Journal of Advances in Microbiology

Volume 24, Issue 10, Page 76-85, 2024; Article no.JAMB.124804
ISSN: 2456-7116

Microbiological Quality of Raw Cow's Milk Produced and Marketed System in the Town of Bongor, Chad

Gondimo Gabdibé Élysée [a], Abdelsalam Adoum Doutoum [b], Alhadj Markhous Nazal [c*], Kemoral Aristide [a], Kouyahbé Fadeunbo [d], Abdelsalam Tidjani [a] and Njintang Yanou Nicolas [e]

[a] Food Science and Nutrition Research Laboratory, Faculty of Human Health Sciences, University of N'Djamena, Chad.
[b] Faculty of Human Health Sciences, Adam Barka University of Abéché, Abéché, Chad.
[c] Faculty of Agricultural and Environmental Sciences, University of Sarh, Sarh, Chad.
[d] Faculty of Letters, Arts and Humanities, University of N'Djaména, Chad.
[e] Faculty of Science, University of Ngaoundéré, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Author GGE conducted the fieldwork with technical and logistical support from authors KA, AAD and AMN directed the progress of the work and its implementation. Author GGE then initiated the writing of the typescript in collaboration with authors KF, AT and NYN proofread and supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jamb/2024/v24i10859

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124804

Original Research Article

Received: 04/08/2024
Accepted: 06/10/2024
Published: 14/10/2024

**Corresponding author: E-mail: markhous2000@yahoo.fr;*

Cite as: *Élysée, Gondimo Gabdibé, Abdelsalam Adoum Doutoum, Alhadj Markhous Nazal, Kemoral Aristide, Kouyahbé Fadeunbo, Abdelsalam Tidjani, and Njintang Yanou Nicolas. 2024. "Microbiological Quality of Raw Cow's Milk Produced and Marketed System in the Town of Bongor, Chad". Journal of Advances in Microbiology 24 (10):76-85. https://doi.org/10.9734/jamb/2024/v24i10859.*

ABSTRACT

Background: Raw cow's Milk, intended for human consumption, is the complete product of the total and uninterrupted Milking of a healthy, well-fed and not overworked dairy cow. However, this raw Milk, even when Milked under adequate hygienic conditions, contains micro-organisms and spoils at room temperature.
Aim: The aim of this study was to assess the sanitary quality of raw Milk produced and marketed in the town of Bongor in Chad.
Methodology: The study was carried out in Bongor, Province of Mayo-Kebbi-Est (Chad). It focused on 30 samples of raw Milk analyzed using the standard microbiology method for counting germs and expressing quality.
Results: Total dissatisfaction was determined in our samples for TAMF, Total Coliforms and *Escherichia coli β-glucuronidase*. The bacterial load of *Staphylococcus aureus* was abnormally high in 80% of cases, 40% of samples were contaminated with Yeasts and Molds, and 23.30% of RSAs were found to compromise quality. *Salmonella spp*, which were absent from our cultures, and *Bacillus cereus*, whose count did not exceed the permitted legal standard, were the only parameters that did not compromise the food quality of the product.
Conclusion: In view of these observations, consumers would be exposed to a real danger to their health if nothing is done to improve the microbiological quality of raw Milk.

Keywords: Raw milk; sanitary quality; pathogens; bongor; Chad.

1. INTRODUCTION

Raw Milk, intended for human consumption, is the entire product of the complete and uninterrupted Milking of a healthy, well-nourished and not overworked dairy cow; it must be collected cleanly and contain no colostrum [1,2]. It is a highly nutritious food, indispensable in the human diet and, thanks to the complexity of its composition, contributes to the dietary balance of populations [3].

Despite the oil boom, Chad remains an Agropastoral country where Milk is a major part of the population's diet [4]. The dairy industry is run mainly by three main ethnic groups of herders: Arabs, Peuhls and Toubous. These nomadic herders' practices archaic livestock rearing without supplementation, and their mobility follows the availability of pasture [5]. This variation in the availability of pasture means that Milk is very plentiful in the rainy season, in contrast to the dry season [2,6].

The production of raw Milk from Milking and its by-products is a chore carried out by women to meet their domestic needs on the one hand, and for commercial purposes on the other [2,6]. These activities are carried out in the traditional way, and all dairy products are marketed completely informally, without any form of quality control [4]. Raw Milk, even when processed under hygienic conditions, contains micro-organisms and spoils at room temperature [7,8]. This natural predisposition to spoilage and to remove germs could be exacerbated among transhumant livestock farmers in Chad, who use traditional methods of Milking, transporting and selling Milk without regard for conventional hygiene conditions and with little concern for consumer health [4,6]. In the light of these factors, this study aims to assess the extent of microbial contamination of raw cow's Milk produced and sold in Bongor, Chad.

