Assessment of the Sanitary and Hygienic Quality of Raw Milk Marketed in the Urban Area of Mostaganem, Algeria

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ABSTRACT

Background: Fresh raw milk is a highly nutritious but perishable product. Its informal sale without control, is detrimental to the health of the consumer. Therefore, assessing the hygienic and sanitary quality of raw milk is an absolute necessity.

Methods: 20 raw milk samples from four regions of Mostaganem City, Algeria, were tested on a microbiological compliance aspect. A serologic analysis was carried out for the indirect detection of brucellosis and a questionnaire was developed to check the hygiene rules applied at the sales store level.

Result: The results indicate an average high contamination of 8.10^9 cfu/ml for aerobic germs at 30°C versus 12.10^4 cfu/ml for thermotolerant coliforms. The presence of *Staphylococcus* with positive coagulase and *anti-brucella* antibodies indicates that these milks are potentially hazardous to human health. Salmonella is absent in all samples. The field investigation shows the proven absence of the most basic hygiene rules for the storageand presentation for sale of raw milk. A quality approach must be put in place at the service of the consumer.

Key words: Contamination, Hygienic and sanitary quality, Microbiological, Raw milk.

INTRODUCTION

Mostaganem is a Wilaya with an agricultural vocation, with a predominantly rural character. Consequently, dairy cattle farming is one of the most important activities for the region's economy (ONS, 2011). Incentives to support the production of raw milk to strengthen its development and increase its collection have also contributed to the emergence of small businesses of raw milk and its derivatives that operate on sale informally, without health and hygiene checks (Baazize-Ammi *et al.,* 2019). These highly sought-after and highly prized products are accessible to the majority of the Algerian population who consume them constantly during traditional and religious festivals. It remains in Algeria, the basis of the low-income consumer's food ration (Rechidi-Sidhoum, 2019). Raw milk is a food rich in nutrients and useful bacteria, but nevertheless remains a fragile product, perishable by its contamination by animal, human and/or environmental sources from its primary production to its commercialization (Sing and Gupta, 2015). The estimated incidence of notifiable diseases in Mostaganem in 2018 is 30.4% for collective food poisoning (CFP) and 0.8% for brucellosis. The evolution of reported cases in the wilaya of Mostaganem from 1999 to 2016, shows that the number of people suffering from brucellosis through consumption of milk and whey is 50% and that it is not correlated with the number of animal cases, it is noted the absence of data on the subject (Rechidi-Sidhoum, 2019). Therefore, the objective of this study is to estimate the bacteriological quality (sanitary and hygienic) of raw cow's milk for human consumption in an urban area, to verify whether the milk offered for sale comes from brucellosis-free dairy cattle herds and to assess the hygienic situation of shops and staff as well as the conditions of storage and presentation for sale of this milk.

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MATERIALS AND METHODS

Study area and period

The study was conducted in the capital of the Wilaya of Mostaganem, located in the northwest region of Algeria. 20 milk samples were collected monthly (from a set of 20 shops), in the spring period (April-May, 2019), during the high season of production and consumption of raw milk and its derivatives by the local population. The distribution of the shops was done according to their distribution in the different districts of the city: North, East, West and South.

Samples

Samples are taken in the morning in their packaging presented for sale. Raw milk is aseptically removed after homogenization, in sterile 250 ml vials and then transported

in a cooler at 4°C to the Laboratory of Sciences and Technics of Animal Production. The assessment of the hygienic situation of the premises, the equipment, the conditions of preservation and presentation for the sale of raw milk is carried out through a questionnaire. The hygiene of the staff (clothing and medical certificates), the origin of the raw milk and the presence or not of the health certificate of the livestock accompanying the sale of milk are noted.

Bacteriological and serological analyses

Bacteriological research has involved aerobic flora at 30°C (AG), thermotolerant coliforms (TTC), positive coagulase *Staphylococcus* (PCS) and Salmonella. The tests are carried out in accordance with official Algerian standards (JORA, 2017). The indirect search for tweezers in raw milk is carried out by the Ring-test. The reaction involves a colored antigen consisting of a suspension of killed brucella and milk antibodies (JORA, 1996).

Methods

Microbiological analyses are carried out under strict aseptic conditions with media and reagents from the Pasteur Institute, Algeria. For the research and counting of these germs, a series of dilutions (from 10^{-1} to 10^{-5}) is carried out beforehand with physiological water from a homogenized raw milk stock solution (Guiraud, 2012).

Search for aerobic germs at 30°C

AG enumeration is performed after appropriate dilutions by inoculation on Plate Count Agar followed by incubation at 30°C for 72 hours.

Search for thermotolerant coliforms

TTC counts are conducted on Violet Red Bile Lactose Agar medium. Enumeration of red colonies with a diameter of at least 0.5 mm is performed after 24 hours incubation at 44°C.

