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Quantitative and qualitative microbial diversity of the raw cow's milk sold by street trading in Meknes, Morocco

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ABSTRACT

Aims: Milk is rich of nutrients that are necessary for the growth of various microorganisms. The aim of the present study was to evaluate the microbial quantity and quality of the raw cow's milk sold through street trading in Meknes, Morocco, and to study the variation and seasonal relationship of microbial diversity during the four seasons of the year.

Methodology and results: Raw cow's milk samples were collected randomly between May 2015 and April 2016 from 3 street trading sale points, two popular neighborhoods (station 1 and station 2) and one popular market, and they were analyzed microbiologically. The results showed that the contamination rates of Total Plate Count (TPC), total coliforms, fecal coliforms, lactobacilli, lactococci and yeasts and molds were 8.8×10⁸ CFU/mL, 8.9×10⁵ CFU/mL, 2×10³ CFU/mL, 4.6×10⁸ CFU/mL, 7.5×10⁸ CFU/mL and 4.1×10³ CFU/mL, respectively. Moreover, *Escherichia coli, Staphylococcus aureus, Clostridium perfringens* and *Listeria monocytogenes* were detected in 66.67% (24/36), 75% (27/36), 36.1% (13/36) and 19.44% (7/36), respectively, while *Salmonella* was not detected in this study.

Conclusion, significance and impact of study: The highest microbiological count in raw milk samples was found in summer, while the lowest was detected in winter (p<0.005). Therefore, the quality of milk marketed in Meknes region of Morocco is deteriorated due to the lack of good hygienic conditions of raw cow's milk sold through street trading.

Keywords: Raw cow's milk, street trading, microbiological quality, season

INTRODUCTION

Milk is the integral product of the total and discontinued milking of a healthy, well-fed and undisturbed dairy female; milk must be collected properly and must not contain colostrum (Pougheon and Goursaud, 2001). It is a nutritive and valuable food product. In many countries, dairy products form a large portion of the daily diet of people, especially infants and children (Koushki et al., 2016). Milk is mainly composed of water, proteins, carbohydrates, lipids, vitamins and minerals (Schechter, 2009) and it is a rich source of nutrients and energy (Koushki et al., 2016). Several microorganisms are responsible for the spoilage of milk and dairy products (lactic acid bacteria) and cause foodborne diseases (Enterobacteriaceae, L. monocytogenes, Staphylococcus aureus, Mycobacterium tuberculosis), the strict anaerobic spore-forming Clostridia, molds and yeast, other Gramnegative pathogens and protozoans (Schaechter, 2009; Paszkiewicz et al., 2015; Koushki et al., 2016; Lan et al.,

2017). However, raw milk microflora contributes greatly to the sensory characteristic and bio-preservation of foods. There are many sources of contamination of milk, including the cow itself, the environment, water, and milking equipments (Lejeune and Rajala-schultz, 2009; Millogo *et al.*, 2009; Griffiths, 2010).

Poor quality of raw milk causes a health threat and economic burden; so many efforts are made to improve its quality (Mallet *et al.*, 2012). During the last decades, the development of food safety measures is applied to ensure safe and qualitative food products by involving Hazard Analysis and Critical Control Points. (HACCP) (Smigic *et al.*, 2012; Schaarschmidt *et al.*, 2016). Worldwide, food-borne illnesses are a major public health problem, with more than 200 diseases caused by improper foods that contain pathogenic bacteria, viruses, parasites or harmful chemicals (WHO, 2015).

In Morocco, food-borne outbreaks are very frequent, occurring in all regions of the country, and more widespread in summer and spring. They are accidental,

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affecting both young people and adults, and are generally nonthreatening although sometimes they are fatal (Ed-Dra *et al.*, 2017a). In 2015, the APPC (Anti-poison and Pharmacovigilance Center) identified 2887 cases of foodborne illnesses in Morocco, from which 60.8% were collective poisoning (CAPM, 2015). The incriminated products were meat and meat products (21.7%), dairy products (9.2%) fish and fishery products (8.7%) and composite foods (7.0%) (CAPM, 2015).

In Morocco, the delivering of milk and milk products from the farm to the consumer involves two types of marketing channels (Bouymajane *et al.*, 2018). The first one involves industrial collection centers. The second one is based on the selling of milk by street traders who collect it from the farms and sell it to traditional dairies (Mahlabates), coffee shops and urban households, using motorbikes and vans as means of transport (Hervieu, 2007). It should be noted that the raw milk marketed by street trader does not undergo any microbiological quality control. Nearly, 30% of the total quantities of milk were intended for cities (Hervieu, 2007).