2. MATERIALS AND METHODS

2.1 Study Framework

The study was carried out in Bongor, Province of Mayo-Kebbi-Est (Chad). The town of Bongor is located in the southern zone of south-western Chad, between the 9th and 11th degrees of north latitude and the 14th and 16th degrees of west longitude, at an average altitude of 328m. It borders Cameroon on the west and has a Sahelo-Sudanese climate overall. It is one of the country's most densely populated cities, with a density of 40 inhabitants per km² and a population of 553,027 according to the second General Population and Housing Census (RGPH2) [9,10].

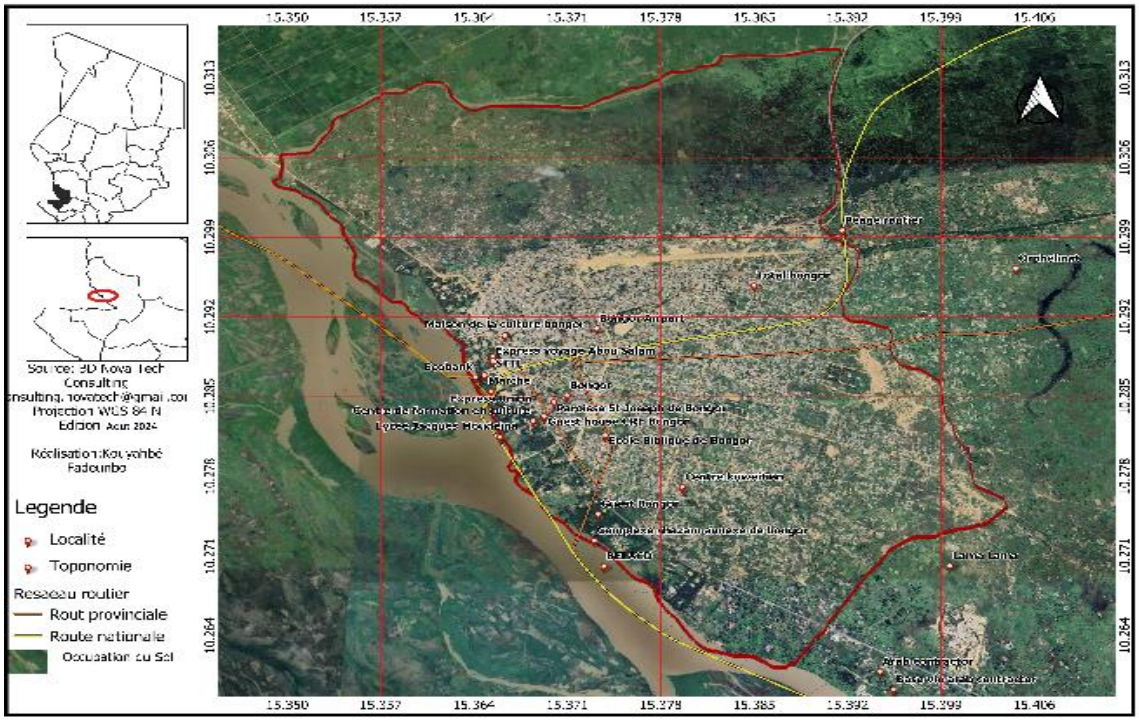

Fig. 1. Map of the town of Bongor (Source: Kouyahbé Fadeunbo)

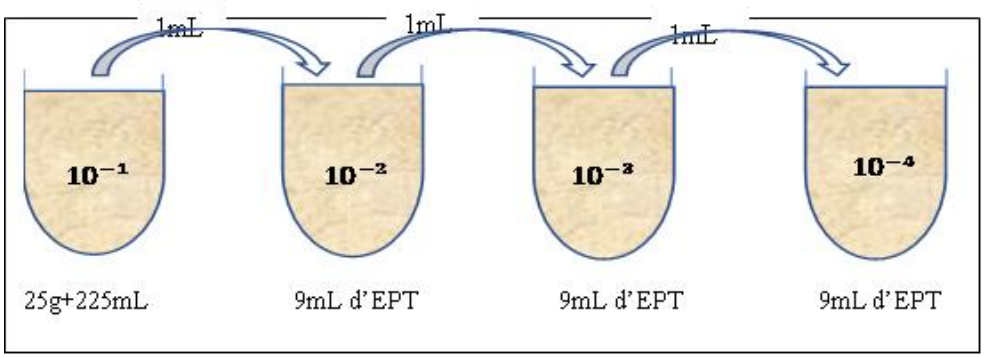

Fig. 2. Preparation of different dilutions

2.2 Study Concept

The This was a cross-sectional analytical study conducted between July and September 2024. The study was carried out in two phases: a field phase to collect samples and a laboratory analysis phase.

