Microbiological quality of the raw cow milk at three rural communes of the eastern region of Morocco

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Abstract

The microbiological safety of raw cow milk from collection centers of three regions in the north-east of Morocco has been studied. It involved 80 samples and was extent over a period of three years. The samples were analysed for the enumeration of Total Plate Count (TPC), total coliforms (TC), fecal coliforms (FC), *Staphylococcus aureus* (S. aureus) as well as the prevalence of pathogens such as *Listeria monocytogenes* and *Salmonella* sp. The means counts per ml of TPC were 1.4×10^6 CFU/ml. The total coliforms and fecal coliforms were also high with respective averages of 2.6×10^3 CFU/ml and 1.9×10^2 CFU/ml. Pathogenic staphylococci have been detected in 23% of samples with an average count of 1.7×10^3 CFU/ml. Samples from the Sidi Bouhria commune were more contaminated than the other two stations especially for TPC and FC ($p < 0.01$). However, no significant difference ($p > 0.05$) was observed in microbial load between the beginning and the end of the study. Basing the regulations applied in Morocco, 75% of samples showed an unsatisfactory quality with respect to the TPC, 52% with respect to fecal coliforms and 21% vis-a-vis *Staphylococcus aureus*. *Salmonella* sp. was not detected in all the samples, while *Listeria monocytogenes* was detected in 3% of samples (1 of 35).

Introduction

Milk, a highly nutritious food for its richness in carbohydrates, proteins, fats, vitamins and minerals, may nevertheless be associated with many serious diseases (Oliver *et al*., 2005). Various types of bacteria that may be present in raw milk, such as *Salmonella, E. coli* and *Listeria monocytogenes*, can cause serious public health problems. These germs may have acquired multiple antibiotic resistances (Dadie *et al*., 2010). Consequence, treatments are complicated and antibiotics become ineffective or less reliable. Children, pregnant women, the elderly and people whose immune system is weakened, are subject to a higher risk (Denny *et al*., 2008; Dominguez *et al*., 2009; Oliver *et al*., 2009). The sale of raw milk is prohibited in some countries such as Canada. While in France, the production and consumption of raw milk delivered as such to the final consumer is subject to strict conditions that farms must meet (Order of 13 July 2012 published in the Official Journal of the French Republic No. 0168 of July 21, 2012).

In Morocco, the circuits of hawking and consumption of raw milk, including the feeding of calves, represents 40% of national milk production.

Hawking of milk is based on informal activity of milk collectors from farmers. The sale of this raw milk in urban centers to traditional dairies, cafes or directly to the consumer, present a problem of competition with the organized sector which represents 60% of national milk production (Akesbi *et al*., 2008). Even more, the sale of raw milk in these uncontrollable conditions poses a serious risk to the consumer (Oliver *et al*., 2009).

Unfortunately for cultural and social reasons, the consumption of dairy products such as ''Jben'', ''Rayeb'', '' Lben'' and butter in traditional dairies continues to have great social importance in urban areas in Morocco. The evaluation of the hygienic quality of raw milk intended for the manufacture of these products is then essential for protecting the health of consumers.

The objective of our study was to evaluate the microbiological quality including the presence of pathogens in raw milk, in three rural communes (RC) of the Eastern Region of Morocco (RC of Sidi Moussa, RC of Sidi Bouhria, and RC of Oued Za).

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Figure1. Location of the study area
CC Sidi Moussa: collect center of Sidi Moussa
CC Sidi Bouhria: collect center of Sidi Bouhria
CC Taourirt: collect center of Taourirt

Materials and Methods

Collection centers involved in this study were supplied by dairy farms located in three different rural communes. These are situated at varying distances from Oujda city, where milk is marketed: the rural commune of Sidi Moussa (12 Km south-west of Oujda city), the rural commune of Sidi Bouhria (44 Km to the west of Oujda city) and the common of Oued Za (110 km to the West of Oujda city) (Figure 1).

Eighty samples of raw cow milk were collected from the three areas mentioned above over a period of three years. The samples, harvested aseptically, were maintained at 6°C in a thermo-electric cooler for less than 24 hours, until analysis.