The aim of the present study was to evaluate the microbial quantity and quality of the raw cow's milk sold through street trading in Meknes, northern central of Morocco.

MATERIALS AND METHODS

Sample collection

From May 2015 to April 2016, a total of thirty-six raw cow's milk samples were randomly collected from three selected street trading sale points, two popular neighborhoods (station 1 and station 2) and the souk, located in Meknes city (northern central Morocco). One liter of raw milk was collected aseptically into a sterile container in the cool box kept at 4 °C and brought to the Laboratory of Microbiology and health of the Faculty of Science of Meknes. The analyses were performed on the same day.

Microbiological analysis

Twenty-five grams of raw milk was added to 225 mL of buffered peptone water (Oxoid) and homogenized for 3 min at 260 rotations per min (rpm), using a Masticator (Stomacher 400 Circulator, Seward). Then, the decimal dilutions were carried out.

Total microbial count, total coliform, fecal coliform, and *E. coli*

The total microbial count was counted after incubation at 37 °C for 48 h (NF V08-051, 1999) on Plant Count Agar (PCA, Biokar). Total and fecal coliform were carried out on Violet Red Bile Glucose Agar (VRBL, Biokar), after incubation at 30 °C for 24 h (ISO 4832, 2006) and 44 °C (NF V08-060, 2009) respectively. *E. coli* colonies were confirmed by the indole test (V08-053, 2002).

Staphylococcus spp.

Staphylococcus spp. were plated out in the Baird Parker medium (BP, Biokar) agar containing 5% of the egg yolk after incubation at 37 °C for 48 h and confirmed by the coagulase test (NF V08-057, 2004).

Anaerobic sulfate-reducing bacteria

Anaerobic sulfate-reducing bacteria (ASR) were carried out, on Tryptone Cycloserine Sulphite (TCS) agar supplemented with D-cycloserine (Biokar) and incubated at 46 °C for 24 h (NM 08.0.125, 2012).

Molds and yeasts

Molds and yeasts were counted after incubation on Sabouraud dextrose agar with chloramphenicol (Biokar) at 25 °C for 5 days (ISO 7954, 2003).

Lactic acid bacteria

Lactococci and lactobacilli were plated on Rogosa and Sharpe (MRS) agar and M17 agar (Biokar) and incubated at 30 °C and 37 °C for 72 h and 48 h, respectively (ISO/ FDIS 15214, 1998).

Salmonella spp.

Salmonella spp. were isolated from different samples by using conventional methods and confirmed by Biochemical tests according to the method described previously (Ed-dra *et al.*, 2017b).

L. monocytogenes

Ten mL of raw milk samples were enriched on 90 mL of half Fraser Broth (Biokar), homogenized and incubated at 30 °C for 24h. Then, 0.1 mL of enrichment broth was inoculated onto 9 mL of Fraser Broth (Biokar) and incubated at 37 °C for 48 h. Approximately 10 μ L were streaked onto Polymyxin Acriflavine Lithium chloride Ceftazidime Aesculin Mannitol (PALCAM) agar plates (Biokar) and incubated at 37 °C for 24-48 h. Presumptive *L. monocytogenes* were purified on Trypticase Soy Agar (TSA, Biokar) and confirmed using Gram staining, catalase, oxidase, hemolysis test, Christie Atkins and Munch-Peterson (CAMP) test and API listeria (NM 08.0.110, 2004).

Statistical analysis

Simple descriptive statistical analyses were performed to evaluate the parameters of the microbial quantity and quality. Furthermore, to evaluate the existence of statistical differences between the parameters evaluated by season and site. Analysis of variance (ANOVA) was carried out using SPSS software (SPSS version 20, IBM Corp, Armonk, NY, USA). A *p*-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The findings of this study, concerning the microbiological analysis of raw cow's milk samples taken randomly from the different points of sale located in two popular neighborhoods and the popular market of the city of Meknes, are reported in Table 1.

Table 1: Microbial load of raw milk collected from street trading in Meknes city of Morocco.