2.3 Sample Collection and Conditioning

Our study involved thirty (30) samples of raw cow's Milk. These samples were taken in sterile 300 ml bottles, a few minutes after manual Milking, from farmers camped with their cattle on the outskirts of the town of Bongor. The samples collected were immediately recapped, labelled and placed in a cool box fitted with ice accumulators at a temperature of 4°C. They were then sent to the laboratory of the Food Quality Control Center in N'Djamena for microbiological analysis. Because of the distance between the two towns, the analyses were carried out 24 hours after sampling. Several microbiological parameters were investigated and enumerated in this study: Total Aerobic Mesophilic Flora (TAMF), Total Coliforms, *Escherichia coli, Staphylococcus aureus, sulphite-reducing anaerobes* (SRA), *Bacillus*

cereus, Yeasts and Molds and *Salmonella spp*.

2.4 Microbiological Analyses

For each sample, 25 g of raw Milk to be analyzed was added to 225 mL of buffered peptone water in a labelled sterile bag. This gave a stock solution of 10^{-1} from which decimal dilutions down to 10^{-4} were made.

Total aerobic mesophilic flora (TAMF) were counted on Plate Count Agar (PCA) medium after 72 Hours incubation at 30°C in accordance with standard NF ISO 4833-1. To assess hygiene, dilutions 10^{-1} to 10^{-3} were inoculated onto VRBL (Violet Red Bile Lactose) medium at 37°C for 24 hours to count total coliforms (NF ISO 4832). *Escherichia coli* β-glucuronidase was detected on TBX (Typtone Bile X-glucuronide) medium at 44°C in accordance with NF ISO 16649-2. After 24 Hours incubation, all blue-green colonies were considered. Baird Parker medium was used to enumerate *Staphylococcus aureus* after 18 to 24 Hours incubation at 37°C in accordance with standard NF ISO 6888-1. To confirm the species, Gram staining and a hydrogen peroxide catalase test were performed. Dilutions of 10^{-1} and 10^{-2} were inoculated onto MYP (Mannitol egg Yolk Polymixine) medium to test for Bacillus cereus. The incubation period was 24 hours at 30°C (NF ISO 7932). For the detection and enumeration of sulphite-reducing anaerobes (SRA), the inoculum was seeded in depth on TSN medium (Tryptone Sulfite Neomycin). These media were incubated at 46°C in an anaerobic jar with a candle that was extinguished in the absence of oxygen. On reading, the characteristic black colonies were considered (NF ISO 15213). The OGA (Oxytetracycline Glucose Agar) medium, inoculated with 10^{-1} and 10^{-2} dilutions for 72 hours at 25°C, was used to investigate yeasts and moulds in accordance with standard NF ISO 21527-1. Finally, standard NF ISO 6579-1 was used for Salmonella testing. Pre-enrichment consisted of incubating the stock solution at 37°C for 24 Hours; then 1000 µL of the pre-enrichment inoculum was inoculated onto MKTTn (Muller Koffmann Tetra Thionate novobiocyne) broth, incubated at the same temperature, and 100 µL of the inoculum was inoculated onto RVS (Rappaport-Vassiliadis) broth for 24 hours at 41.5°C. After this time, two agars were used together for selective identification: Hektoen agar and XLD (Xylose Lysine Deoxycholate) agar. Black colonies grown on the previous agars were subcultured onto TSA (Tryptone Soy Agar) medium at 37°C for 24 hours. The API 20E Gallery (Biomérieux tool) was inoculated with strains grown on this medium and identified using apiwebTM software after entering the seven (7) digit numerical code on the keyboard.

The results of the enumeration of the various colonies of the germs sought were then expressed in colony-forming units per ml or per g (CFU/ml).

2.5 Quality Standards Used

To assess the microbiological quality of Milk, the results of our analyses are expressed in terms of the reference standards of the Luxembourg Food Safety Directorate (DSA/Luxembourg). These standards are listed in the Table 1.

2.6 Statistical Analyzes of Data

The microbiological analysis data were entered into Excel 2016; this workbook was also used to design the graphs. Finally, SPSS software version 26.0 was used to express statistical means and extremes.

