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Asian Journal of Animal and Veterinary Advances

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OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2016.854.860

Research Article
Assessment of Microbiological Quality of Raw Milk Produced at Tizi Ouzou Area (Algeria)

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Abstract

Background: The microbiological quality of raw milk is relatively a new subject that interest more actors of milk production in Algeria. Until now, the raw milk collected presented a high rate of microbial contamination, prejudicial for human consumption and dairy processing. **Objective:** The aim of this study is to assess the hygienic and sanitary quality of raw milk produced throughout three segments where milk is handled in Tizi Ouzou area (Algeria). **Methodology:** For this, 174 samples of raw milk were collected throughout farms, collectors and local markets and submitted for microbiological analysis. **Results:** The results of this study showed that microbial contamination of raw milk increase along the dairy chain. The mean $\log_{10}$ of TBC at farm level was 6.73 ± 0.25 $\log_{10}$ CFU mL^{-1}. This value increase to reach a load of 6.81 ± 0.19 and 7.2 ± 1.05 $\log_{10}$ CFU mL^{-1} at collection centers and market points, respectively. In addition, cases of contamination by *S. aureus* and antibiotic residues were observed. **Conclusion:** This study suggest that improving of microbial quality of raw milk requires the establishment a quality policy with the training of farmers on good hygienic practices.

Key words: Raw milk, microbiological quality, microbial contamination, dairy products, hygienic quality, antibiotics residues, dairy chain, hygienic practices, *Staphylococcus aureus*

Received: August 13, 2016 Accepted: September 22, 2016 Published: November 15, 2016

Citation: Yacine Titouche, Ahcène Hakem, Djouza Salmi, Benalia Yabrir, Nadia Chenouf, Achour Chergui, Amel Chenouf and Karim Houali, 2016. Assessment of microbiological quality of raw milk produced at Tizi Ouzou area (Algeria). Asian J. Anim. Vet. Adv., 11: 854-860.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The development of dairy farming in Algeria remains a target for the government but the imports of milk powder from the industrialized countries are necessary to satisfy the needs of population, which is in full demographic expansion. The national milk production is still low, covers only half of the annual consumption. The annual value of imports was estimated between 600 and 800 millions of dollar[1]. One of the main constraints of this sector is the low production of the imported breeds and a poor organization of the collection system[2]. In order to improve the levels of local production and integrate them in dairy industry, the government initiated a program of rehabilitation and promotion of the dairy production. The results of this strategy were encouraging: Increasing of local production, creation of a new dairies and diversification of dairy products. However, the collection level of raw milk remains low and the milk collected is often loaded with bacteria, harmful for human consumption and for industrial processing. The data of ministry of health, population and hospital reforms revealed that milk and milk products were implicated on food poisoning[3]. The existence an informal system, escaping to the sanitary control, suggest that an important part of this milk production present a potential hazards for public health. Currently, some dairies plan to collect a raw milk that present a good microbial quality. Therefore, payment measures according to the microbiological quality have been introduced. In parallel, some dairies are opting to distribute products that are used during milking procedures and during cleaning of equipment involved during raw milk storage. Many studies have been conducted in order to assess the hygienic quality of raw milk. Aggad *et al*.[4] evaluated the hygienic quality of mik at Western of Algeria at the reception (Dairy units). Ghazi and Niar[5] and Hakem *et al*.[6] focused their studies at farm level. Adjlane-Kaouche *et al*.[7] evaluated the quality of milk at farms and collectors centers. However, to our knowledge's, there is no study which have been conducted to evaluate the hygienic quality at the end of dairy chain. The mains objectives of this study is to identify the relevant sources of microbial contamination of raw milk produced locally and to assess the hygienic quality of this product along the chain production.