	Mean (CFU/ mL)	Min (CFU/ mL)	Max (CFU/ mL)	%
Total plate count	8.8×10 ⁸	2×10⁵	9.6×10 ⁹	-
Total coliforms	8.9×10⁵	25	5.8×10 ⁶	-
Fecal coliforms	2×10 ³	10	9.2×10 ³	-
Yeasts and molds	11	4.4×10 ⁴	4.1×10 ³	-
Lactobacilli	4.6×10 ⁸	5×10 ⁴	8.1×10 ⁸	-
Lacbacocci	7.5×10 ⁸	5.5×10 ⁴	8.8×10 ⁸	-
C. perfringens	-	-	-	36.1
E. coli	-	-	-	66.67
S. aureus	-	-	-	75
L. monocytogenes	-	-	-	19.44
Salmonella spp.	-	-	-	0

Total plate count

Our study showed a high prevalence of total plate count (TPC) of 8.8×10⁸ CFU/mL (Table 1), which is much more noticeable in summer than in winter (Table 2). This means that it is higher than the limit set by Moroccan standards (3×10⁵ CFU/mL) (Ministry of Agriculture, 2004). Several factors may be involved such as the physical state of the cow, unhygienic milking equipment, microbiological quality of water and milk storage conditions. The TPC results found ranged from 2×10⁵ to 9.6×10⁹ CFU/mL. In our study, the TPC load is higher than that found previously in dairy products in Rabat city of Morocco (6.9×108 CFU/mL) (Hadrya et al., 2012), in Malaysia (12×10⁶ CFU/mL) (Fook et al., 2004), in China (2×106 CFU/mL) (Lan et al., 2017), and Burlington (<10⁵ CFU/mL) (D'Amico and Donnelly, 2010). In USA the TPC varies between 2.7×10⁴ CFU/mL and 2.1×108 CFU/mL (Brooks et al., 2012). However, the milking parlor, the milking process, milking machines, milk pipelines, transport tankers, and cooling units should be cleaned to improve the quality of milk (Özer, B. and Yaman H., 2014).

Total coliforms and *E. coli*

The average counts of total coliforms were 8.9×10^5 CFU/mL (Table 1). This value was higher than that reported in Malaysia (17 CFU/mL) (Fook *et al.*, 2004) and USA (between 50 CFU/mL and 1.7×10^3 CFU/mL) (Brooks *et al.*, 2012), and lower than that reported in Sudan

(between 1.2×10^9 and 1.5×10^{10} CFU/mL) (Ibtissam and Mahboba, 2007). On the other hand, *E. coli* was detected in 66.67% of samples (Table 1), which was similar to that found in Malaysia 64.5% (Fook *et al.*, 2004) and higher than that reported in China 45% (Lan *et al.*, 2017). However, the presence of *E. coli* and coliform in food products is considered as an indicator of fecal contamination (Fook *et al.*, 2004; Ghafir *et al.*, 2008). It should be noted that the total coliforms and *E. coli* levels detected in samples taken during summer and autumn are higher than those of winter and spring (Table 2).

Staphylococcus aureus

Staphylococcus aureus is responsible for mastitis in dairy herds, involving the inflammation of the mammary glands and sporadic shedding of *S. aureus* cells into the raw milk (Barkema *et al.*, 2006); it is recognized that *S. aureus* produces pathogenic enterotoxins which cause human diseases (Hill *et al.*, 2012). In our study, the prevalence of *S. aureus* was 75% (Table 1) and the frequency of its isolation from the taken samples was high (Table 1). This value is greater than that found in China (45%) (Lan *et al.*, 2017) and in another study in Morocco (30%) (Ismaili *et al.*, 2016).

Salmonella spp.

The results of our study showed the absence of *Salmonella* in the analyzed samples (Table 1). These results are similar to those reported in raw milk from Switzerland, Finland, and Belgium (Piret *et al.*, 2015). Nevertheless, *Salmonella* has been detected in raw milk collected from many countries, such as China (1.25%) (Lan *et al.*, 2017), Italy (0.3%) (Piret *et al.*, 2015) and Canada (6%) (Griffiths, 2010).

Listeria monocytogenes

Listeria monocytogenes can grow under different conditions such as a wide range of pH, refrigerated temperatures, water activity, and high salinity; it is responsible for listeriosis (Yoon et al., 2016). Our results showed the presence of L. monocytogenes in 19.44% of analyzed samples (Table 1). However, it was detected in other studies with a lower contamination rate, in Europe from 2.2% to 10.2 % (Wendie et al., 2013), New Zeland 0.68% (Hill et al., 2012), Malaysia 4.4% (Fook et al., 2004), China 1.8% (Lan et al., 2017), Finland 5.5% (Lan et al., 2017), and Poland 2.58% (Paszkiewicz et al., 2015). In addition, the incidence of L. monocytogenes in winter and spring is higher than that of autumn and Summer (Table 2), this effect may be explained by the variation of factors influencing the growth of L. monocytogenes such as a wide range of temperature (-0.15 to 45 °C), pH (4.3 to 9.4), high water activities (>0.92) and salt (10% NaCl) (Liu, 2008).