Monitoring the quality of raw milk samples covered the pH and microbiological analyses. The pH was measured using a pH meter type (Cyberscan pH1500) calibrated at pH 7.02 and 4.00. Microbiological analyses concerned: enumeration of the Total Plate Count (TPC) in accordance with ISO 4833 standard method (Anon, 2003), enumeration of total coliforms in accordance with ISO 4832 standard method (Anon, 2006) and fecal coliforms using V 08-060 standard method (Anon, 2009). The enumeration of Staphylococcus aureus was performed in accordance with ISO 6888-2 standard method (Anon, 1999), the detection of Salmonella spp using ISO 6579 standard method (Anon, 2002) and Listeria monocytogenes in accordance with ISO 11290-1 standard method (Anon, 1991). Sample preparation and decimal dilutions were done in accordance with ISO 6887-5 standard method (Anon, 2010).

Interpretation of results was made according to the Order No 624-04 of 17 Safar 1425 (8 April 2004) related to the microbiological standards to be met by animal and animal-origin products (official journal No. 5214 of May 2, 2004). For comparative purposes we also included the regulation applied in Luxembourg (Table 2).

Results and Discussion

The results of the analysis showed pH values ranging from 6.0 to 6.9 units with an average of 6.5 pH units (Table 1). 65% of the samples are in the normal range of pH of raw cow's milk which is of the order of 6.5 to 6.8 (Mathieu, 1998). The variability of results is low (coefficient of variation (CV) = 3%) and the estimated average is reliable. Also there was no significant difference between the pH of the milk from the three communes, or between the beginning and end of the study period ($p > 0.05$).

The pH results we recorded are similar to those obtained in previous studies (Labioui *et al*., 2009; El Marnissi *et al*., 2013). The percentage of samples exceeding the standard range could be attributed to the metabolic activity of the microbial flora due to the poor hygienic conditions of milk handling, as reported by other authors (Alais, 1984; Mathieu, 1998). These authors reported that pH values are also related to the content of casein, minerals and ions.

The results of microbiological analyses (TPC, TC, FC, *Staphylococcus aureus*) showed that raw milk collected during the study period present a significant contamination (Table 1). Enumeration of the TPC fluctuates from 7×10^3 to 1.5×10^8 CFU/ml, with an average of 1.4×10^6 CFU/ml, this average value exceeds 1 to 1.2 log unit of that of the minimum threshold of tolerance (3×10^5 CFU/ml) allowed for the production of raw milk destined for direct consumption (Table 2). Only 25% of samples have a lower load than the minimum threshold, and are therefore of satisfactory quality (Table 1).

This high charge of the total flora and the large number of samples exceeding the recommended limits reveals a serious failure in good hygiene practices during milking and milk handling after collection. In this sense we noted that the majority of the raw milk arrives at the collection center to a temperature above 10°C. (Information provided by the collection center)

Similar results of the total flora in raw milk were obtained in other studies in Morocco. Hadrya *et al*. (2012) reported values ranging from 2.7×10^5 to 7.0×10^9 CFU/ml at the city of Kenitra. Labioui *et al*. (2009) recorded values ranging from 2.6×10^6 to 12.0×10^6 CFU/ml. Chye *et al*. (2004) Showed that the raw milk collected from farms in Malaysia had an average load of 1.2×10^7 CFU/ml. These authors also noted that all parts of Malaysia seem to provide milk with a total microbial load exceeding the limit (10^6

Table 1. Charge (CFU/ml) of different microorganisms of raw cow milk recorded in all three communes of the eastern region of Morocco during the all study period

	TPC	TC	FC	S. aureus	pH
Min.	7.0×10^3	<1	<1	<1	6.0
Mean	1.4×10^6	2.6×10^3	1.9×10^2	1.7×10^3	6.5
Log (Mean)	(6.16)	(3.42)	(2.28)	(3.22)	
Max.	1.5×10^8	2.5×10^6	1.0×10^6	4.0×10^4	6.9
CV	16%	54%	75%	12%	3%
% Of unsatisfactory samples.	75%	---	52%	21%	35%

(Min.: minimal value, Max.: maximal value, Mean: mean value, CV: variation coefficient, TPC: total plate count, TC: total coliform, FC: fecal coliform; S.aureus: *Staphylococcus aureus)*

Table 2. acceptability threshold required in raw cow milk according to local standards in Morocco and Luxembourg

TPC	Fecal Coliforms	Staphylococcus aureus	Salmonella spp	Listeria monocytogenes	Source
3×10^5 CFU/ml	10^2 CFU/ml	--	Absence in 25ml	---	Morocco [a]
5×10^4 CFU/ml	---	100 CFU/ml	Absence in 25g	10^2 CFU/ml	Luxembourg [b]

a: microbiological criteria applied in Morocco (Official Bulletin No. 624-04, 17 Safar, 1425 (8 April 2004))

b: Microbiological criteria for foodstuffs in Grand-Duche de Luxembourg

CFU/ml) established by the Department of Veterinary Services of Malaysia. According to these authors this finding may be due to the infected udder, unsanitary equipment or procedures of milking, and/or improper microbiological quality of water used for cleaning of utensils and animals, as well as conditions of milk storage.