Table 1. Microbiological quality criteria for raw milk [11]

Microbiological parameters	Required standards
Total Aerobic Mesophilic Flora	≤ 5.10^4
Total Coliforms	≤ 10^2
Escherichia coli	≤ 10
Staphylococcus aureus	≤ 10^2
Sulphite-Reducing Anaerobes	≤ 10^2
Bacillus cereus	≤ 10^2
Yeasts and Molds	≤ 10^4
Salmonella spp	Absence in 25 g of Milk

3. RESULTS AND DISCUSSION

3.1 Average Loads and Extreme Values for the Germs Tested

Table 2 summaries the mean and extreme values (maximum and minimum) for each parameter tested. The table shows that all of the samples were contaminated with TAMF, with an average of 3.10^6 CFU/ml; this high level of contamination means that the average is equal to the extremes for this parameter.

The average total coliform content was $1.2.10^5$ CFU/ml for a bacterial load ranging from $2.4.\ 10^3$ CFU/ml to $1.5.\ 10^5$ CFU/ml. The average *E. coli* content was $1.3.\ 10^4$ CFU/ml and a minimum of $2.1.\ 10^3$ CFU/ml, indicating a high level of contamination by this pathogenic germ. Of the 30 samples analyzed, the *Staphylococcus aureus* load ranged from 10^2 to $1.5.\ 10^5$ CFU/ml, giving an average of $9.5.\ 10^4$ CFU/ml, well above the standard (N≤ 102). In all the samples analyzed, *Bacillus cereus* presented an acceptable load, as the maximum and limit loads used to assess quality converged; the same observation was made for Salmonella, which did not grow in our cultures (the numerical codes from the gallery did not correspond to the Salmonella strains). Yeasts and Molds showed extreme loads ranging from $4,10^1$ CFU/ml to $9.7.\ 10^4$ CFU/ml and an average of $1.3.\ 10^4$ CFU/ml. Finally, the SRA showed an average contamination of $0.4.\ 10^2$ CFU/ml with a maximum of $3.3.\ 10^2$ CFU/ml and a minimum of $0.1.10^2$ CFU/ml.

3.2 Prevalence of TAMF

The germs that make up TAMF were highly contaminated in our cultures for all the samples analyzed; as a result, total dissatisfaction with the quality with regard to the legal assessment standard was observed (Fig. 3).

3.3 Prevalence of Total Coliforms

The evaluation of the hygienic quality with regard to this parameter gave rise to the breakdown according to which all the samples analyzed (100%) were unsanitary and therefore unfit for human consumption. This is illustrated in the graph below (Fig. 4).

Table 2. Averages and extremes of microbiological parameters (CFU/ml)

Parameter	TAMF	TC	*E. coli*	*S. aureus*	SRA	*B. cereus*	L.M.	*Salmonella spp*
Average	3.10^6	$1,2.10^5$	$1,3.10^4$	$9,5.10^4$	$0,4.10^2$	$0,92.10^2$	$1,3.10^4$	00
Minimum	3.10^6	$2,4.10^3$	$2,1.10^3$	10^2	$0,1.10^2$	10	4.10^1	00
Maximum	3.10^6	$1,5.10^5$	$1,5.10^4$	$1,5.10^5$	$3,3.10^2$	10^2	$9,7.10^4$	00

TAMF = Total Aerobic Mesophilic Flora TC = Total Coliforms E. coli = Escherichia coli
S. aureus = Staphylococcus aureus SRA = Sulphite-Reducing Anaerobes;
B. cereus = Bacillus cereus L.M. = Yeasts and Molds