MATERIALS AND METHODS

Study design and collections of samples: This study was conducted during the dry season (April-September, 2015) at three stages where milk is handled in Tizi Ouzou area (Algeria): Farm tank, collector milk and local market. In this season, the study included 25 farms, 25 collectors and 4 local markets. The choice of this farms and collectors is based to their memberships to the different dairies. For this, five dairies were chosen. A total of 174 samples of raw milk were collected. Three samples of raw milk were collected from each farm and from each collector and six samples of raw milk were taken from each local market. Seventy five milk samples at farm consisted of milk tank before delivery to collector, while 75 samples from the collector's tank at the end of collection. The samples were immediately cooled at 4°C in order to minimise the growth of spoilage microorganisms and transported them to the laboratory where tests were carried out on the same day. Data on milking procedure and other farm management that influence the microbiological quality of milk at farm level were collected using a structured questionnaire with closed end questions in a face a face farmer interview. These factors included cow cleanliness and hygiene during milking procedures, time taken to deliver milk to the collection centres, frequency of milking, type of utensil used for storage of milk at farm, equipment maintenance and cleaning, measures used in mastitis control as the use of antibiotic treatment. For this, initial visits were made to each farm where farmers were interviewed and the easiness and the lack of clarity of questions was revised.

Microbiological analysis: The microbiological analysis were carried out at the Laboratory of Analytical Biochemistry and Biotechnology of the University of Tizi Ouzou (Algeria). Samples of raw milk were analyzed for enumeration of Total Bacteria Count (TBC), Fecal Count (FC), *Clostridium perfringens*, detection of *S. aureus* and *Salmonella* sp. Samples of raw milk were submitted to the detection of *Brucella* antibodies using the Ring Test (RT) and antibiotic residues by the microbiological method.

Enumeration of Total Bacterial Count (TBC) and coliform Fecal Count (FC): The total viable bacterial counts (TBC) and coliform Fecal Counts (FC) were determined using the pour plate method. Briefly, 1 mL of each decimal dilution of the sample (10^{-1}-10^{-8}) was added to a sterile petri dishes and mixed with 20 mL of molten Plate Count Agar (PCA, Biokar, French) cooled to approximately at 45°C. The set agars were incubated aerobically at 30°C for 72 h. The counts of Fecal Coliforms (FC) was performed as for the total bacterial count but using a VRBL agar medium (brilliant green and phenol red agar, Biokar, French) and incubation of plates agar was realized at 44°C for 24 h. Counts were expressed as colony forming units per mL of milk.

Enumeration of *Clostridium perfringens*: The detection and enumeration of *Clostridium perfringens* was carried out in a selective medium TSN (Tryptone Sulfite Neomycin, Conda Pronadisa, Spain) by inoculation of 1 mL of raw milk and dilution (10^{-1}) into 15 mL of this medium after their heating at 80°C /10 min (to destroy vegetative forms). The incubation was performed at 46°C for 24-48 h.

Detection of *Staphylococcus aureus*: Detection of *S. aureus* was realized at mannitol salt agar (Conda Pronadisa, Spain) after enrichment at Giolitti Cantoni broth (Conda Pronadisa, Spain) supplemented with potassium tellurite (Pasteur Institute, Algeria). Plate agar were incubated for 24-48 h at 37°C and observed for bacterial growth. Suspect colonies subcultured on the same selective media plates and incubated at 37°C for 24 h in order to obtain a pure cultures. Pure cultures were further examined for morphological (convex elevation, smooth margin), staining and cultural characteristics and for biochemical characteristics (fermentation of mannitol, catalase, DNAase and coagulase production on rabbit plasma).

Detection of *Salmonella* sp.: For the detection of *Salmonella* sp., a pre-enrichment step was carried out in buffered peptone water (Conda Pronadisa, Spain) followed by an enrichment on Rapapport Vasiliadis Broth (Conda Pronadisa, Spain). The isolation was performed on Hektoen agar (Conda Pronadisa, Spain) after incubation at 37°C for 24 h.