On their side, Nero *et al.* (2004) found that 75.7% of the samples have a load of TPC>10^5 CFU/ml, while Arcuri *et al*., (2006) found that 46% of herds have a load of TPC <10^5 CFU/ml. Similarly D'Amico and Donnelly (2010) found that 86% of samples had a load of TPC <10^5 CFU/ml. The enumeration of coliforms showed a very significant contamination with averages of 2.6×10^3 CFU/ml for total coliforms and 1.9×10^2 CFU/ml for fecal coliforms (table 1). The variability of results was very high for these indicators of contamination with a coefficient of variation of 54% for TC and 75% for FC. This high and variable contamination would support a lack of control of hygiene and handling of raw milk between breeders.

The presence of coliform bacteria does not necessarily indicate a direct fecal contamination of milk, but more specifically exhibits poor hygiene and sanitation practices during milking and other manipulations. On the other hand the presence of fecal coliforms (FC) in milk is highly associated with the risk of contamination with other enteric pathogens. (Van Kessel *et al*., 2004)

Intermediate loads of FC, higher than ours and varying between 8.1×10^6 and 1.1×10^8 CFU/ml, were reported by Hadrya *et al*. (2012) at dairies in the Kenitra city. Moreover, Labioui *et al.* (2009) noted

an average contamination in total and fecal coliforms of 2.0×10^4 and 5.2×10^3 CFU/ml, respectively. The difference between these results may be due to a difference in awareness of farmers to control hygiene, transport and storage conditions. Climate and environmental conditions could be involved in this difference; the city of Oujda is situated in an arid climate, whereas the city of Kenitra has a humid climate.

Chye *et al.* (2004) reported average loads of 1.7×10^4 CFU/ml for TC and 6.8×10^3 CFU/ml for Escherichia coli (FC). The mean of fecal contamination (FC) of raw milk recorded in the region exceeded the minimum threshold of tolerance (10^2 CFU/ml) applied to raw milk for human consumption (Table 2). Also, more than half of the samples (52%) analysed have a load of FC exceeding the minimum recommended (Table 1).

Staphylococcus aureus was isolated in 23% of the samples with an average of 1.7×10^3 CFU/ml (Table 1). This value is higher than that allowed by the Grand Duchy of Luxembourg (m = 100) (Table 2). Furthermore, 21% of the samples (all stations combined) showed values greater than 10^2 CFU/ml (table1). In comparison with other studies in Morocco, our results are lower than those reported by Hadrya *et al*. (2012). These authors found a mean of 1.4×10^4 CFU/ml with percentage of unsatisfactory samples ranging from 15 to 35%. In Brazil, Costa Sobrinho *et al*. (2012) found that 87% of the samples were greater than 10^2 CFU/ml. Those authors showed that 46.1% of the samples had values greater than 103 CFU/ml.

In Norway, Jorgensen *et al.* (2005) detected *S.*

Table 3. Variations loads (CFU/ml) of different microorganisms depending on the areas studied

		Sidi Moussa	Sidi Bouhria	Oued Za
TPC	Min.	3.85	6.70	5.00
	Mean	**8.3x10^5**	**1.9x10^7**	**1.5x10^6**
	(MeanLog ± SD)	(5.92 ± 0.96)	(7.28 ± 0.41)	(6.18 ± 0.59)
	Max.	8.18	7.85	6.81
	(CV)	(16%)	(6%)	(10%)
TC	Min.	0.00	2.95	0.00
	Mean	**10^3**	**1.1x10^5**	**5.1x10^3**
	(MeanLog ± SD)	(3.01 ± 1.84)	(5.03 ± 1.29)	(3.71 ± 1.44)
	Max.	6.40	6.18	5.90
	(CV)	(61%)	(26%)	(39%)
FC	Min.	0.00	0.00	0.00
	Mean	**1.1x10^2**	**2.8x10^3**	**1.5x10^2**
	(MeanLog ± SD)	(2.04 ± 1.59)	(3.45 ± 1.88)	(2.19 ± 1.70)
	Max.	5.90	6.00	5.15
	(CV)	(78%)	(55%)	(78%)
Staph. aureus	Min.	0.00	0.00	0.00
	Mean	**2.4x10^3**	**8x10^2**	**2.3x10^3**
	(MeanLog ± SD)	(3.4 ± 0.7)	(2.9 ± 0.5)	(3.4 ± 0.4)
	Max.	4.60	3.30	3.65
	(CV)	(22%)	(17%)	(12%)