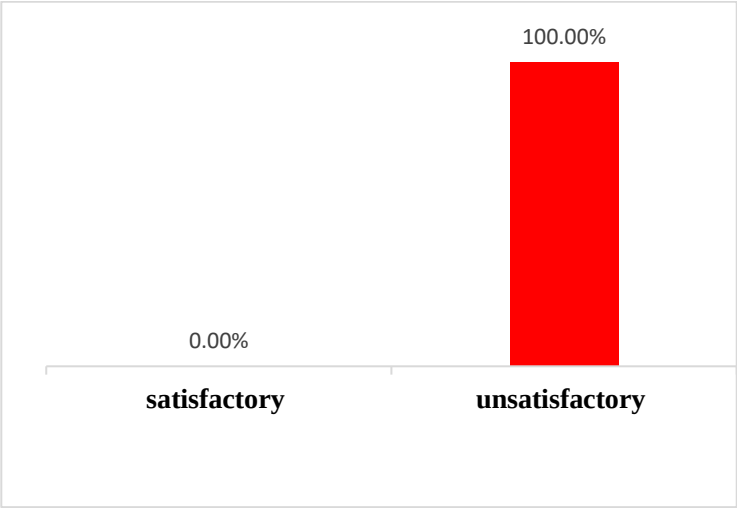

Fig. 3. Compliance rate of raw Milk in relation to TAMF

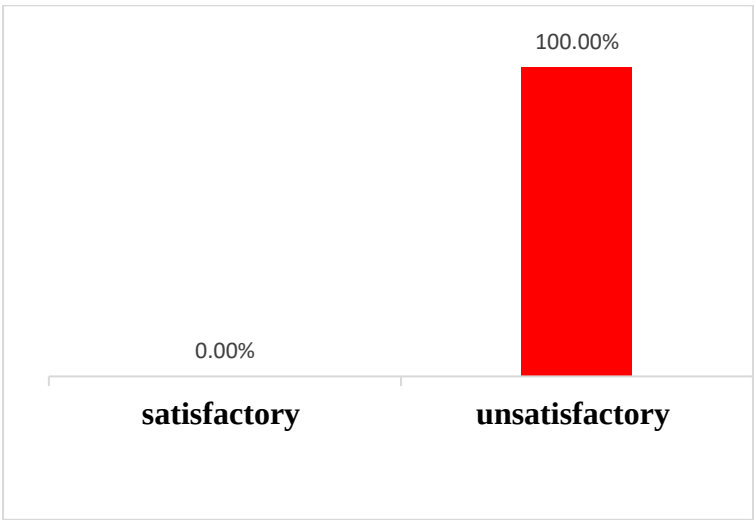

Fig. 4. Compliance rate of raw milk with respect to total coliforms

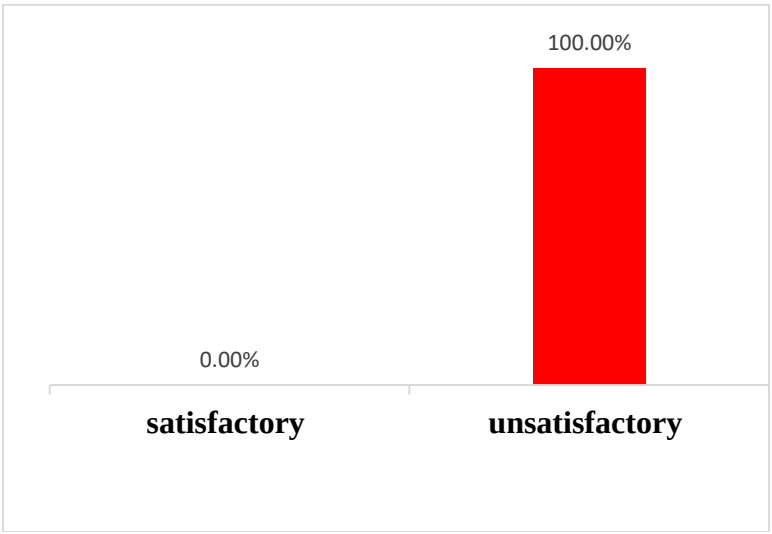

Fig. 5. *Escherichia coli* compliance rate for raw milk

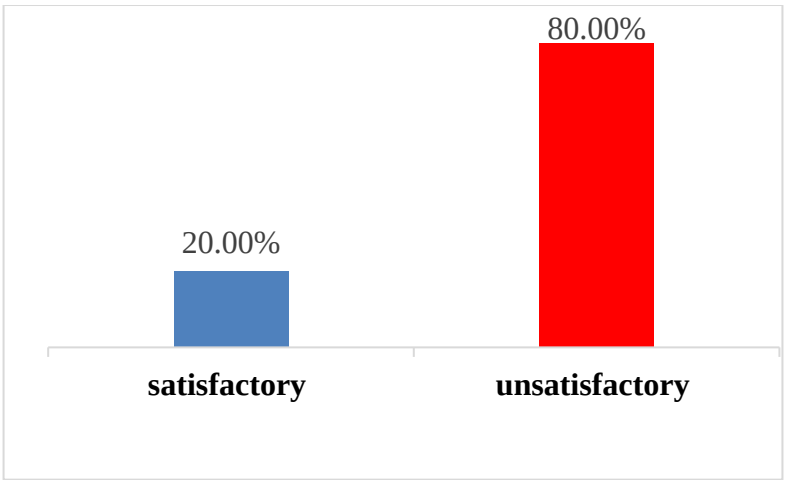

Fig. 6. Compliance rate of raw Milk with respect to *Staphylococcus aureus*

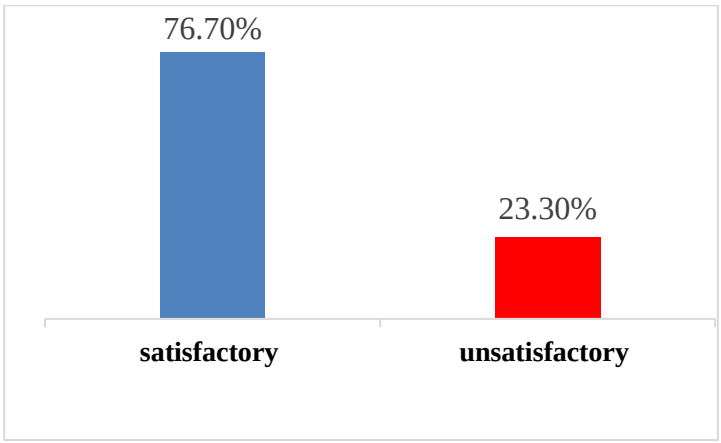

Fig. 7. Compliance rate for raw Milk in relation to SRA

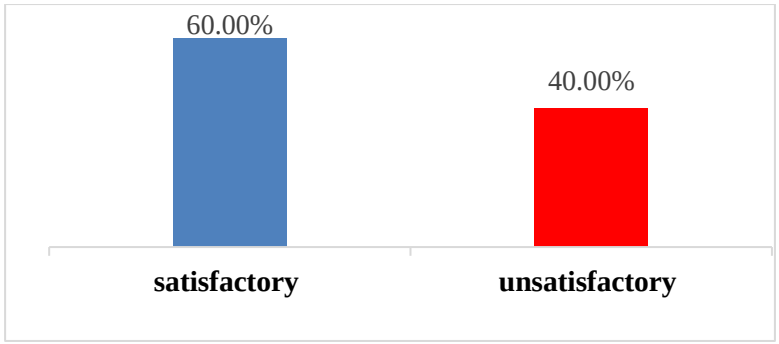

Fig. 8. Raw Milk compliance rate for yeasts and molds

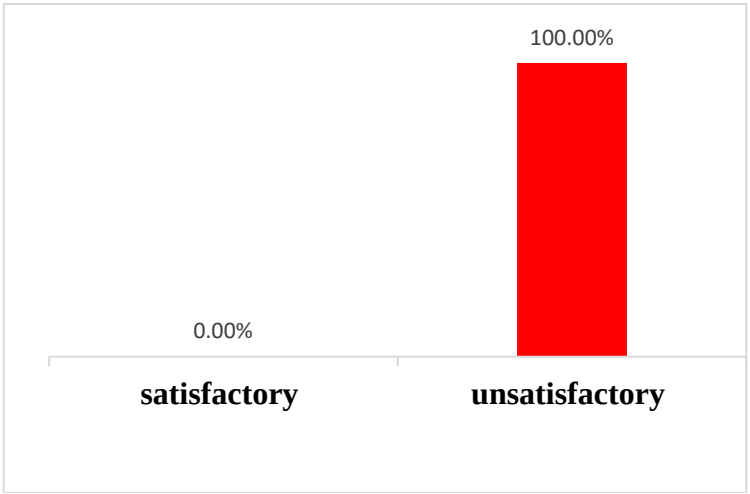

Fig. 9. Raw Milk compliance rate with regard to *Salmonella spp.*

3.4 Prevalence of *Escherichia coli*

Indicative of fecal Contamination and therefore of poor hygiene, the bacterial load of *Escherichia coli* β-glucuronidase was abnormally high compared with the legal standard (N≤ 10) for all the raw Milk considered in this study.

3.5 Prevalence of *Staphylococcus aureus*

Investigations into this bacterium revealed 80% (24/30) of dissatisfaction with quality compared with only 20% of satisfaction, i.e. 6/30 samples suitable for human consumption.

3.6 Prevalence of Sulphite-Reducing Anaerobes

By expressing the different SRA bacterial loads observed in our cultures as a function of the standard (N≤ 10^2), we came to the conclusion that 76.7% of the samples were compliant compared with 23.3% non-compliant.

3.7 Prevalence of Yeasts and Molds

Fig. 8 shows Yeast and Mold contamination at a rate of 40%, i.e. 12/30 samples unfit for human consumption; the other 60% were satisfactory, i.e. did not contain flora suggesting contamination.

