

Dept. of Food Hygiene,
Faculty of Veterinary Medicine, South Valley University

OCCURRENCE OF *LISTERIA SPECIES* IN RAW COW'S MILK & ICE CREAM SOLD IN QENA CITY

(With 3 Tables and 2 Figures)

By

LAILA M. EL-MALT and KARIMA G. ABDEL-HAMEED

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**تواجد ميكروبات الليستيريا في اللبن البقري الخام والأيس كريم
المباع في مدينة قنا**

ليلى مصطفى الملط ، كريمة جلال عبد الحميد

تمتد الإصابة بميكروبات الليستيريا على اتساع العالم وتنتشر هذه الميكروبات في البيئة وجميع الحيوانات والإنسان. والإصابة بالليستيريا تعتبر من الإصابات الناتجة من تناول الأغذية الملوثة. وهذا يتطلب مجهودا بحثيا نشطا. لذا تم جمع مائة عينة من مدينة قنا مقسمة بين خمسين عينة من اللبن الخام وخمسين عينة من الأيس كريم المنتج على مدى واسع ومن المنتج على حيز ضيق وتم جمع هذه العينات من المحلات التجارية الصغيرة والكبيرة والبائعين وتم فحص العينات لمعرفة مدى تواجد الميكروبات من أنواع الليستيريا المختلفة بها. تم التعرف على أصناف الليستيريا في خمسة عشر عينة بنسبة ثلاثين في المائة من كل عينات اللبن الخام المفحوصة. وتواجدت هذه الأصناف في ثمانية عشر عينة من الأيس كريم بنسبة اثنين وسبعين في المائة. وتم التعرف على الليستيريا من النوع ولشميراي في عينة واحدة بنسبة اثنان في المائة في اللبن الخام وفي ثلاثة عينات بنسبة إثني عشر في المائة في الأيس كريم المنتج على حيز ضيق (محلي). وتواجدت الليستيريا أنكو في عینتين بنسبة أربعة في المائة في اللبن الخام وفي ثلاثة عينات بنسبة إثني عشر في المائة في الأيس كريم المحلي. وكانت نسبة تواجد الليستيريا موراي بواقع أربعة عينات بنسبة ثمانية في المائة في اللبن الخام بينما وصلت العينات الايجابية لهذا النوع إلى أربعة بنسبة ستة عشر في المائة في الأيس كريم المحلي. تم العثور على ميكروب الليستيريا جراي في خمس عينات من اللبن الخام بنسبة عشرة في المائة. وتواجد هذا الميكروب في ست عينات بنسبة أربعة وعشرين في المائة في الأيس كريم المحلي. وفيما يخص الليستيريا مونوسيتوجين فقد تم عزلها من ثلاثة عينات من اللبن الخام بنسبة ستة في المائة. وتواجدت الليستيريا سيليجراي في عينتين من الأيس كريم المحلي بنسبة ثمانية في المائة. تم مناقشة هذه النتائج التي تشير إلى وجود تأثير ضار على الصحة العامة ويجب إتخاذ الطرق المناسبة للحد من تلوث اللبن الخام والأيس كريم وخاصة ما ينتج محليا بهذه الميكروبات الهامة.

SUMMARY

Listeria infections caused by microorganisms of the genus *Listeria*, occur worldwide and in a variety of animals and man. Listeriosis was recognized as a food –borne human disease that prompted intense research activity. Thus a total of 100 samples including (50) raw cow's milk and (50) of both ice cream small and large scale producers purchased from retail local markets , supermarkets and street vendors in Qena city. These samples examined to investigate the occurrence of *listeria species*. *Listeria* spp. could be detected in 15(30%) and 18(72%) of examined raw milk and small scale ice cream samples respectively, while failed to be detected in large scale ice cream samples. *L. welshimeri* was identified in one sample (2%) in raw milk, and 3 (12%) in small scale ice cream, while *L. innocu* present in 2 samples (4%) of raw milk, and in 3 samples (12%) of small scale ice cream. *L. murray* was isolated from 4 samples (8%) of raw milk and 4 samples (16%) of small scale ice cream. *L. gray* was detected in 5 samples (10%) of raw cow's milk and 6 samples (24%) of small scale ice cream. Suspected *L. monocytogenes* has been recovered in 3 samples (6%) of raw milk and *L. seeligeri* was detected in 2(8%) of small scale ice cream. The public health importance and the recommended sanitary measures were discussed.

Key words: *Listeria* spp., milk, ice cream, public health.

INTRODUCTION

Listeria monocytogenes is a Gram positive, psychrotolerant food borne pathogen that is widely distributed in nature and it is the causative agent of Listeriosis, a serious invasive disease in both domestic animals and humans (Schlech *et al.*, 1983; Farber and Peterkin, 1991; and Low and Donachie, 1997). Several outbreaks of Listeriosis associated with the consumption of milk and dairy products since 1980 and thirty percent over all mortality rate of these outbreaks were recorded (Griffiths, 1989).

L. monocytogenes may directly contaminate milk as a consequence of Listerial mastitis, encephalitis or *Listeria*–related abortion in cattle, and asymptomatic cows can also shed *L. monocytogenes* in their milk for many months (Hird and Genigeogis, 1990). More over under unhygienic milking practices indirect contamination of bulk milk is likely to occur if *L. monocytogenes* is

present in feeds, faeces, udder surface or bedding (Fedio and Jackson, 1992).