		Total plate count			Total coliforms			Fecal coliforms			Clostridium perfringens		
-		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
WINTER	Station 1	2.6×10⁵	1.6×10 ⁶	7.3×10 ⁵	25	6.0×10 ³	2.0×10 ³	12	50	34	0	0	0
	Station 2	2.3×10⁵	2.0×10 ⁶	8.4×10 ⁵	30	5.6×10 ³	2.0×10 ³	10	70	33	0	0	0
	Popular market	3.0×10 ⁵	3.0×10 ⁶	1.3×10 ⁶	40	7.4×10 ³	2.6×10 ³	14	3.0×10 ²	1.3×10 ²	0	0	0
SPRING	Station 1	3.1×10 ⁵	9.2×10 ⁶	3.8×10 ⁶	2.8×10 ²	5.5×10 ⁴	2.0×10 ⁴	50	4.1×10 ²	2.1×10 ²	0	0	0
	Station 2	2.0×10 ⁵	7.8×10 ⁶	4.0×10 ⁶	2.3×10 ²	6.3×10 ⁴	2.2×10 ⁴	30	2.4×10 ²	1.4×10 ²	0	40	13.3
	Popular market	5.0×10 ⁵	8.0×10 ⁶	4.0×10 ⁶	3.6×10 ²	2.0×10 ⁵	6.8×10 ⁴	40	8.0×10 ²	4.6×10 ²	0	0	0
SUMMER	Station 1	6.0×10 ⁷	9.0×10 ⁹	3.5×10 ⁹	5.5×10⁵	4.8×10 ⁶	2.1×10 ⁶	5.5×10 ²	9.0×10 ³	6.0×10 ³	50	70	61.6
	Station 2	5.5×10 ⁷	8.7×10 ⁹	3.2×10 ⁹	4.0×10 ⁵	4.2×10 ⁶	1.7×10 ⁶	5.4×10 ²	8.8×10 ³	5.6×10 ³	50	68	61
	Popular market	8.0×10 ⁷	9.6×10 ⁹	3.2×10 ⁹	6.6×10 ⁵	5.6×10 ⁶	2.3×10 ⁶	6.3×10 ²	9.2×10 ³	6.0×10 ³	45	67	57.3
AUTUMN	Station 1	7.0×10 ⁶	1.6×10 ⁸	2.1×10 ⁸	6.7×10 ⁴	4.0×10 ⁶	1.5×10 ⁶	1.1×10 ²	3.0×10 ³	1.1×10 ³	0	30	10
	Station 2	9.0×10 ⁶	5.0×10 ⁸	1.9×10 ⁸	5.1×10 ⁴	1.5×10 ⁶	7.3×10 ⁵	1.5×10 ²	1.5×10 ³	6.2×10 ²	0	20	6.6
	Popular market	8.0×10 ⁶	6.2×10 ⁸	1.9×10 ⁸	9.0×10 ⁴	5.8×10 ⁶	2.2×10 ⁶	7.0×10 ²	8.6×10 ³	3.4×10 ³	0	25	8.3

Table 2: Bacteriological quality of raw cow's milk collected from street trading depending of the season (CFU/mL), statistically.

