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Presence of *Listeria monocytogenes* in raw milk and traditional dairy products marketed in the north-central region of Morocco

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The objective of this study was to determine the prevalence of *Listeria monocytogenes* in raw milk and its two traditional dairy derivatives, the *Lben* (traditionally fermented skimmed milk) and *Jben* (traditional fresh cheese) marketed in the city of Fez in the center northern of Morocco. A total of 288 samples from three dairy products including one third of raw milk, one third of "Lben" and one third of "Jben" were collected from eight traditional dairies belonging to four sectors of the city of Fez between October 2009 and September 2010. Isolation and identification of *L. monocytogenes* were carried out according to official standard ISO procedure 11290-1 and the acidity of the various samples was performed according to the Moroccan standard. The overall prevalence of *L. monocytogenes* contamination was 5.90%. It was present in eight raw milk samples, five of *Lben* samples and in four *Jben* samples. The results reveal a variation of contamination from one sector to another with a higher contamination in the samples collected in the fall and winter. This suggests a link between management practices feed, hygienic conditions and *L. monocytogenes* contamination. The contamination levels achieved justify the control of the feeding cattle, milk pasteurization and the enforcement of the general principles of food hygiene in order to reduce consumer's exposure to *L. monocytogenes*.

Key words: *Listeria monocytogenes*, raw milk, traditional dairy products, food safety, Morocco.

INTRODUCTION

Currently, *Listeria monocytogenes* is considered one of the most important pathogens responsible for food-borne infection. It is often incriminated in outbreaks of human listeriosis (Ryser and Marth, 2007).

Pregnant women, infants, immunocompromised and the elderly people are at greatest risk for listeriosis (Gillespie et al., 2010). This infection is regularly monitored and reported in Europe and North America, but in Africa and other developing countries, only a few sporadic cases have been reported (Tazi, 1981; Boukadidda et al., 1994). In Morocco and other countries of North Africa, the studies on the incidence of human listeriosis are rare.

Evidence is accumulating to vindicate that the organism

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is a food-borne pathogen, and dairy products have been implicated. In dairy industry, *Listeria* can contaminate directly or indirectly the products and the environment through contaminated raw milk, resulting in huge losses both in terms of public health and economy. This sensitizes the scientific and medical communities to focus on the safety of these products.

Due to the interest given to *L. monocytogenes* as emerging pathogens and to assess its possible role as agents of foodborne listeriosis in Morocco, several studies have been conducted on the prevalence of these bacteria in meat products (Kriem et al., 1998; Cohen et al., 2007; Ennaji et al., 2008) and in the marine environment (Bou-M'handi and El Marrakchi, 2003). On the other hand, little information is available related to the prevalence of *L. monocytogenes* in milk and dairy products available in Morocco.

The pasteurization of milk has been recognized to ensure effective consumer safety against *L. monocytogenes* (World Health Organization, 1988). However, Morocco has a wide range of traditional dairy products based on raw milk that have been known and highly appreciated by consumers for centuries. These foods have a loyal clientele, despite some apparent alterations and incidents in which consumers may be victims.

Accordingly, the aim of the present study was to investtigate the prevalence of *L. monocytogenes* in raw milk and its two traditional derivatives, the *Lben* (traditionally fermented skimmed milk) and *Jben* (traditional soft white cheese) commercialized in the city of Fez in the central north of Morocco.

MATERIALS AND METHODS

Sample collection

In the period October 2009 to September 2010, a total of 288 samples of which 96 samples were raw milk, 96 *Lben* and 96 *Jben* were collected in Fez, located in the northern center of Morocco. Dairy products samples were collected from eight traditional dairies, belonging to four administrative sectors of the city (S1, S2, S3 and S4). Each dairy receives about 80 to 100 liter per day L day^{-1} from small farms located in four regions around the city (*Ras El Mae, Sidi Harazem, Boughioul/Sefrou and Al Gharb*). In order to collect information on farm characteristics, feeding practices and milk production, visits were organized to some farms. In this study, we randomly chose two dairies by sectors that were monthly sampled for a whole year. Milk sold in the traditional dairies is consumed without any heat treatment either in the production of traditional juice (fruits + raw milk) or for use in the manufacturing of traditional dairy derivatives (*Lben*, *Jben*, etc.). The samples were transported in iceboxes to Regional Laboratory of Epidemiological Diagnosis and Environmental Hygiene in Fez and analyzed immediately upon arrival.

Isolation and identification of *L. monocytogenes*

Isolation and identification of *L. monocytogenes* were carried out according to official standard ISO procedure 11290-1 (ISO, 2004).

