






Research Article

# Microbiological Quality of Raw Camel Milk Consumed in the Zinder Region (Niger)

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## Abstract

Camel milk is a highly distinctive product for camel-rearing populations. However, camel milk production must be strictly controlled due to the potential risks it may pose to human health. The objective of this study is to evaluate the microbiological quality of processed milk camels from the Zinder region. The study which was conducted in Niger in the department of Tesker (Zinder), was conducted on 34 samples from camels depending on the availability of breeders and also the docility of the females. Seven (7) categories of germs were searched for through microbiological analysis FMAT, Total Coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* ssp, *Colostridium perfringens* and fungal flora. For the assessment of the microbiological quality of milk, the results of our analyses are expressed according to the reference standards of the Luxembourg Food Safety Directorate (DSA/Luxembourg). These standards are listed in the table below. The microbiological quality of camel milk samples is not compliant to the standards for germs Total Aerobic Mesophilic Flora (85.29%), *Staphylococcus aureus* (61.76 %) and Yeasts (94.11%) and Molds (38.23%) and with respective average loads in CFU/ml: 1251.79 10<sup>5</sup>; 34.12 10<sup>2</sup>; 612.74 10<sup>3</sup>; 10.15 10<sup>3</sup>. Total Coliform Germs, *E. coli*, *Salmonella* ssp and *Clostridium perfringens* were absent in all samples analyzed. These results demonstrate a lack of compliance with good production hygiene practices during milking. 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, Camel, Microbiological Quality, Pathogenic Germs, Tesker, Niger

## 1. Introduction

Camel milk (*Camelus dromaderius*) is a highly distinctive product for populations that raise dromedaries. It is the main food resource for nomadic peoples who usually consume it

raw or fermented. It plays an important role in human nutrition.

Global camel milk production is estimated at 5.4 million

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tons, of which approximately 55% is taken by camels [1, 2]. Individual productions vary between 1000 and 2700 liters per lactation in Africa, but can reach 7 000 to 12 000 liters in South Asia [3, 4]. In recent decades, a growing interest and remarkable increase in demand for dromedary milk and dairy products have been observed worldwide due to the superior medicinal value and beneficial health effects. This emergence has led the dairy industry to provide various camel dairy products to consumers with superior nutritional and functional quality [5]. According to the statistical report of the livestock sector, Niger has 1 858 799 heads of dromedaries in 2020 and is considered a livestock country par excellence [6]. Niger occupies a prominent place in the field of livestock farming, because of the best routes it offers and the existence of salty lands. Livestock farming is of the extensive and transhumant type and remains dominated mainly by camel farming, with some small ruminants and cattle [7].

The Zinder region is characterized by a massive presence of dromedaries with a population of 259,990 heads for a milk production of 15 465 tons [6]. Camel milk is a strategic product for Niger, given its economic and socio-cultural importance, which contributes not only to the food and nutritional security of households, but also to the creation of wealth and jobs through income and related production, processing and marketing activities. But the production of camel milk must be strictly controlled because of the potential risks they may present to human health. Indeed, strains pathogenic to humans and animals, which may have acquired multiple resistance to antibiotics, can proliferate there [8]. An assessment of the hygienic quality of the milk makes it possible to search for the natural microflora and pathogenic microorganisms.

The objective of this study is to evaluate the microbiological quality of processed milk camels from the Zinder region. The search for microbial germs in raw camel milk helps to improve health quality.

## 2. Materials and Methods

### 2.1. Study Framework

This study took place in Nguelbilo (N 14 ° 54'13.4"; E 10 ° 31'10.5" and N 14 ° 54'50.3"; E 10 ° 31'21.6"), a peri-urban area located 35 km west of the rural commune of Tesker in the Zinder Region (Niger). The choice of the sampling site was made based on its accessibility, understanding of the local dialect and the availability of breeders to collaborate.

### 2.2. Concept of the Study

This is a cross-sectional analytical study conducted between July and September 2023. This study was carried out in two phases: a field phase for sample collection and a laboratory analysis phase.