3.8 Prevalence of *Salmonella spp*

The results of the test for *Salmonella spp* in the raw Milk samples showed that all the samples were free of these pathogenic germs.

3.9 Discussion

TAMF is an indicator of overall microbiological quality and also reflects the state of freshness or deterioration of raw Milk. Our results show an average of 3. 10^6 CFU/ml for all the samples contaminated by this flora. The same observation was made in Algeria by [12]. However, other researchers also assessing the quality of raw Milk obtained a lower contamination rate than ours; [2] in Abéché reported 46.66%, [13,6] in Pala and Moundou obtained 20% and 76.70% respectively. This high level of TAMF contamination is thought to be the result of poor hygiene practices by farmers on their farms.

Assessment of bacteriological quality in terms of fecal contamination markers revealed total dissatisfaction, i.e. 100% of samples were unfit for human consumption, for both total coliforms and *E. coli*. This result is similar to that observed in Moundou in Chad by [6]. In the same country, however, [2] in Abéché obtained only 46.66% dissatisfaction; [14] reported 83.33% contamination in Togo in their work on the nutritional and hygienic quality of raw cow's Milk. This high level of coliform contamination of raw Milk could be explained by the neglect of hygiene measures, the failure to wash hands and udders before Milking, and the use of Milk collection utensils in pens strewn with dung, all in the open air and at room temperature [15]. The carelessness of farmers and, above all, their illiteracy could be a determining factor [16,4]. Consumption of this raw Milk could constitute a real danger for the consumer, especially if it is an enterohaemorrhagic strain of *E. coli*, particularly the best-known serotype O157:H7 [8].

Of the 30 samples analyzed, a total absence of Salmonella was observed. These results are similar to those of other authors in Chad who also did not detect Salmonella in their samples during their work. This was the case for [4] in N'Djamena, [2] in Abéché and finally [6] in the town of Moundou. However, in Maroua, Cameroon, [15] reported 20% Salmonella contamination under normal Milking conditions. The absence of *Salmonella* in raw Milk in Chad could be linked to the activity of lactic acid bacteria, which are inhibitory to pathogens through competition or bacteriocin production, and also to the fact that *Salmonella* are lactose negative [17,4].

As a pathogenic germ, the average *Staphylococcus aureus* count was 9.5. 10^4 CFU/ml for a contamination rate of 80%, i.e. 24/30 samples unfit for consumption with regard to this parameter. Our results are in line with those reported by [13] in Pala who found 83.33% and also with those of [18] who, assessing the bacteriological and sanitary quality of raw bovine Milk in Algeria, reported 80.1% contamination by *Staphylococcus aureus*. In contrast, and especially below the contamination rate reported in our study, [15] obtained a dissatisfaction rate of 70% under normal Milking conditions, [2] reported only 20% non-conformity and in Togo, [14] did not detect any load above the accepted standard. The hypotheses that could be formulated to explain Staphylococcus contamination include manual Milking, which exposes the Milk to the sometimes unwashed hands of the Milker, the Milking environment, which is the herd enclosure itself, the utensils used, which were previously washed with water from the marsh, and all the maneuvering that took place in the open air in the field environment. Furthermore, staphylococcal mastitis in dairy cows could predispose the Milk to contamination by this bacterial agent [15,18].

Sulphite-reducing anaerobes were detected at an unsatisfactory rate of 23.30%, with 7 contaminated samples. This indicates the relative health of dairy cows, which may have carried infections linked to these germs. Lower results than ours were obtained in Algeria by [18], who reported a contamination rate of 12.5%, and by

[19] in the same country, who did not detect Clostridium in their cultures.

The average yeast and mold count was 1.3 .10^4 CFU/ml, with a contamination rate of 40%. These are useful germs in the dairy industry if they are contained in the Milk in a reasonable proportion. However, abnormally high levels can cause spoilage of the Milk or even its by-products, leading to defects in taste or appearance [20,17].

