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Hygienic quality of bovine raw milk samples from Oulmes local breeds in Morocco

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Abstract: *Milk composition and safety are essential factors for both commercial and human health reasons. Here we characterized the microbiological quality of raw cow milk collected in the Oulmes region (Middle Atlas, Morocco).Forty milk samples were collected during autumn 2013, winter 2014, and spring 2014.Microbiological analysis showed that the mean mesophilic aerobic flora count was $15.7x10^2$ CFU/ml and the mean total coliform count ranged from $5.5x10^2$ to $22.3x10^2$ CFU/ml; therefore, approximately 5% of samples were contaminated by fecal coliforms. Staphylococcus was absent in autumn samples. 50.5% of analyzed samples contained lactic acid bacteria while 20% contained yeasts and molds. Salmonella spp., Listeria monocytogenes, and anaerobic sulfite reducing bacteria were absent in all analyzed samples.*

Keywords: *milk, Oulmes race, microbiological analysis, pathogenic bacteria.*

I. Background

The dairy cattle industry is an important part of Moroccan agriculture that generates income and employment opportunities. However, genetic modifications, the import of dairy cattle, and artificial insemination (nearly 300,000 heifers from 1970 to 2006) have a profound effect on live stock structure [1]. Thus, local breeds have decreased from 90% of total in 1975 to around 53% in 2004 [2]. Of the main Moroccan breeds, Oulmes-Zaer was identified as early as 1912 and recognized by ministerial decree in 1982. This original race, indigenous to the Middle Atlas of Morocco, is reared for milk and meat production and is well adapted to the harsh environment [2].

The farming system in the Oulmes region is traditional and adapted to the region's breed and topography. Oulmes-Zaer is the only local breed to have benefited from a breeding program, which has resulted in a suckling breed well suited to mountainous areas due to its small size and ability to adapt to production conditions (traditional, extensive).It also has genetic growth potential, prompting interest in farmers about this genetic potential and a desire to preserve it [2,3,4].

To the best of our knowledge, there have been no studies on milk hygiene in this region. Thus, the main objective of this research was to evaluate the microbiological quality of local bovine raw milk produced in the Oulmes steppe eco region to highlight the impact of livestock and hygiene practices on milk quality.

II. Methods

II.1 Study sites

This study was conducted in rural Oulmes, located 150 km northwest of Rabat city. Oulmes is in the high mountains of the Middle Atlas in the Berber Tamazight area. This mountainous zone provides highlands located 1260 m above sea level, 33°25'0"N and 6°1'0"W. This region is known for its local cattle breed, the blonde Oulmes-Zaer, a traditional and original Middle Atlas race reared for both milk and meat production [5].

II.2. Sample collection

One sample was collected from each of40 cows. Cows were sampled over three periods during one year: the first between early October and late November 2013, the second between mid-January and late February 2014, and the third between late March and late May 2014. This sampling strategy was selected in the knowledge that the lands cover changes during each season, which might have an impact on milk hygienic quality.

Samples were collected aseptically from all animals in natural pasture. Raw milk was obtained in the morning by hand milking directly into sterile bottles without preservative before being stored at 4°C. All samples were analyzed immediately after arrival at the laboratory [6].

II.3. Microbiological evaluation

Samples were analyzed for their microbiological quality and safety and the prevalence of selected bacterial pathogens. Samples were serially diluted as follows: 1 ml of milk was suspended in 9 ml of peptone water and milk dilutions between 10^{-1} and 10^{-3} studied [7, 8]. The selective media used for isolation and enumeration of colonies are described in Table1.

The isolation and enumeration of mesophilic aerobic bacteria, total coliforms, fecal coliforms, lactic acid bacteria, *Salmonella* spp., *Listeria monocytogenes*, *anaerobic sulfite reducing bacteria*, *staphylococcus*, and yeasts and molds were carried out according to international standards [9, 10, 11, 12].

Table 1. Selective media used for isolation and incubation.