Twenty five grams (25 g) of each sample were weighed into sterile stomacher bags, diluted with 225 ml of *Listeria* pre-enrichment Fraser broth (Biokar Diagnostics, BK115HA) homogenized and incubated at 30°C for 24 h. A 1 ml portion from this pre-enrichment culture was transferred to 9 ml enrichment broth (Complete Fraser broth, BIO-RAD), and incubated at 37°C for 24 h. A loopful of the enrichment culture was plated onto *Listeria* Ottaviani et Agosti agar [ALOA] (AES CHEMUNEX, AEB620088, Rennes, France), and PALCAM (Biokar Diagnostics, BK145F). Plates were incubated at 37°C for 24 to 48 h. Morphologically in ALOA *Listeria* grow as blue-green regular round colonies (detection of β-glucosidase by using a specific chromogenic substrate). *L. monocytogenes* also show an opaque halo which helps to easily differentiate them from other species of *Listeria*. The halo is due to the activity of a phospholipase involved in the infection process of pathogenic *Listeria*. PALCAM colonies *Listeria* are green with grayish reflections, or green with a black halo (esculin hydrolysis).

Typical colonies were taken as possible *Listeria* spp. Isolates from five suspect colonies on ALOA and PALCAM, respectively per sample were purified on tryptic soy agar with 0.6% yeast extract (Oxoid) (TSEYA) and identified using the following tests: Gram's staining, catalase reaction with 3% H_2O_2, oxidase test, motility at 25°C, test for glucose/lactose fermentation-iron utilization-gas production, fermentation of sugars (rhamnose, xyloseand mannitol), CAMP tests, methyl red-Voges Proskauer (MR-VP) reactions, nitrate reduction, indole production, urease production and β-haemolysis. Identified isolates of *L. monocytogenes* were confirmed in Institut Pasteur of Morocco with API *Listeria* (Biomerieux, 10300, Lyon, France).

Chemical analysis

The pH of the different samples was determined using a pH meter (Adwa" model "AD100"). The titratable acidity was measured by titration with 1 N NaOH in the presence of phenolphthalein and expressed in degrees Dornic according to Moroccan standard (NM 08.4.005, 2007).

Statistical analysis

Data were analyzed using statistical software package Epi Info version 6. The percentages of positive results were compared using the chi-square and Statistical significance was indicated by $P \leq 0.05$.

RESULTS

The results of the occurrence of *L. monocytogenes* and physicochemical parameters in analyzed samples of raw milk and traditional dairy derivatives are summarized in Table 1. Out of 288 samples of dairy products examined, 17 (5.90%) were found to be contaminated with *L. monocytogenes*. The prevalence of *L. monocytogenes* in raw milk, *Lben* and *Jben* samples was found to be 8.33, 5.20 and 4.16%, respectively. The average pH of analyzed samples was 6.6 for raw milk, 4.5 for *Lben* and 4.3 for *Jben*. The average titratable acidity measured in this study was 16, 80 and 95°D for raw milk, *Lben* and *Jben* respectively.

On the other hand, the prevalence of *L. monocytogenes* in samples from S1, S2, S3 and S4 was 4.16, 11.11, 2.77 and 5.55%, respectively. The highest prevalence of *L. monocytogenes* contamination was observed in autumn (11.11%) and winter (11.11%), while no positive

Table 1. Prevalence of *L. monocytogenes* isolated from raw milk and dairy derivatives.

Parameter		Raw milk	*Lben*	*Jben*	All
Number of analyzed samples		96	96	96	288
Number of positive samples	n	8	5	4	17
Incidence of *L. monocytogenes*	%	8.33	5.20	4.16	5.90
Average pH		6.6	4.5	4.3	-
Average titratable acidity (°D)		16	80	95	-

Table 2. Prevalence of *L. monocytogenes* in all samples of dairy derivatives sold in different sectors of Fez city between October 2009 and September 2010.

Sector	Collection season	Number of samples	Number of *L. monocytogenes* positive sample (%)
Sector 1	Autumn	18	2 (11.11%)
	Winter	18	1 (5.55%)
	Spring	18	0
	Summer	18	0
	Subtotal	**72**	**3 (4.16%)**
Sector 2	Autumn	18	4 (22.22%)
	Winter	18	3 (16.66%)
	Spring	18	0
	Summer	18	1 (5.55%)
	Subtotal	**72**	**8 (11.11%)**
Sector 3	Autumn	18	0
	Winter	18	2 (11.11%)
	Spring	18	0
	Summer	18	0
	Subtotal	**72**	**2 (2.77%)**
Sector 4	Autumn	18	2 (11.11%)
	Winter	18	2 (11.11%)
	Spring	18	0
	Summer	18	0
	Subtotal	**72**	**4 (5.55%)**
All		**288**	**17 (5.90%)**

samples were detected in spring (Table 2). Between seasons isolation rates of *L. monocytogenes* were statistically significant (χ^2 = 14.19; P < 0.05). However, the difference between sectors was statically non significant (P > 0.05).