Search for *Staphylococci* with positive coagulase

This research is carried out by Chapman's medium enrichment method for incubation at 37°C for 48 hours. Fluorescent golden colonies are retained for the count. For orientation to *Staphylococcus aureus*, one colony is placed in a tube containing Brain-Heart Infusion Broth and then incubated at 37°C for 18 hours. Then, 1 ml of this solution is mixed with 1 ml of rabbit plasma and incubated at 37°C for 4 hours. The presence of *Staphylococcus aureus* results in the coagulation of the solution.

Salmonella testing

Their search requires pre-enrichment in buffered peptone water for incubation at 37°C for 18 hours. Enrichment of this solution is carried out on a sodium acid selenite medium and then incubated at 37°C for 24 hours. Isolation is then carried out on *Salmonella-Shigella* medium for incubation at 37°C for 24 hours. *Salmonella* that does not ferment lactose have colourless, transparent colonies with or without a black center.

Serological research of brucellosis

It is a qualitative immunological reaction that indirectly highlights the anti-*brucella* antibodies present in bovine milks (Pal *et al.,* 2020). The Ring-milktest (MRT) consists of a suspension of a strain of *Brucella abortus* killed by phenol and colored by hematoxylin. After a60 minutes incubation at 37°C, the test is positive when the agglutinates adhere to the lipid globules that gather with the cream ring by staining it blue-violet.

Hygienic situation of the premises

A survey using a questionnaire is carried out to assess the hygienic situation of the premises, equipment, staff and the way in which milk is sold.

Interpreting of results

Interpretation of the analyses is carried out on the values set by Algerian normative references for brucellosis (JORA, 1996) and microbiology (JORA, 2017). The decision rule will be conditioned by the level of quality: satisfactory, acceptable, unsatisfactory or toxic. The statistical analysiswas made possible by the use of SYSTAT SOFTWARE MYSTAT 12. The threshold of statistical significance is estimated at P<0.05.

RESULTS AND DISCUSSION

Bacteriology

The classification of the overall estimate of the germs studied according to the standards setting the microbiological criteria of foodstuffs (JORA, 2017) is presented in (Table 1). There is a significant level of contamination of the sampled milks. Taking into account the different regions of the urban area of Mostaganem, contamination is much more important for aerobic germs at 30°C and for thermotolerant coliforms.

Aerobic germs at 30°C

The microbiological analysis of the collected milks shows that there is a significant difference in the estimated number of AG at 30°C between the different geographical regions studied (p>0.05). The average obtained for all samples is 8.10^9 cfu/ml, which is largely higher than the acceptability thresholds required by Algerian regulations (JORA, 2017) and the estimates made by different authors.

These results reflect a poor state of freshness of these raw milks since 100% of the samples are of unacceptable quality at the level of the 04 sampling areas (Table 1). This is probably related to poor practices of milking and packaging of raw milk in cattle farms (Guiraud, 2012 and Sing and Gupta, 2015) or to uncontrolled situations during their transport and sale in stores, especially in this period when the ambient temperature is quite high. We note, a great variability in the enumeration of these bacteria for all samples as described in various researches (Gupta *et al.,* 2020 and Hamiroune *et al.,* 2016). The region has the highest rate, as the farms that supply this region with milk are located in mountainous areas far from the urban area.

Table 1: Estimation of bacterial flora (Average count in cfu/ml).

Bacterial flora	Standards cfu/ml (JORA, 2017)	Studied urban area							
		North		East		West		South	
		1st month	2nd month	1st month	2nd month	1st month	2nd month	1st month	2nd month
AG	3.10^6	10^{9*}	3.10^{9*}	2.10^{9*}	8.10^{9*}	3.10^{9*}	6.10^{9*}	10^{8*}	2.10^{9*}
CPS	10^3	0	0	10	10^{4*}	0	0	0	3.10^{3*}
TTC	5.10^3	4.10^3	5.10^{4*}	2.10^3	12.10^{4*}	10^2	8.10^{3*}	3.10^2	2.10^{4*}
Salmonella	Absence/25 ml	0	0	0	0	0	0	0	0

1st month: April 2019, 2nd month: May 2019; AG: Aerobic germs at 30°C; CPS: Coagulase positive *Staphylococcus*; TTC: thermo-tolerant coliforms. *(P>0.05): Significant difference.