Germs	Medium	Temperature (°C)
Mesophilicaerobicflora	Plate count agar	30
Total coliforms	Violet red bile lactose agar	37
Fecal coliforms	Violet red bile lactose agar	44
Lactic bacteria	De Man, Rogosa, and Sharpe agar	37
Yeasts and molds	Sabouraud Dextrose agar with chloramphenicol	30
Anaerobic sulfite reducing bacteria	Sulfite polymyxin sulfadizine agar	37
Salmonella spp.	Peptone water Selenite cysteine Salmonella shigella	37
Listeria monocytogenes	Enrichment broth Oxford	37
Staphylococcus	Baird Parker Agar	37

III. Result and discussion

Microbiological quality is important for milk preservation and/or transformation [7, 13,14]. The presence of contaminating fecal microorganisms is an indicator of the product's hygiene. Forty raw milk samples were analyzed over three seasons (autumn, winter, and spring) as shown in Table 2.

Table 2. Microbiological analysis of raw milk samples over three seasons

Total flora	Season	Mean (CFU/ml)
Total aerobic mesophilic flora	Autumn Winter Spring	2×10^3 1×10^3 2×10^3
Total coliforms	Autumn Winter Spring	6×10^2 Abs 3×10^3
Fecal coliforms	Autumn Winter Spring	Abs Abs 2×10^1
Staphylococcus	Autumn Winter Spring	Abs 2×10^3 2×10^3
Lactic acid bacteria	Autumn Winter Spring	2.5×10^3 2×10^3 3×10^3

Abs: absent

The total aerobic mesophilic flora number is considered the best indicator of the hygienic quality of raw milk and is, therefore, the commonest microbiological analysis in this setting. Raw milk was contaminated during the three seasons, with an average of $2x10^3$, 10^3, and $2x10^3$ CFU/ml for autumn, winter, and spring, respectively. However, this contamination can be regarded as minimal compared to French standards or quality assessment of American raw milk, which have maximum tolerated loads of $5x10^5$ and $3x10^5$ CFU/ml, respectively [15].

These results are in contrast to Hadrya et al. [16], who showed that the total aerobic mesophilic flora load in commercial milk significantly differs between seasons. However, of note, the maximum value of $2x10^3$ CFU/ml was obtained during the period with the highest temperatures, and the minimum value of 10^3 CFU/ml was obtained during a low temperature period. According to Quebec government regulations on microbiological standards for dairy products, the total mesophilic bacteria count in milk should be lower than $1x10^5$ CFU/ml [17]. According to these standards, these milk samples would not be deemed contaminated.

Fecal coliform levels showed a similar pattern: there was a total absence of these organisms during autumn and winter with an average spring value of $2x10^1$ CFU/ml. Total coliforms were similarly absent in winter but were present at an average of $3x10^3$ and $6x10^2$ CFU/ml in spring and autumn, respectively.

A previous Moroccan study showed that there are generally high numbers of coliforms in raw milk [18]. Ounine et al. [19], in their study of milk in the Gharb region, reported counts of $1.07x10^7$ and $1.99x10^6$ CFU/ml for total and fecal coliforms, respectively, which are higher than the current study.

In a study conducted in Benin, Farougou et al. [6] found an average of $9.2x10^2$ CFU/ml total coliforms, higher than the average levels that we found in spring ($3x10^2$ CFU/ml),perhaps due to the climate, especially temperature. Conversely, total coliforms density was different between seasons, similar to Hadya et al. [16].

However, we note that the number of *Staphylococcus* in Oulmes raw milk was lower than that observed in the Tadla region, where the values ranged between $0.8x10^3$ and $5x10^3$ CFU/ml [20]. Here, *Staphylococcus* were completely absent in autumn, with an average of $2x10^3$ CFU/ml in winter and spring. Therefore, Oulmes raw milk can be considered a staple food with no measurable health risk.

The average lactic acid bacteria load in Oulmes raw milk samples was $3x10^3$ CFU/ml, with higher average counts in the spring ($3x10^3$ CFU/ml) followed by autumn ($2.5x10^3$ CFU/ml) and winter ($2x10^3$CFU/ml). These results are much lower than those detected by Afif et al. and Rhiat et al. [20, 21], i.e., $13.01x10^6$ and $8.32x10^5$ CFU/ml, respectively.