Detection of *Brucella* antibodies and antibiotic residues: The detection of *Brucella* antibodies was made using *Brucella* Milk Ring Test (MRT). This test was performed by a dding 30 µL of *Brucella abortus* antigen (biovar 1, strain 99) (Veterinary Laboratories Agency, UK) to a volume of 3 mL of milk that has been stored at 4°C for at least 24 h. The tubes were incubated at 37°C for 1 h. If the blue colour in the cream layer at the top of the fluid is deeper than the remaining milk, the test is considered positive. If the intensity of colour in the cream layer is equal to or less than that in the milk portion, the test is considered negative. The detection of antibiotic residues in raw milk samples was performed according to the official European method for the detection of antibiotic residues in milk (Commission decision 91/180/EEC of 14 February, 1991), which is applied in European community since 1 January, 2002 (the European 91/180, CEE, EC Regulation Nr. 1664/2006). Two tests were successively used: Acidification test based on a possible inhibition growth of *B. stearothermophillus* variety *calidolactis* ATCC 10149, as indicated by the pH indicator (purple bromocresol, Sigma Aldrich, Germany), followed by a confirmation test, corresponding to the realization of three agar diffusion tests using three bacteria strains: *Bacillus stearothermophilus*, *Bacillus subtilis* and *Bacillus megaterium*.

Other analysis: Milk samples of collectors were submitted to the measurement of their density using a densimeter at a standardized milk temperature, in order to reveal a possible adulteration of milk.

Statistical analysis: The results of microbiological analysis (TBC and FC) were transformed into $\log_{10}$ in order to create a normality distribution. Data were analyzed using analysis of variance (ANOVA) with STATISTICA version 8.0. Differences were considered significant at $p<0.05$. The results are presented as square Mean±Standard Error of mean.

RESULTS

Hygienic practices during milking: The results of this study showed clearly the disregard and lack of interest accorded by farmers and collectors to hygienic practices. In most farms concerned by this study, the milking was conducted on salty conditions and the use of pre-dipping was absent. All famers clean the udder and teats using their hands or collective cloths, which can leads to increase the microbial contamination of milk and increase the risks of mastitis spread. Farmers let the cows suckle the calf before milking them, in order to eliminate foremilk from the udder, which is generally the most contaminated in bacteria. At farm level, the equipment used to store milk are an plastic or aluminum matter but a few farmers use a refrigerated tanks to maintain a low temperatures in order to prevent a high microbial contamination. The delivery time of milk to collections centres was considered very long. It was varied between 3 and 7 h, depending to the perimeter of collection and the number of farms associated with each collector. The majority of dairy farms are located far away the milk collection centres, causing a delay in the delivery of milk to the collection centres where cooling facilities are available. All collectors cleaned their isothermal tanks before each use, but the cleaning solution varies from one to another. Most of them, used a cold water, supplemented with soap and detergents. It was suggest that the microbiological quality of water using during cleaning was doubtful, contributing to contaminate the milk.

Table 1: Density of raw milk at the different collectors

	Collectors affiliated with dairies				
	1	2	3	4	5
Density	1.028±0.0005[b]	1.029±0.0003[a]	1.029±0.001[a]	1.029±0.001[a]	1.028±0.0005[b]

On the same line, the values followed by different letters are significantly different at p<0.05

Table 2: Total Bacterial Count (TBC) and coliform Fecal Count (FC) of raw milk at farms and collectors level

	Farms		Collectors	
	Log_{10}TBC	Log_{10} FC	Log_{10}TBC	Log_{10} FC
Dairies	(CFU mL^{-1})		(CFU mL^{-1})	
1	6.74±0.58[a]	1.73±0.83[a]	6.79±0.72[a]	1.53±0.47[a]
2	7.09±0.61[a]	1.40±0.41[a]	6.93± 0.36[a]	1.94±0.93[a]
3	6.62±0.58[a]	1.25±0.24[a]	6.86± 0.35[a]	2.42±0.87[a]
4	6.41±0.55[a]	1.68±0.59[a]	6.49± 0.48[a]	2.75±0.37[a]
5	6.77±0.69[a]	1.59±0.34[a]	6.99± 0.27[a]	2.67±0.95[a]
Mean	6.73±0.25	1.53±0.20	6.81±0.19	2.26±0.51