DISCUSSION

This study reveals that raw milk, *Lben* and *Jben* that was consumed in the city of Fez in the period studied were contaminated with *L. monocytogenes* with an overall prevalence of 5.90%. Our results are comparable to other surveys conducted in other countries on raw milk and dairy products. Boubendir et al. (2011) reported 5.76% prevalence of *L. monocytogenes* in bovine raw milk produced in the North Eastern Algeria. Similar results found by Guerra et al. (2001), where the incidence of contamination was 5% in milk and dairy products sold in mainland Portugal. Gaya et al. (1998) reported also a low incidence of 3.6% of *L. monocytogenes* in raw milk produced in Spain. Whereas in China, the prevalence of *L. monocytogenes* in raw milk is very low (0.23 to 1.2%) (Ning et al., 2013). Common sources of *L. monocytogenes*

in raw milk have been reported to be fecal (Husu, 2010) and environmental contamination during milking, storage and transport, infected cows in dairy farms and poor silage quality (Bemrah et al., 1998). *L. monocytogenes* have also been isolated from soil, decaying foliage, and bird and wildlife feces (Weis and Seeliger, 1975).

This study reveals a disparity of seasonal contamination with *L. monocytogenes* and more prevalent in one of the sectors (Table 2). These differences are probably due to seasonal and sectoral variations in the type and quality of feed given to cows. Silage was widely used as animal feed in certain zones around the city of Fez. Contamination of feeds is influenced by the conditions of preparation and storage practices.

Farmers in that area lack training in good agricultural practices for silage making, resulting in a product of poor quality (personal observation), may explain the high contamination of milk with *L. monocytogenes* in autumn and winter when silages are fed. Broseta et al. (2003) also reported that contamination of raw milk with *L. monocytogenes* is usually more common in the winter, most likely because silage feeding in many parts of the world is more common in that season. In addition, a large number of studies have indicated that clinical listeriosis in ruminants is often associated with feeding poor-quality silage (Boerlin et al., 2002).

The absence of *L. monocytogenes* in spring and low contamination in summer is most likely due to the pasture-base feeding during that period. Consistent with this hypothesis, Fenlon (1996) also found that it is uncommon for grazing animals to shed detectable levels of *L. monocytogenes.*

In addition to the initial charge of *L. monocytogenes* in raw milk used in the manufacture of traditional dairy products, the *Lben* and *Jben* can be contaminated during the manufacturing process in traditional dairies. It has been emphasized that the contamination of cheeses or dairy products with *Listeria* spp. is most likely due to post-process contamination from environmental sources and cross-contamination in the dairy plant and/or retail stores or inadequate processing (FAO/WHO, 2004) and colonization of *L. monocytogenes* in refrigerators in retail stores (Sergelidis et al., 1997). *L. monocytogenes* can survive a number of cheese-making processes and can remain viable in the final product for a considerable length period of time (Griffiths, 1989).

A lower prevalence of *L. monocytogenes* contamination in the *Lben* (5.20%) and *Jben* (4.16%) was noticed compared with raw milk (8.33%). This may be due to the acidic nature of *Lben* and *Jben* which have a low pH and high titratable acidity (Table 1), that can inhibit the growth of *L. monocytogenes.* The richness of Moroccans *Lben* and *Jben* by lactic acid bacteria (Ouadghiri, 2009) can also inhibit the growth of *L. monocytogenes*. In this context, Benkerroum et al. (2003) reported that bacteriocins produced by the lactic acid bacteria reduce counts of *L. monocytogenes* in cheese and yoghurt. Furthermore, several authors have confirmed that the growth or survival of *L. monocytogenes* in a food product depends on a variety of physico-chemical parameters, including pH, a_w and NaCl content (Conner et al., 1986; Learson et al., 1999).

Previous studies have specifically shown that *L. monocytogenes* does not grow at a pH below 5.3 when the a_w is lower than 0.93 (Tienungoon et al. 2000), or at a pH below 4.46 regardless of the a_w (Cole et al., 1990; Farber et al., 1996). In our study, the average physico-chemical parameters associated with *Lben* and *Jben* are as such that they should limit (if not prevent) the growth of *L. monocytogenes*.

In conclusion, raw milk and traditional dairy derivatives marketed in the north central region of Morocco has been contaminated with *L. monocytogenes*. The highest prevalence of contamination was recorded in autumn and winter and in an area where silage feeding is important.

The poor hygienic conditions during milking, transport, storage of milk and its use in the manufacture of *Lben* and *Jben* in traditional dairies, which do not respect the principles of food hygiene, can also be in favor of the contamination with *L. monocytogenes.* The results show that there is a risk of infection by strains of *L. monocytogenes* for consumers of raw milk and traditional dairy products marketed in the city of Fez.

So, we suggest that the general principles of food hygiene should still be enforced in order to minimize count of *L. monocytogenes* in milk and dairy products during the handling, storage and manufacturing in traditional dairies. Control of the feeding cattle and milk pasteurization can also limit the contamination with *L. monocytogenes*.

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