		Yeasts and molds			Lactococci			Lactobacilli		
		Min	Ma×	Mean	Min	Ma×	Mean	Min	Ma×	Mean
WINTER	Station 1	11	33	24.3	6.2×10 ⁴	3.0×10⁵	1.9×10⁵	2.5×10⁵	6.0×10⁵	1.9×10⁵
	Station 2	12	3.2×10 ³	1.2×10 ³	5.0×10 ⁴	5.0×10 ⁵	2.0×10⁵	5.0×10 ⁴	5.0×10 ⁵	2.0×10⁵
	Popular market	15	24	20.6	2.5×10⁵	6.0×10 ⁵	4.5×10⁵	2.5×10⁵	6.0×10 ⁵	4.5×10⁵
ŋ	Station 1	2.5×10 ³	70	44.33	5.6×10 ⁴	8.5×10 ⁶	3.0×10 ⁶	7.4×10 ⁴	8.0×10 ⁵	3.0× 10 ⁶
SPRING	Station 2	30	4×10 ³	1.7×10 ³	5.8×10 ⁴	7.0×10 ⁶	2.5×10 ⁶	5.8×10 ⁴	7.0×10 ⁶	2.5×10 ⁶
	Popular market	20	3×10 ³	1.26×10 ³	7.4×10 ⁴	8.0×10 ⁵	5.5×10⁵	7.4×10 ⁴	8.0×10 ⁵	5.5×10⁵
R	Station 1	7.7×10 ³	3.3×10 ⁴	1.2×10 ³	2.5×10 ⁷	8.1×10 ¹⁰	2.7×10 ¹⁰	2.6×10 ⁷	7.7×10 ⁹	2.7×10 ¹⁰
JMI	Station 2	7.4×10 ³	3.2×10 ⁴	1.2×10 ⁴	1.5×10 ⁷	7.5×10 ¹⁰	2.5×10 ¹⁰	1.5×10 ⁷	7.5×10 ¹⁰	2.5×10 ¹⁰
SUMMER	Popular market	8.0×10 ²	4.4×10 ³	1.6×10 ⁴	4.2×10 ⁹	2.6×10 ⁷	2.7×10 ⁹	2.6×10 ⁷	7.7×10 ⁹	2.7×10 ⁹
AUTUMN	Station 1	3×10 ³	4.5×10 ³	3.8×10 ⁴	5.4×10 ⁶	8.0×10 ⁷	3.8×10 ⁷	1.0×10 ⁶	6.4×10 ⁷	3.8×10 ⁷
	Station 2	2×10 ³	3×10 ³	2.5×10 ⁴	3.6×10 ⁶	7.0×10 ⁷	3.3 ×10 ⁷	3.6×10 ⁶	7.0×10 ⁷	3.3×10 ⁷
	Popular market	4×10 ³	6×10 ³	5.0×10 ³	3.3×10 ⁷	1.0×10 ⁶	3.8×10 ⁷	1.0×10 ⁶	6.4×10 ⁷	3.8×10 ⁷

Table 3: Prevalence of lactic acid bacteria, yeast and molds in raw milk collected from street trading depending of the season (CFU/mL), P<0.05.

Clostridium perfringens

Clostridium perfringens is a Gram-positive, sporulating anaerobic bacterium responsible for a wide spectrum of diseases in humans and animals (Mcclane, 2001; Ed-Dra *et al.*, 2017c). In this study, the percentage of contamination by *C. perfringens* was 36.1% (Table 1), with a high frequency in summer and autumn (Table 2). This result is higher than that found in Egypt (20%) (Rowayda *et al.*, 2015) and in India (10%) (Gurmu *et al.*, 2013).

Lactococci, lactobacilli, yeasts, and molds

Lactic acid bacteria improve the diversity of gut microbiota, and its metabolic activities promote human and animal health and/or prevent diseases (Umu et al., 2017). In this study, the means of lactococci and lactobacilli detected in raw cow's milk were 7.5×108 CFU/mL and 4.6×10⁸ CFU/mL respectively (Table 1). This value is higher than that found beforehand in the camel milk in the south of Morocco (lactococci: 4.25×107 CFU/mL; lactobacilli: 3.55×107 CFU/mL) (Alaoui Ismaili et al., 2016). Molds and Yeasts were found at average levels of 4.1×10³ CFU/mL, this result is lower than that found previously in Morocco (from 3.13×10⁶ CFU/mL to 1.60×105 CFU/mL (Maha et al., 2016). It is worth noting that the highest prevalence of molds and veasts. lactococci and lactobacilli (Table 3) was recorded during summer and autumn and the lowest in winter and spring.

CONCLUSION

In this study, the microbiological analysis revealed poor raw cow's milk quality sold through street trading. Therefore, raw milk that is intended for direct consumption cannot be considered microbiologically safe. Currently, in Morocco, there is no legislative requirement regarding the selling of raw milk and dairy products by street traders. However, the microbiological quality of raw milk is believed to be influenced by handling practices during milking, hygiene and sanitation personnel, storage, transport equipment, healthy cows, water quality and seasonal variation.

Some measures may be applied to evaluate the microbiological quality of raw milk. These include the establishment of milk hygiene standards, information to the producers, street trading and consumers, and action to strengthen the regulatory framework of the activity of street traders.

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