On the same column, the values followed by different letters are significantly different at p<0.05

Table 3: Total Bacterial Count (TBC) and coliform Fecal Count (FC) of raw milk at local markets level

Local markets	Log_{10}TBC (CFU mL^{-1})	Log_{10} FC (CFU mL^{-1})
1	5.70±0.35[a]	1.18±00[a]
2	7.28±0.64[b]	1.89±0.61[a]
3	7.73±0.49[b]	1.81±0.85[a]
4	8.09±0.48[b]	1.18±00[a]
Mean	7.20±1.05	1.51±0.38

On the same column, the values followed by different letters are significantly different at p<0.05

Table 4: Incidence of *S. aureus*, *Clostridium perfringens* and *Salmonella* sp. on raw milk

	No. of samples	No. of *Staphylococcus aureus* (%)	No. of *Clostridium perfringens* (%)	No. of *Salmonella* sp. (%)
Farms	75	13 (17.33)	2 (2.67)	0 (0)
Collectors	75	18 (24)	3 (4)	0 (0)
Local markets	24	12 (50)	0 (0)	0 (0)
Total	174	43 (24.71)	5 (2.87)	0 (0)

Table 5: Incidence of antibiotic residues and *Brucella* antibodies on raw milk

	No. of samples	No. of antibiotics residues (%)	No. of *Brucella* antibodies (%)
Farms	75	57 (76)	0 (0)
Collectors	75	60 (80)	0 (0)
Local markets	24	8 (33.33)	0 (0)
Total	174	125 (71.83)	0 (0)

Density of raw milk: Overall milk samples of collectors had a density below the preconized value (1.030). The minimum value of density was observed from collectors affiliated with both dairy 1 and dairy 5 with a value of 1.028±0.0005. While the maximum value was recorded at collectors affiliated with dairy 2, with a mean value of 1.029±0.0003 (Table 1).

Microbiological quality of raw milk: The microbiological analysis of milk samples taken at three levels of production chain revealed a high microbial contamination. The mean log_{10} of TBC at farms level was 6.73±0.25 log_{10} CFU mL^{-1}, followed by collector's tank milk and local market milk with mean values of 6.81±0.19 and 7.2±1.05 log_{10} CFU mL^{-1}, respectively (Table 2). The counts of TBC varied depending to the affiliation of farms and collectors to the different dairies (Table 2). The average count of TBC and FC at local markets are 7.2±1.05 and 1.51±0.38 log_{10} CFU mL^{-1}, respectively (Table 3).

The prevalence of *Staphylococcus aureus* on milk was 24.71%. This rate varies along the production chain. It was highest at markets level than collector's tank milk or farms level (Table 4). The contamination rate of milk by *Clostridium perfringens* was very low (2.87%). No contamination case by *Salmonella* sp. was revealed throughout the dairy chain (Table 4). A high contamination by antibiotic residues was observed for all samples taken along the production chain (Table 5). Out of 174 milk samples taken from three stages, 125 (71.83%) were contaminated. No contamination case by *Brucella* antibodies was detected (Table 5).

DISCUSSION

The density of milk collected from different dairies was below to the value preconized by Algerian norms (1.030), suggesting an adulteration. The normal density of milk is around 1.030-1.035. It varies according to the dry matter and inversely proportional to the fat content. Adulteration of milk by addition of water may introduce chemical or microbial health hazards as well as reducing the nutritional and processing quality and marketing value of milk[8]. The microbiological analysis of raw milk samples taken from various segments of production chain showed a high microbial contamination. The total bacteria count was more important in local market and collector's tank than farms level. The average load of TBC on farms was 6.73±0.25 log_{10} CFU mL^{-1}. This load increase to reach a values of 6.81±0.19 and 7.2±1.05 log_{10} CFU mL^{-1} at collector's tank and local markets, respectively. This study corroborates the