4. CONCLUSION

This study assessed the hygienic quality of raw cow's Milk produced and consumed in the city of Bongor. Apart from *Salmonella spp,* which were completely absent, and *B. cereus*, which showed no abnormal bacterial load, all the samples involved were contaminated with TAMF, Total Coliforms, *E. coli*, *Staphylococcus aureus*, Yeasts and Molds and SRA. These results make raw Milk unsuitable for human consumption, deprive it of its expected safety and reduce its potential for industrialization. The degree of contamination detected in this study reflects a relative lack of hygiene. This needs to be improved by introducing good hygiene practices throughout the production chain in order to protect the consumer.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The authors sincerely thank the administration of Food Quality Control Center for allowing the research work to be carried out in their structure. They express their deep gratitude to the staff of the microbiology laboratory for their technical support and frank collaboration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Villain AC. What is milk? French Journal of Allergology. 2010;50:124–127.
2. Doutoum AA, ERBI D, Al cherif HM, Hingue NV, Hamadou A, Nazal AM, Doutoum SAB, Tidjani A. Evaluation of the hygienic and microbiological quality of raw milk from cows intended for consumption or processing in the Town of Abéche in Chad. Food Science Nutr Res. 2023; 6(2):1-9.
3. Agbo GM, Dokui F, Dedome SL, Salifou CFA, Toleba SS, Tchobo FP. Physical and nutritional quality of milk from Borgou cows fed with salt licks developed in Benin. Rev. Mar. Sci. Agron Vet. 2023;11(3):346-350.
4. Bechir M, Tidjani A, Makhlouf H, Abderahim I, Schelling E. Food safety of camel milk sold in N'Djamena, Chad. Scientific Review of Chad. 2016;97-116.
5. Koussou MO. Dynamics of changes in the livestock sector in Chad: The case of the dairy sector in N'Djamena. PhD thesis, Agro Paris Tech, Paris, 2008;242.
6. Gondimo GE, Doutoum AA, Nazal AM, Abdallah HO, Hamadou A, Djamalladine MD, Tidjani A. Microbiological quality of raw milk produced and marketed in the Town of Moundou, Chad. J. Food. Stab. 2023;6(5):27-37.
DOI: 10.36400/J.Food.Stab.6.5.2023-053.
7. AFSCA. Information brochure for consumers; 2015.
8. Silveira A, Pinheiro CJ, Loh LC, Benusic M. Public health risks of raw milk consumption: lessons from a case of pediatric hemolytic uremic syndrome. Canada Communicable Disease Report. 2023;49(9):413–7.
Available:https://doi.org/10.14745/ccdr.v49i09a03f
9. Gouataine RS, Baohoutou L. Highlighting rainfall variability on the Mayo-Kebbi plain, southwest Chad. Rev. Ivoir. Sci. Technol. 2015;25:93–109.
10. Gouataine RS, Dadoum MD, Doubragne I. Irrigated rice cultivation and socio-economic changes in the Logone Valley in Bongor (Chad). Canadian Journal of Tropical Geography. 2021;8(2):33-38.
11. The Government of the Grand Duchy of Luxembourg. Microbiological criteria for foodstuffs, guidelines for interpretation; 2018.
12. Hamiroune M, Berber A, Boubekeur S. Bacteriological quality of raw milk from local and improved cows sold in the regions of Jijel and Blida (Algeria) and impact on public health. Ann. Méd. Vét. 2014;158:137-144.

13. Gondimo GE, Doutoum AA, Abdullahi HO, Djamalladine MD, Valery BM, Tidjani A. Microbiological and physicochemical quality of raw milk marketed in the City of Pala in Chad. Journal of Food Studies. 2023;12(1):13. DOI:https://doi.org/10.5296/jfs.v12i1.21103
14. Seme K, Pitala W, Osseyi GE. Nutritional and hygienic quality of raw milk from suckler cows in the maritime region of southern Togo. European Scientific Journal. 2015;11(36).
15. Maïworé J, Baane MP, Amadou AT, Ouassing AD, Ngouné LT, Montet D. Influence of milking conditions on the physicochemical and microbiological qualities of raw milk collected in Maroua, Cameroon. Afrique Science. 2018:14(4): 235 – 248.
16. Koussou MO, Grimaud P, Mopaté LY. Evaluation of the physicochemical and hygienic quality of bush milk and local dairy products marketed in dairy bars in N'Djamena, Chad. Revue Élev Méd. Vét. Pays Trop. 2007;60(1-4):45-49.
17. Renard J. About raw milk. Walloon milk and dairy products sector; 2014.
18. Baazize -ammi D, Gharbi I, Dechicha AS, Kebbal S, Guetarni D. Bacteriological and sanitary quality of raw milk from cattle in direct and indirect circuits in the central region of Algeria. Rev. Mar. Sci. Agron. Vet. 2019;7(2):267-272.
19. Matallah S, Matallah F, Djedidi I, Mostefaoui KN, Boukhris R. Physicochemical and microbiological qualities of raw milk from cows raised extensively in the North-East of Algeria. Livestock Research for Rural Development; 2019.
20. Laithier C. Microflora of raw milk. Cheese sectors promoting their terroir; 2011.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/124804