### 2.3. Sample Collection and Packaging

Samples were collected in sterile 60 ml bottles, while respecting good hygienic conditions. A total of 34 samples were collected from 34 camels using convenience sampling. The samples collected were immediately recapped, labeled and placed in a cooler equipped with ice accumulators at a temperature of 4 °C and sent to the Central Livestock Laboratory in Niamey (Niger) for microbiological analyses. Due to the distance between the two cities, the analyses took place 24 hours after sampling. Several microbiological parameters were sought and counted in this study: Total Aerobic Mesophilic Flora, Fecal Coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, Yeasts and Molds and *Salmonella* spp.



*Figure 1. Direct collection.*



*Figure 2. Packaging of samples from the bottle.*

## 2.4. Preparation of Decimal Dilutions

The preparation of decimal dilutions is carried out in accordance with ISO 8261 (2001), this step allows the mother solution to be diluted until an exploitable microbial concentration is obtained. From the mother solution, a series of decimal dilutions are carried out, required to determine the number of bacteria in the sample, by transferring 1 ml of the previous dilution into 9 ml of physiological water, for each transfer a separate sterile tip is used. All dilutions must be shaken immediately before carrying out the transfer in order to ensure that the microorganisms present are distributed uniformly.

## 2.5. Microbiological Analyzes

The enumeration of total aerobic mesophilic flora was carried out according to the international standard ISO 4833-2013. A volume of 0.1 ml of the inoculum of each of the dilutions used (10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>) was taken and inoculated on the petri dishes containing the previously prepared PCA (Plate Count Agar) medium. After inoculation in a single test, these boxes, in an inverted position, are incubated at a temperature of 37 °C for 24 hours. The count is done using an electric meter

from the colonies.

The search for fecal coliforms and *E. coli* was carried out according to the ISO 7251: 2005 standard on the “*E. Coli-Coliforms*” medium. Chromogenic Medium » which allowed simultaneous detection and differentiation between *E. coli* and coliforms in a single medium. The mother solution was diluted to 10<sup>-6</sup>, the inoculation was done in bulk, by aseptically carrying 1 ml of the 10<sup>-3</sup> and 10<sup>-4</sup> dilutions into the bottom of the petri dishes and then poured a quantity of 10 to 15 ml of *E. Coli-Coliforms* Chromogenic Medium agar. The plates are incubated at a temperature of 44 °C for 24 hours. The green colonies represent *E. coli* and the purple ones represent coliforms.

For the isolation and enumeration of *Staphylococcus aureus*, the ISO 6888-2: 2003 standard was used. A surface inoculation with 0.1 ml of the 10<sup>-2</sup> and 10<sup>-3</sup> dilutions of each stock solution is placed on Baird Parker selective medium and then spread using a single-use spreader. Incubation is carried out at 37 °C for 24 hours. *Staphylococcus aureus* is characterized by the formation of black, shiny, convex colonies, surrounded by a halo of egg yolk lightening. Inside the halos, an opaque zone may appear due to the action of a lecithinase.

**Table 1.** Microbiological quality criteria for raw milk (Luxembourg, 2018).

Germes	Culture medium	Incubation Time and Temperature	Microbiological criteria in CFU/g
TAMF	Plate Count Agar	72 H at 30 °C	≤ 10 <sup>5</sup>
Fecal Coliforms <i>E. coli</i>	<i>E. Coli-Coliforms</i> Chromogenic Medium	24 h at 44 °C	≤ 10 <sup>-6</sup>
<i>Clos. perfringens</i>	Tryptone Sulfite Neomycin	24 h at 46 °C	≤ 50 / ml
<i>St. aureus</i>	Baird-Parker	24 h at 37 °C	Absence
Yeasts - Molds	Sabouraud	96 – 120 H at 37 °C	Absence
Salmonella spp.	MKTTN	24 h at 37 °C	Absence

The search for *Clostridium perfringens* according to ISO 7937: 2004, was done with the Tryptone Sulfite Neomycin (TSN) medium which is a selective agar, it exploits the fact that *Clostridium perfringens* is resistant to polymyxin, neomycin (antibiotics) and that it has the power to reduce sulfites. The mother solution is diluted up to 10<sup>-6</sup> with buffered peptone water and the inoculation was done in bulk, aseptically carrying in depth 1 ml of the 10<sup>-1</sup> and 10<sup>-2</sup> dilutions at the bottom of the Petri dishes. Then poured a quantity of 10 to 15 ml of the TSN agar. Once the agar is solidified, the dishes are placed in an anaerobic jar and the incubation was done in the incubator between 44 or 45 °C for 24 hours. Colonies producing a diffusible black pigment are sulfite-reducing anaerobic bacteria and possibly *Clostridium perfringens*.

