018)

Settings	Standards
TAMF	$\leq 5.10^4$
Total coliforms	$\leq 10^2$
E.coli	≤ 10
Staphylococci	$\leq 10^2$
Bacillus cereus	$\leq 10^3$
Yeasts and molds	$\leq 10^4$
Salmonella spp	Absence in 25g of raw milk

Table 2: results of microbiological analyzes

Samples	Total Aerobic Mesophilic Flora	Total coliform	*Escherichia coli*	Staphylococci	*Bacillus cereus*	yeasts and molds	*Salmonella spp .*
01	$5.4.10^{5}$	$3.2.10^{2}$	08	8.10^{2}	80	5.10^{2}	Absent
02	$1.9.10^{6}$	10	06	60	90	85	Absent
03	$1.2.10^{5}$	$5.3.10^{2}$	20	35	75	90	Absent
04	3.10^{4}	$5.5.10^{2}$	05	40	86	72	Absent
05	$6.2.10^{4}$	$4.4.10^{2}$	50	5.10^{2}	20	5.10^{2}	Absent
06	$4.4.10^{4}$	$1.1.10^{3}$	09	46	78	6.10^{2}	Absent
07	$3.1.10^{4}$	$4.2.10^{2}$	25	50	95	60	Absent
08	2.10^{5}	$1.7.10^{3}$	07	72	80	48	Absent
09	3.10^{6}	$8.8.10^{3}$	10	9.10^{3}	30	78	Absent
10	$9.4.10^{4}$	$5.7.10^{3}$	09	56	70	95	Absent
11	$1.1.10^{5}$	$5.5.10^{3}$	$1.7.10^{2}$	$1.3.10^{3}$	98	$4.5.10^{5}$	Absent
12	$2.1.10^{5}$	$4.6.10^{2}$	20	35	15	55	Absent
13	4.10^{4}	$7.6.10^{2}$	$4.9.10^{2}$	29	74	87	Absent
14	3.10^{6}	$5.3.10^{2}$	39	22	89	98	Absent
15	$1.6.10^{4}$	$5.1.10^{2}$	50	75	99	64	Absent
16	$7.1.10^{4}$	$1.4.10^{3}$	26	44	78	25	Absent
17	$1.4.10^{6}$	$6.5.10^{2}$	07	28	81	93	Absent
18	$9.3.10^{5}$	$6.8.10^{3}$	70	61	67	88	Absent
19	$6.6.10^{5}$	$1.1.10^{3}$	09	99	89	65	Absent
20	$9.1.10^{4}$	$3.8.10^{3}$	07	65	73	90	Absent
21	$1.2.10^{5}$	$2.3.10^{2}$	10	91	60	99	Absent
22	$3.4.10^{5}$	$2.3.10^{2}$	05	65	34	70	Absent
23	3.10^{6}	$5.4.10^{2}$	06	28	88	94	Absent
24	$2.3.10^{5}$	7.10^{3}	09	98	88	65	Absent
25	$5.7.10^{5}$	$2.3.10^{3}$	$1.7.10^{2}$	60	77	48	Absent
26	$1.7.10^{4}$	$6.1.10^{2}$	80	34	69	99	Absent
27	$5.7.10^{5}$	$1.1.10^{3}$	08	$6.7.10^{3}$	90	87	Absent
28	$3.5.10^{4}$	$2.8.10^{3}$	38	$1.5.10^{5}$	65	75	Absent
29	7.1.104	$5.2.10^{3}$	05	10^{4}	62	86	Absent
30	$3.2.10^{5}$	$1.9.10^{3}$	$1.8.10^{2}$	96	89	90	Absent

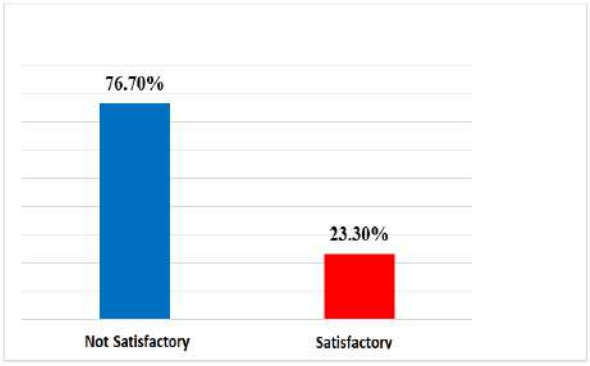

Figure 1: microbiological quality according to TAMF

According to the standard considered, 76.70% or 23/30 samples presented a not satisfactory TAMF load compared to a satisfactory proportion of 23.30% or 7/30.

3.1.2. Total coliform

The total coliform content was compared to the standard for quality assessment. The resulting observations are presented in figure 2.