Positive coagulase *Staphylococcus*

PCS is detected in samples from the Eastern region (10^4 cfu/ml), with a low count and in samples from the Southern region (3.10^3 cfu/ml), qualifying it as a health risk (JORA, 2017). Positive samples represent 10% of the cases studied. PCS is an indicator of direct contamination by human pathogenic strains from healthy carriers or dirty hands of untreated sick animal workers and failure to follow good hygiene practices (Baazize-Ammi *et al.,* 2019) but also of indirect contamination, by animal infections (subclinical mastitis) (Hamiroune *et al.,* 2016) and by the various manipulations of objects used during milking accentuated by the dusty environment of the barn and rearing areas of the producing herd (Sing and Gupta, 2015). PCS causes inflammation of the udder which has an economic impact on milk production (Pal *et al.,* 2020). Baazize-Ammi *et al.* (2019) report in the same context significantly higher average loads in milks from small-scale farms collected (9.10^2 cfu/ml) compared to milks from large-scale production (8×10^4 cfu/ml). This can be explained by the high prevalence of staphylococcal mastitis on small, uncontrolled farms and by the lack of hygiene observed during milking.

Thermo tolerants coliforms (TTC)

The estimation of the number of TTC shows that there is a significant difference between the different milk samples studied in the urban area of Mostaganem (p>0.05). It is observed a high count in the eastern region with an average of 12.10^4 cfu/ml (Table 1).

Samples of unsatisfactory quality represent more than 50% and come from the 4 regions studied. TTCs being commensal bacteria of the gut can survive as sapprophytes outside and contaminate the animal's body during uncontrolled milking operations by transfer of fecal material from soil, manure and dust containing microorganisms (Guiraud, 2012). Comparable results exceeding the contamination threshold are obtained by Baazize-Ammi *et al.* (2019) who find that 40% of the milk analyzed on the farm is loaded with TTC. In approximately 54.5% of cases, removal of first sprays of milk before milking is lacking, milking machines are not clean and 63.6% of milkers wear inappropriate and soiled clothing.

Salmonella

Salmonella were noted to be absent in all samples (JORA, 2017). Because their numbers are generally low in products, they are often difficult to find and identify. According to Singh *et al.* (2018), since the animal gut is the most important reservoir of Salmonella, animal feces are the main sources of milk contamination during milking operations. Due to their consistent pathogenicity, Salmonella are not tolerated in a food such as milk (Guiraud, 2012). The results of this research corroborate with those of Bousbia *et al.* (2018) who found milk free of this germ. The investigations of Singh *et al.* (2018) revealed the presence of *Salmonella* spp, resistant to many antibiotics with a higher prevalence in raw milk (11.9%) compared to other products (7.4% in milk collected from vendors and 14.2% in dairies and retail stores).

Indirect search for brucellosis

Anti-brucella antibodies were revealed in two samples see (Table 2) (10% of the cases studied). This suggests that milk came from brucellosis-infected cattle farms. In Algeria, CFP due to the ingestion of raw milk or its fresh derivatives is the cause of several cases of human brucellosis (Rechidi-Sidhoum, 2019). In Sidi Bel-Abbès, 190 people were affected in 2019 compared to 211 cases detected in 2018; in Ghardaïa 198 cases in 2017 compared to 1547 in 2016 with 36 cases of bovine brucellosis in seven outbreaks across the wilaya during the latter year. For Mostaganem, only 3 cases were reported in 2020, this figure does not reflect the true incidence values given the high number of bovine brucellosis cases in the region(DSPRH, 2016-2021).

In developed countries, indirect, digestive contamination through consumption of raw milk or artisanal cheeses, perhaps observed in tourists who travel sharing the way of life of local populations or in those returning from a country where animal brucellosis is endemic, 84% of recorded cases were imported (Kamwine *et al.,* 2017).

Table 2: Ring-test positive milk samples.

Positive test	North	East	West	South
Anti-*brucella* antibodies	2	0	0	0

Survey of the state of shops

The survey on the situation of the premises showed for all shops (100%), the lack of control of milk, insufficient control of the rules of hygiene of the premises and equipment. The storage and sales equipment does not meet standards and equipment is inadequate or defective (plastic cans, storage bags used for the sale of milk as it is), milk is stored at room temperature (no refrigeration) (Baazize-Ammi *et al.,* 2019). It was noted the absence of sanitary facilities, running water and the wearing of gowns by traders who did not present a health certificate and documents relating to the origin of the milk. The investigation revealed that the amount of milk retailed was that which was refused by the dairies.

CONCLUSION

In view of the results obtained from microbiological and serological criteria, it appears that the quality of the milks studied does not comply with the required national standards, with a potential danger associated with CFP. The non-conformity of these milks is related to poor storage conditions and sale in unsuitable stores and not subject to the commercial standards required in Algeria, accentuated by those of production practices on the farm and transport. The presence of anti-brucella antibodies in milk indicates the lack of rigorous control of cattle farms that are infected with brucellosis. This preliminary assessment should alert all players in the informal sector to the need to implement a strategy to improve the quality of milk, both by respecting good production practices up to the sale and by establishing a rigorous control protocol at all levels.

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