*Salmonella* spp detection was carried out according to ISO 6579 2002. 1 ml of the stock solution was placed aseptically in a tube containing MKTTN medium and incubated at 37 °C for 24 hours. Tubes showing microbial disturbance are considered positive. From a positive enrichment tube, a drop is taken with a sterile loop and streaked on the edge of a Petri dish containing previously melted HEKTOEN agar. Then a count is carried out, which consists of noting the presence or absence of *Salmonella* in the dishes which, if present, give a bright color.

Yeasts and molds are isolated and counted on the Sabouraud selective agar medium according to ISO 6611: 2004. Dilutions 10<sup>-3</sup> and 10<sup>-4</sup> were chosen for the determination of yeasts and molds, a surface inoculation is carried out by transferring 0.5

ml of each dilution. After incubation at room temperature for 4 to 5 days, a count of the Yeasts and Molds present in the Petri dishes is carried out.

The results of the counting of different colonies of the germs sought were then expressed in colony-forming units per ml or per g (CFU/ml). For the assessment of the microbiological quality of milk, the results of our analyses are expressed according to the reference standards of the Luxembourg Food Safety Directorate (DSA/Luxembourg). These standards are listed in the table below.

## 2.6. Statistical Analyzes of Data

Microbiological analysis data were collected and recorded with Microsoft® Excel 2016. SPSS software version 26.0 was used for the expression of statistical means and extremes.

## 3. Results

### 3.1. Average Loads and Extreme Values of the Germs Sought

Table 2 summarizes the average and extreme values (maximum and minimum) of each parameter sought. The results of the germ count highlighted an average load of  $12.51 \cdot 10^7$  CFU/100 ml in Total Aerobic Mesophilic Flora with extreme values between 0 and  $134.2 \cdot 10^7$  CFU/100 ml. For *Staphylococcus aureus*, the extreme values obtained are of the order of 0 to  $550 \cdot 10^2$  CFU/100 ml with an average load of  $34.12 \cdot 10^2$  CFU/100 ml. As for yeasts, the extreme values of the loads obtained are between 0 and  $410 \cdot 10^4$  CFU/100 ml with an average load of  $61.27 \cdot 10^4$  CFU/100 ml. Molds are present with an average load of  $10.15 \cdot 10^3$  CFU/100 ml corresponding to values that oscillate between 0 and  $90 \cdot 10^3$  CFU/100 ml. In all the samples analyzed, fecal coliforms, *Escherichia coli*, *Salmonella* ssp and *Clostridia* perfringens were absent.

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

Parameters	TAMF	<i>St. aureus</i>	Yeasts	Molds
Average	$12.51 \cdot 10^7$	$34.12 \cdot 10^2$	$61.27 \cdot 10^4$	$10.15 \cdot 10^3$
Minimum	0	0	0	0
Maximum	$134.2 \cdot 10^7$	$550 \cdot 10^2$	$410 \cdot 10^4$	$90 \cdot 10^3$



**Figure 3.** Colonies of TAMF.



**Figure 4.** Colonies of *Staphylococcus aureus*.



**Figure 5.** Colonies of Yeasts.



**Figure 6.** Colonies of Molds.

### 3.2. Prevalence of Total Aerobic Mesophilic Flora

Contamination of raw camel milk by total aerobic mesophilic flora (TAMF) resulted in 85.29% non-conformity, i.e. only five samples (14.71%) showing values below those set by the evaluation criteria (figure 7).



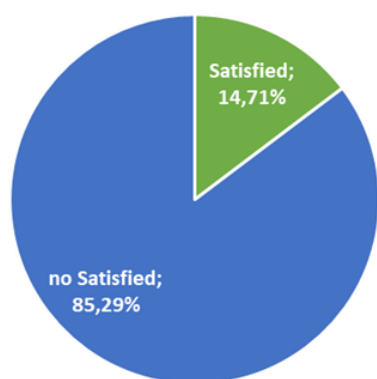


Figure 7. Raw milk compliance rate compared to TAMF.