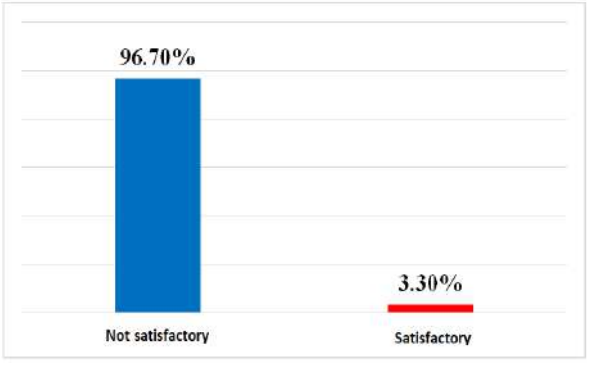

Figure 2: microbiological quality based on total coliforms

Total coliforms had a not satisfactory load of 96.70% compared to only 3.30% quality satisfaction.

3.1.3. Escherichia coli

The quality as a function of *E. coli* gave the distribution presented in figure 3. The content of this bacillus compromised the quality in 53.30% of cases and was satisfactory in 46.70% of cases.

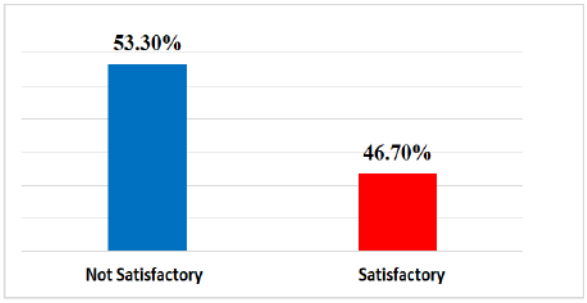

Figure 3: quality based on *E. coli*

3.1.4. Staphylococcus aureus

The different *S. aureus loads* of milk samples presented in Table 2 were used to obtain the distribution presented in figure 4. 23.30% of samples presented an abnormally high load of staphylococci compared to 76.70% having a normal load.

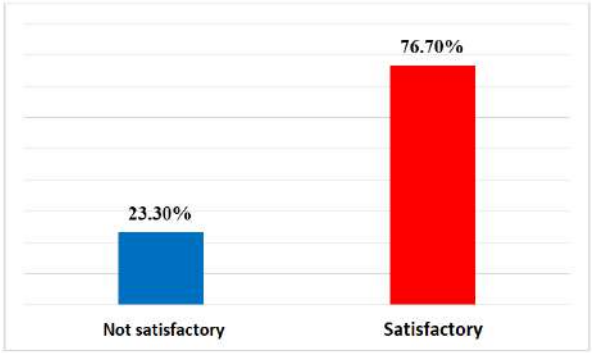

Figure 4: quality according to *S. aureus*

3.1.5. Yeasts and molds

The expression of yeast and mold loads as a function of normal values helped to obtain the distribution presented in figure 5.

A proportion of 3.30% or 1/30 of samples had a not satisfactory charge compared to 96.70%, i.e. 29/30 samples were satisfactory.