As noted indicated above, high levels of lactic acid bacteria were present in the spring. Spring is also a warm season in this region, providing the necessary conditions for lactic bacteria proliferation and thus increased acidity. Lactic acid bacteria may also be present on the surface of the teats or, more generally, on the bodies of cows [22].

The average load of yeasts and molds was $3x10^3$ and $2x10^2$ CFU/ml, respectively, in spring, with a total absence in autumn and winter. These values were low compared to those reported by Rhiat et al. in milk from the Gharb region [21], who found an average of $9.71x10^3$ CFU/ml. The molds were less abundant than the other flora consistent with Benalia et al. and Tormo et al.[23, 24]

We examined bacteria that are a known health risk such as *Salmonella* spp., *anaerobic sulfite reducing bacteria*, and *Listeria monocytogenes*. None of these organisms were found in any sample, the same as reports from Switzerland and Morocco [25, 20].

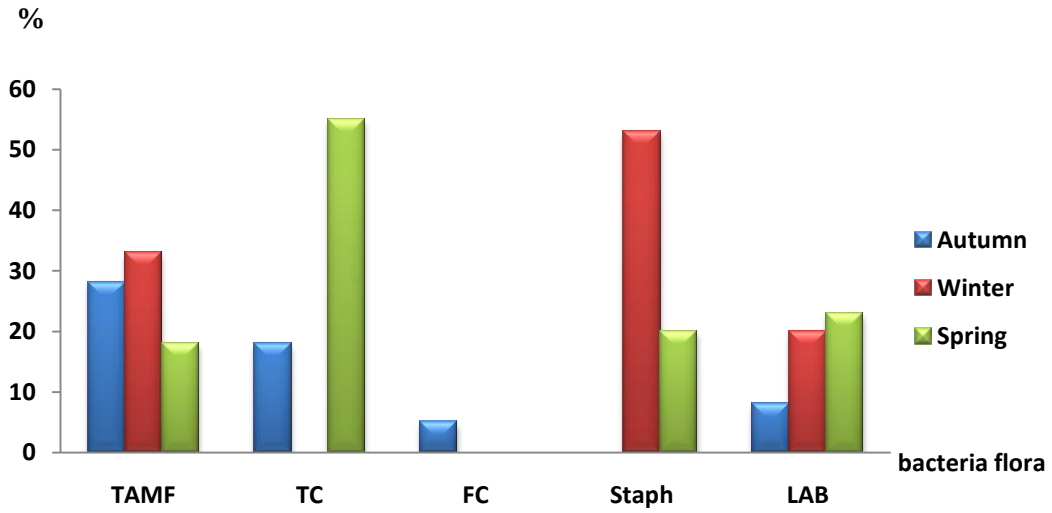

Figure 1: Hygienic quality of bovine raw milk during the three studied seasons.

Overall, Oulmes local bovine raw milk had low microbial flora levels over the three seasons (autumn, winter and spring) compared to the microbial limit guidelines reported by Alais and Blanc, Bonfoh et al. in Mali, Chye et al. in Malaysia, Reglement 2073/2005/CE, Ferhan and Salik in Pakistan, Benalia in Algeria, and Ounine et al. and Afif et al. in Morocco, [15, 19, 20, 23, 26, 27, 28, 29]. Therefore, Oulmes bovine raw milk appears to be safe.

IV. Conclusion

Overall milk quality is multi factorial. This is particularly true in the arid steppe environment, where direct environmental factors and indirect factors such as vegetation cover, the livestock itself, and hygiene practices during collection must all be taken into consideration. The microbiological quality of raw milk from

Oulmes cattle appears satisfactory, with lower contamination values compared to several studies in Morocco and beyond. Nevertheless, quality policies are required to promote good farming practices including animal cleanliness, consideration of the immediate environment, and safe trafficking.

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