results of other research in smallholder areas in Algeria where counts of TBC exceeded largely the load fixed by Algerian norms (10^5 CFU mL^{-1}). Ghazi and Niar[5] announced that more than 91.78% of milk samples have a charge above 10^5 CFU mL^{-1}. Adjlane-Kaouche *et al.*[7] reported a load of TBC of 6.42±0.43 log_{10} CFU mL^{-1} for milk collected in the mid-Northern region of Algeria. This charge increase to reach an average value of 6.81±0.19 log_{10} CFU mL^{-1} during the collection circuit and a value of 7.2±1.05 log_{10} UFC mL^{-1} at market points. The high load of TBC may be explained by the intense microbial multiplication, favored by the no application of hygienic practices during milking and storage of milk. The use of plastic buckets during the milking and storage, the high milk temperature, the long distance between farms and collection centres and the lack of cold chain between farms to markets may be the main factors contributing to rapid bacterial multiplication[8]. The cleaning procedure during milking, cleaning of milking equipment and the hygiene in the handling of milk after milking requires attention to avoid contamination of milk Millogo *et al.*[9]. Bonfoh *et al.*[10] have noted that poor cleaning of containers which are an contact with raw milk, leaves a residual contamination levels (4.1 log_{10} CFU mL^{-1}). According to Faye and Loiseau[11], the microbial load of milk results from both the age of product (shelf life) but also it was linked to temperature of storage. The enumeration of total bacteria count is the most common method using by milk processing units to evaluate the hygienic quality of raw milk and therefore, it is an important indicator of hygienic conditions during milking at farm level[12]. The average load of TBC of milk at local market was 7.2±1.05 log_{10} UFC mL^{-1}. This value was higher than recorded by El-Marnissi *et al.*[13] who reported a charge of 4.5×10^5 CFU mL^{-1} for the milk sold in the city of Fes in Morocco. However, Millogo *et al.*[9] reported a charge of 10^7 CFU mL^{-1}, on milk sold in the markets of Burkina Faso.

The average contamination of raw milk at farm level by fecal coliforms was 1.53±0.20 log_{10} CFU mL^{-1}. This value was lower than announced by Aggad *et al.*[4] who reported a load of 10^3 CFU mL^{-1} and higher than announced by Ghazi *et al.*[14] who reported an average of 17 CFU mL^{-1}. The charge of fecal coliforms increase to achieve a load of 2.26±0.51 log_{10} CFU mL^{-1} at collection centres. The abundance of fecal coliform bacteria in raw milk reflects the non-observance of sanitary measures required during milking, and probably the existence of cross contamination during storage of milk. The main vectors are usually related to the integument of the teat skin, soiled by feces and milking equipment poorly cleaned[12].

This study revealed that 24.71% of milk samples were contaminated by *S. aureus*. The frequencies of isolation varied from segment to another. It was important on local market and collectors than farm level. Aggad *et al.*[4] and Ghazi and Niar[5] announced an important isolation frequencies that are in the range of 54.54 and 82%, respectively. While, Adjlane-Kaouche *et al.*[7] reported a frequency of 33.33% at the end of milk collection. The higher prevalence of *S. aureus* on milk explain the role that this bacteria play on microbial contamination of food animals. The *S. aureus* is known to be responsible for a significant proportion of subclinical and chronic mastitis in dairy cows. Adding to that, nasal carriage of *S. aureus* in humans and pets is the main reservoir of this germ, which could contribute to this contamination. Kamal *et al.*[15] reported that hand swabs of dairy workers and food handlers revealed high frequency of Coagulase Positive (CP) *S. aureus* colonizing their skins. Eighty percent of hand swab sample were positive for CP *S. aureus*; consequently, they may constitute another sustainable source of CP *S. aureus* contamination of dairy products. This situation does not remove a mutual transmissions between humans, animals and the food matrix. The absence of a real pasteurization step, the presence of cross contamination and the absence of a continuous refrigeration system in most local markets, as well as the bad practices (conservations storage at ambient temperatures, especially in summer) could be explain the contamination by *S. aureus*. In case of consumption of raw milk, there can be a real hazard, the main threat being the fact that about 10% of mastitis staphylococci are known to be producers of enterotoxin. This toxin may be produced when *S. aureus* counts exceed[16] 10^5 CFU mL^{-1}.