SD: standard deviation;
Values between bracket correspond to decimal log values;
Coefficient of variation (CV) = (standard deviation / mean) x 100

Table 4. Variations of mean loads (CFU/ml) of different microorganisms between the beginning and the end of study

		TPC	TC	FC	S. aureus
Beginning of the study	Mean	1.9x10^6	2.0x10^3	6.7x10^2	2.7x10^3
	% NS	81%	--	58%	15%
End of the study	Mean	2.9x10^6	4.9x10^3	9.9x10^1	1.8x10^3
	% NS	86%	--	49%	31%

NS%: percentage of unsatisfactory samples

aureus in 75% of the samples of raw cow milk used for the production of raw cheese. 56.8% of these samples exceeded the permissible value of 10^2 CFU/ml.

On the other hand, Jakobsen *et al.* (2011) detected *S. aureus* in 47.2% of samples of raw cow milk; this frequency is higher than that of the United States (D'Amico *et al.*, 2008) which was 27.4%. The presence of *S. aureus* in our samples still supports a non-compliance with good hygienic practices during milking operation by farmers. The presence of *S. aureus* and the possibility of production of staphylococcal toxin could be a potential risk to public health and thus represent a major concern for the safety and quality of dairy products traditionally prepared from raw milk.

The comparative study between the different areas studied (Sidi Bouhria, Sidi Moussa, and Oued Za) showed variability in the results (Table 3). The coefficients of variation ranged from 6 to 16% for the TPC, 26 to 61% for TC, 55 to 78% for FC and 12 to 22% for *S. aureus* (Table 3). This variability in the results implies the existence of a great variability in the control of sanitary conditions and good practices during milking and also to the level of awareness of different breeders to sanitary practices. Samples from Sidi Bouhria area were more contaminated than the two other areas, especially for TPC and FC (p<0.01). However, this difference was not significant between Sidi Moussa and Oued Za (p>0.05).

The comparison of our results between the beginning and the end period of our study (Table 4) showed no significant variation for the TPC, TC and *S. aureus* (p> 0.05). However, we recorded a slight decrease of FC of about 0.9 log unit. Furthermore, the percentage of unsatisfactory samples remained high for the FC and TPC. This percentage has actually increased in the case of *S. aureus* (Table 4)

L. monocytogenes has been researched in 35 of the 80 samples studied. The results obtained showed that only 1 of 35 samples tested positive. *Salmonella* were not isolated on the 80 samples analyzed. There are no Moroccan standards for *L. monocytogenes* in raw milk (Table 2); however, the absence of *Listeria* sp. in 25 g was defined as standard for cheese made from raw milk. D'Amico et al. (2008) showed that *L. monocytogenes* was detected in only 3 of 133 raw milk samples, and no samples yielded *Salmonella* spp. Similar results were obtained by D'Amico and Donnelly (2010) in raw milk used for small-scale artisan cheese production in Vermont, these authors showed that none of the pathogens was detected in 101 samples analyzed.

The presence of *L. monocytogenes* in our samples at a very low level (1/35) may be due to the initial microbial load of raw milk, which was usually above 5 log units CFU/ml. This high load of microorganisms influences the survival or growth of *L. monocytogenes* in raw milk. Also the antagonistic activity of lactic acid bacteria against *L. monocytogenes* has been described in dairy products (Suh and Knabel, 2001; Carvalho *et al.*, 2006; Nero *et al.*, 2008).

Conclusion

The results we found indicate that the quality of raw milk produced by farmers in the eastern region of Morocco is associated with high levels of total and fecal coliforms contamination and a significant incidence of *S. aureus*. Otherwise an absence of *Salmonella* spp. and a very low incidence of *L. monocytogenes* were observed. The control of the microbiological quality of raw milk is certainly very useful, but still insufficient. Therefore, it is recommended that the training, guidance and advice on the use of appropriate hygiene practices during milking and processing of raw milk must be provided. The owners of farms and their workers implicated in trafficking operations and handling of raw milk have to apply firmly these guides of good practice.

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