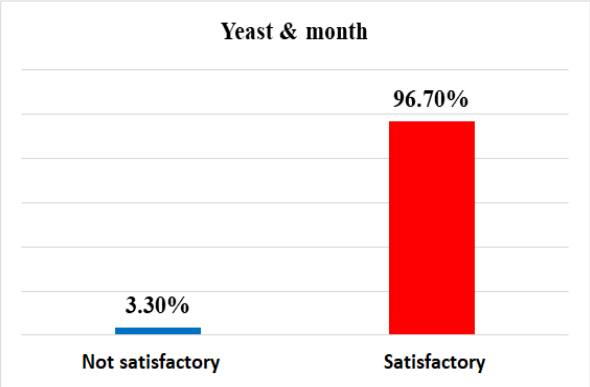

Figure 5: quality depending on yeasts and molds

3.2. Discussion

The result of the TAMF enumeration showed a dissatisfaction of 76.70% with the quality according to the standard (N <5.10 ^{4} CFU/ mL) for an average load of 5.9.10 ^{5} CFU/ mL. This is a parameter defining the hygienic quality of food and sign of exogenous contamination. These results are in line with those obtained by Gondimo *et al.* (2023) in Pala, Chad as well as those of Maïworé *et al.* (2018) in Maroua, Cameroon. The first authors found 70% dissatisfaction with their raw milk; the second in ordinary and improved milking conditions found respectively 90 and 70% dissatisfaction in TAMF. In contrast to our observations, the work of Koussou *et al.* (2007) in the city of N'Djamena revealed a total dissatisfaction in TAMF despite the season considered. The difference between these two results could be explained by the method used to collect samples or the analysis technique used. In this study, the samples were collected from breeders' camps just after milking, but in their case, they were collected from artisanal producers' tanks. It can also be suspected that this strong contamination of milk by germs is linked to the illiteracy of breeders and their very low level of technicality. Apart from sample 2, all the other samples analyzed had an abnormally higher load of coliforms, representing a dissatisfaction of 96.70% according to the standard (N < 10 ^{2}). These results are similar to those of Elhadj *et al.* (2015) in Algeria who found only a single sample meeting the standard and to those of Kouamé-sina *et al.* (2010) in Ivory coast who observed a prevalence of 94.1%, i.e. a single sample also not meeting the quality standard. In this same order, the results of the work carried out by Maïworé *et al.* (2018) showed an abnormal proportion of total coliforms amounting to 90%. Indeed, the poor quality of raw milk in Chad has been reported by other authors (Koussou *et al.,* 2007). Knowing that the sector is ran by breeders who are not educated and above all resistant to education, this could be a factor exposing the milk to several sources of direct or indirect contaminations. Unwashed udders, milkers' hands, utensils and the unsanitary milking environment could explain the results observed (Koussou, 2008; Kouamé-sina *et al.,* 2010; Gondimo *et al.,* 2023). The abnormal prevalence of *E. coli* stood at a proportion of 53.30%. This result is higher than that of our previous work carried out in Pala during which a dissatisfaction of 30% was observed and rather lower than those of Maïworé *et al.* (2018) *in* ordinary trafficking conditions. In contrast to our results, Aggad *et al.* (2009) in Morocco observed a normal *E. coli* load in all samples. On the other hand, Kouamé-sina *et al.* (2010) in Abidjan detected a prevalence of 70.50% in mixed raw milk. This observation was made with the result of the total coliform load reflecting very poor hygiene measures causing exogenous microbial contamination. Milking is done in the open air; utensils washed in exposed water, sometimes water from a pond, hands of

milk extractors are often dirty. These might be the factors causing contamination (Maïworé *et al.,* 2018). Furthermore, one could think that the bacterial load is reduced by the fact that milk harvest depends on the calf's feeding since the first streams of milk are generally very contaminated. It should also be noted that some authors reported a seasonal effect linked to strong microbial contamination and the season in question is the summer during which this study was carried out (El Marnissi *et al.,* 2013). The quality assessment with regard to *S. aureus* showed a proportion of dissatisfaction of 23.30% with an average of $1.1.10^{3}$ CFU/mL. This result is similar to those observed by Kouamé-sina *et al.* (2010) in Ivory Coast who detected a rate of 20% in mixed milk. On the other hand, it was lower than the result of our previous work (Gondimo *et al.,* 2023) and that of Aggad *et al.* (2009) which were respectively 83.3% and 54.28%. It has been reported that *S. aureus* contaminating raw milk comes mainly from the water used to rinse utensils, from the hands of milkers and finally from the udder especially in cases of mastitis (Kouamé-sina *et al.*, 2010; Markhous *et al.,* 2019). The mechanical milking carried-out in the study area, the wind that circulates there carrying dust and the unsanitary work environment due to dung littering on the floor favor such contamination by *S. aureus*. For *Salmonella spp*, results showed a total absence in all samples analyzed during this study. This result corroborates those of Gondimo *et al.* (2023) in Pala, Chad, Kouamé-sina (2010) in Abidjan, Ivory Coast, El marnissi *et al.* (2013) in Morocco and finally Matallah *et al.* (2021) in Algeria. The hypothesis that could be formulated to explain this observation is that the high acidity of milk would have neutralized these pathogenic germs. Analyses carried-out in parallel on these same samples showed a high content of titratable acidity, which suggests an intense activity of lactic acid bacteria. However, a study carried-out by Doutoum *et al.* (2013) on raw milk in Chad revealed a high load of lactic acid bacteria which could inhibit microbial activity amongst which possible pathogenic germs. As for yeasts & molds, dissatisfaction of only 3.30% was observed, i.e. only one sample did not meet the standard. Yeasts and molds are rather germs sought by the dairy industry for aromas and taste. However, they are spoilage germs. The proportion observed in this work was lower than that found by Gondimo *et al.* (2023) in Pala, Chad who observed a dissatisfaction rate of 7%.

5. Conclusion

The present study aimed at evaluating the microbiological qualities of raw milk produced and marketed in the city of Moundou. Microbiological analyzes revealed that raw milks sold to Moundou are contaminated with total coliforms. Almost half of the samples analyzed contained fecal coliforms. The *Salmonella Spp* and *Bacillus cereus* for their part were not present in the samples analyzed. The quality of the milk in general was considered to be poor and risky for consumers. It would therefore be wise to improve in the microbiological quality of raw milks for the health and safety of consumers. Public authorities, through the Ministry of Livestock should initiate efforts to create awareness among stakeholders in the sector regarding hygiene measures in order to restore this commodity to its place of choice in the diet.

Conflict of interest

The authors declare that they have no competing interests.

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breeders for cooperating. They also express their gratitude to the staff of the microbiology laboratory for their collaboration.

Ethics

This Study does not involve Human or Animal Testing.

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