The prevalence of *Clostridium perfringens* in milk samples remains low (2.87%), this is probably related to the sampling period, which is itself linked to the alimentation of dairy cows. The dairy cows feed on fresh grass in pastures instead of silage, which constitute an important source of bacterial spores. These results are similar to those announced by Afif *et al.*[17] who reported a contamination rate around 0%. Nevertheless, Aggad *et al.*[4] reported that sulphite-reducing Clostridia were detected in 29% of raw milk, with a lower load than that prescribed by Algerian standard (50 spores mL^{-1}). No contamination by *Salmonella* sp. was revealed. These results are similar to those announced by Hakem *et al.*[6] and Afif *et al.*[17] who indicate the absence of *Salmonella* in milk samples. The results of this study revealed that all milk samples were not contaminated by *Brucella* antibodies. The absence of *Brucella* antibodies can probably be explained by the results of companions of vaccination and screening to eradicate brucellosis outbreaks in Algeria areas. These results do not corroborate with those announced by Hakem *et al.*[6] who reported a contamination rate of 7.84%. Yabrir *et al.*[18] unregistered a contamination rate of 13.73% in sheep milk at

central of Algerian steppe. The ring test sometimes gives a false positive results if the milk sample contains colostrum or consisting a milk obtained from mastitis cow. For this, other serological test are necessary to detect a *Brucella* cows.

The detection of antibiotic residues in 71.83% of milk samples reflects the abusive use of antimicrobial agents by smallholder producers. Ours results do not corroborate with those announced by Ben-Mahdi and Ouslimani[19], who indicated a low contamination rate (9.87%). Adjlane-Kaouche *et al.*[7] found a contamination level of 30%, using a Delvotest method. Variable frequencies of antimicrobial residues in milk have been reported by other studies. Srairi *et al.*[20], Kouame-Sina *et al.*[21], Adesiyun *et al.*[22] and Kivaria *et al.*[16] were announced a contamination rates of 25, 24, 7 and 6.5%, respectively. The high contamination rate by antibiotic residues can probably be explained in part by the massive and uncontrolled use of intra-mammary pharmaceutical preparations during treatments and preventions applied against mastitis cows and the no respect of withdrawal periods. On the other hand, this rate is due to an intentional addition of germ growth inhibitors such as antibiotics in order to stabilize the microbial quality of milk[23]. Among other reasons which may be explain this high contamination, the errors committed by the farmers such as accidental mixing of milk obtained from a cow treated with other untreated and accidental mixing of medicated feed with the ration of lactating cows[24]. The presence of antibiotics residues in milk is a public and economic concern, because of the risk of impaired health in persons who consume milk and the interference with manufacturing of dairy products by the antibiotics present in milk[16].

CONCLUSION

The sanitary quality of milk meets many challenges, it is firstly, a necessary condition to ensure the health of consumers and secondly the issue of quality is essential in the sector because it largely determines the economic development of dairy sector. This study was revealed a high microbial contamination of milk along the production chain, which indicated that consumption of raw milk pose a health hazards. The high bacterial count in milk can attributed for many factors. Among them, the lack of hygiene practices during milking, storage and handling of milk, the increase of delivery time of milk to collection centres and the absence of a continuous system of refrigeration in order to minimize the bacterial multiplication. The improving of milk quality needs the training and education of farmers, collectors and milk sellers in milk hygiene and the application of quality policy based on payment of milk according to the bacteriological quality.

ACKNOWLEDGMENT

The authors would like to acknowledge the smallholder dairy farmers and collectors for their cooperation and for samples collection.

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