### 3.3. Prevalence of Staphylococcus Aureus

*Staphylococcus aureus* was found in 61.76% of the raw camel milk samples analyzed (Figure 8).

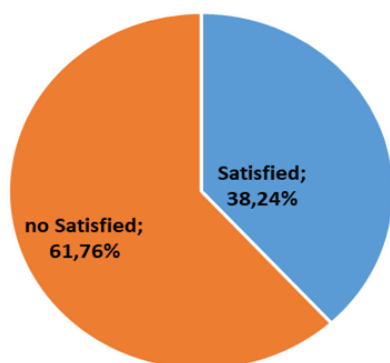


Figure 8. Compliance rate of raw milk with respect to *Staphylococcus aureus*.

### 3.4. Contamination of Sesame by Yeast

Yeast germs showed high contamination in our cultures for most of the samples analyzed. Indeed, yeasts induced 94.12% of samples that did not meet the criteria (Figure 9).

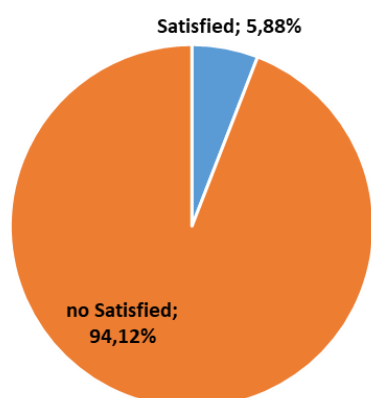


Figure 9. Compliance rate of raw milk compared to yeasts.

### 3.5. Prevalence of Molds

Molds induced 38.24% non-conformity in raw camel milk samples. More than the majority of samples (61.76%) were compliant with the standard (Figure 10).

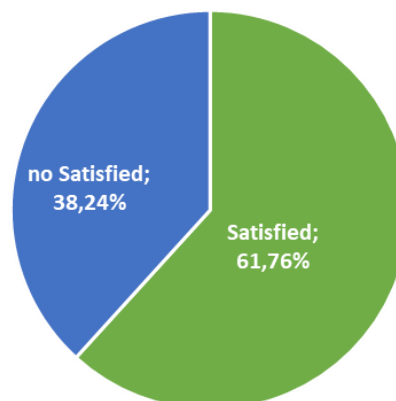


Figure 10. Rate of induced conformity with respect to mold.

### 3.6. Prevalence of Total Coliforms, E. coli, Salmonella spp and Clostridia Perfringens

The search for Total Coliforms, E. coli, Salmonella spp and Clostridia perfringens in 34 samples during this study revealed the total absence of these germs. All samples were compliant (100%).

## 4. Discussion

The results of microbiological analyses showed that the samples of raw camel milk were contaminated by most of the germs tested for. They were heavily contaminated by total mesophilic flora, yeasts and *Staphylococcus aureus*.

The average load of total mesophilic aerobic flora was  $1251.79 \times 10^5$  CFU/ml. This value is higher than the standard used in this study and also higher than the average value of  $7.1 \times 10^4$  CFU/ml found in camel milk raised in a semi-intensive system in the locality of Ghardaïa in southern Algeria by a study Mosbah *and al* [9]. Kaoudja and Mecheri obtained a result of the total mesophilic aerobic flora lower than ours ( $193 \times 10^4 \pm 0.35$  CFU/ml) [10]. On the other hand, in camel milk collected in the Oued Souf region in Algeria, Arbia and Chiheb obtained an average load of  $1.312 \times 10^5$  CFU/ml of the total mesophilic aerobic flora [11]. The enumeration of the total mesophilic aerobic flora reflects the general microbial quality of a natural product [12]. The absence of colonies of total mesophilic aerobic flora in certain samples could be due to the handling conditions Amhoury, *and al* and Mosbah *and al* state that the search for microorganisms of the total mesophilic aerobic flora allows to judge the hygienic state of food products [9, 13].

The raw milk samples analyzed contain a variable load of staphylococci, ranging from 0 to  $550 \times 10^2$  CFU/ml with an average value of  $34.12 \times 10^2$  CFU/ml. The latter does not comply with the standard. This load shows that there is a high contamination of some samples. Mosbah *and al* obtained a microbial load of  $7.1 \times 10^4$  CFU/ml. This value is lower than ours [9].

Kaoudja & Mecheri obtained results in accordance with the standard, as all the samples analyzed were free of *Staphylococcus aureus* [10]. Alem & Hamoulili and Hammi & Litim obtained  $0.62 \times 10^2$  CFU/ml and  $9.10^4$  CFU/ml respectively, a result lower than ours [14, 15]. Aouachria obtained a *Staphylococcus aureus* load  $< 10$  CFU/ml [16]. This result is not in accordance with the standard and lower than ours.

*Staphylococcus aureus* are the most common agents of food poisoning. Contamination of milk by *Staphylococcus aureus* could perhaps be the consequence of a mammary infection [15, 17]. However, poor milking practices such as the failure to clean udders and hands before and after milking by almost all camel households in this area could also be incriminated for the high contamination rate of the samples analyzed. In perspective, a complementary study can be carried out to locate the origin of this contamination.

The average yeast load was  $612.74 \times 10^3$  and the average mold load was  $10.15 \times 10^3$  CFU/ml. These average loads are higher than the standard. Hammi, I., & Zineb obtained a combined fungal load of yeasts and molds ( $37 \times 10^2$  CFU/ml) lower than ours and slightly higher than the standard [15]. Alem & Hamoulili obtained an average value of yeasts and molds of  $0.31 \times 10^2$  CFU/ml [14]. This result is lower than ours and closer to the standard. On the other hand, Aouachria reported a total absence of yeasts and molds in his samples [16].

all samples analyzed, Total Coliforms, *E. coli*, *Salmonella ssp* and *sulfite-reducing Clostridia* were absent. This situation shows that our milk samples were not contaminated by these germs. The coliform count is considered a good indicator of fecal contamination.

Kaoudja and Mecheri obtained a value ( $116 \times 10^4 \pm 0.64$  CFU/ml) of total Coliforms significantly higher than ours [10].

Alem & Hamoulili, Hammi, I., & Zineb and Aouachria recorded a load of  $4,958 \times 10^4$  CFU/ml;  $1.3 \times 10^4$  CFU/ml and  $1.8 \times 10^2$  CFU/ml in Total Coliforms in their samples respectively [14-16].

Aouachria reported a total absence of *E. coli* and of *Salmonella ssp* [16]. These results are consistent with ours. On the other hand, Alem and Hamoulili obtained a microbial load of *Salmonella ssp* of  $7.9 \times 10^4$  CFU/ml [14]. The samples analyzed by Mosbah *and al* were all negative for *Salmonella ssp* [9].

## 5. Conclusions

The study shows that the microbiological quality of camel milk samples does not comply with the standards for the germs Total Aerobic Mesophilic Flora, *Staphylococcus aureus* and fungal flora. The results for Total Coliforms, *E. coli*, *Salmonella ssp* and *Clostridia perfringens* were in accordance with the standards. This situation shows that milking practices influence udder infection and the microbiological quality of milk. Consumers of camel milk are therefore likely to contract the *Staphylococcus aureus* germ which causes skin infections, pneumonia, heart valve infections and bone infections. The contamination of camel milk by this germ then constitutes a public health problem. It is urgent that studies be conducted to determine the effect of good milking practices on the hygienic-sanitary and nutritional qualities of camel milk in urban, peri-urban and rural areas; seasons and areas at high risk of contamination and sanitation processes to obtain good quality milk.

## Abbreviations

Clos. perfringens	Clostridium Perfringens
E. coli	Escherichia Coli
St. aureus	Staphylococci Aureus
TAMF	Total Aerobic Mesophilic Flora
CFU	Colony Forming Units

## Author Contributions

**Abdelsalam Adoum Doutoum:** Data curation, Formal Analysis

**Hisseine Mahamat Allamine:** Data curation, Formal Analysis

**Alhadj Markhous Nazal:** Conceptualization, Data curation, Methodology, Resources, Writing – original draft, Writing – review & editing

**Abdoulaye Idriss Sakha roun:** Conceptualization, Methodology, Resources, Writing – original draft

**Chaibou Mahamadou:** Data curation, Project administration, Validation, Visualization

## Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest.

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