Listeriosis in human are usually associated with the hemolytic species of *Listeria* (*L. monocytogenes*, *L. ivanovii* and *L. seeligeri*) however, *L. welshimeri* pathogenicity to human has been well documented (Cocolin *et al.*; 2002). *L. monocytogenes* is the only species of the genus *Listeria* that has been involved in known food borne outbreaks of serious disease such as septicemia, abortion, meningioencephalitis, meningitis and gastrointestinal illness specially in immunocompromised elderly individual, pregnant women and their neonates (Longhi *et al.*, 2003). Many surveys have been conducted and showed that *L. monocytogenes* and other *Listeria* spp. could be isolated from milk and dairy products worldwide (Davison *et al.*, 1989; El-Sherbini & Abdallah, 2003 and El-Prince & Sayed 2004.). Likewise *Listeria* spp. are well equipped to survive food processing technologies. They tolerate high concentration of salt and relatively low pH; also they are able to multiply at refrigeration temperature (Lou & Yousef, 1999).

The reported prevalence estimates for *L. monocytogenes* in ice cream from many countries ranges from zero to 20 % (Greenwood *et al.*, 1991; Pak *et al.*, 2002; Molla *et al.*, 2004; Meldrun *et al.*, 2005; El-Sharef *et al.*, 2006 and Kiss *et al.*, 2006). Furthermore *Listeria* spp. have been isolated from pasteurized milk and ice cream in which contamination occurred post production or recontaminated during further handling (Jeong and Frank, 1994 and El-Prince & Sayed, 2004).

Because most of the consumed milk and dairy products are not prepared under hygienic conditions by ignorant producers, utilization of milk that is most often not boiled enough in the production of their products. This work was conducted to investigate the incidence of *Listeria species* in raw milk and ice cream in Qena city.

MATERIALS and METHODS

Collection of samples

A total of 100 samples including 50 raw cow's milk and 50 of both small scale and large scale ice cream samples were collected from retail local markets, supermarkets and street vendors in Qena city. Samples were investigated as soon as possible in the laboratory for the presence of *Listeria* spp. Milk samples were examined by Storch test to detect heat treated samples according to Lampert (1975).

Isolation of *Listeria* spp.:

Detection of *Listeria* spp. was done as described by Hitchins (1992) where one ml of milk or one gram of ice cream samples was aseptically added to 9 ml of *Listeria* enrichment broth (Biolife) and incubated at $35 \pm 1^\circ\text{C}$ for 48 hours. After incubation a loopful of enrichment broth was streaked on the surface of palcome medium and incubated at $35 \pm 1^\circ\text{C}$ for 24 – 48 hours (Cutris *et al.*, 1989).

Identification of *Listeria* species:

Identification was carried out according to Warburton *et al.*, (2003) including Gram stain, catalase test, carbohydrate fermentation, B-haemolysis on blood agar and CAMP test.

Serotyping of *L. monocytogenes* strains:

Biochemically identified isolates were serotyped with *Listeria* antisera type one (Difco) by using slide agglutination test according to Difco (1984).

RESULTS

Table 1: Incidence of *Listeria* species in the examined samples of raw cows milk and ice cream.

Types of samples	No of tested samples	Positive samples	
		NO	%
Raw cows milk	50	15	30 %
Large scale ice cream	25	zero	zero %
Small scale ice cream	25	18	72 %

Table 2: Distribution of *Listeria* species in positive samples of raw cow's milk and ice cream.

<i>Listeria species</i>	Raw cow's milk		Large scale ice cream	Small scale ice cream	
	No.	%		No.	%
<i>L. monocytogenes</i>	3	6%	zero	zero	zero
<i>L. seeligeri</i>	zero	zero	zero	2	8%
<i>L. welshimeri</i>	1	2%	zero	3	12%
<i>L. innocua</i>	2	4%	zero	3	12%
<i>L. murraye</i>	4	8%	zero	4	16%
<i>L. Gray</i>	5	10%	zero	6	24%

Table 3: Serotyping of *Listeria monocytogenes* isolates from raw cow's milk samples.

Types of sample	No. of suspected isolates	Serotype (1) pattern
Raw cow's milk	3	1

Fig. 1: Occurrence of *Listeria* species in the examined raw cows milk and ice cream samples.

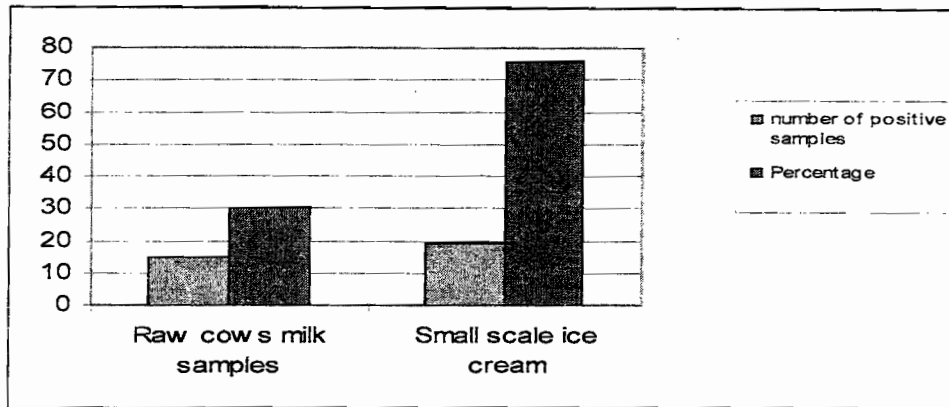
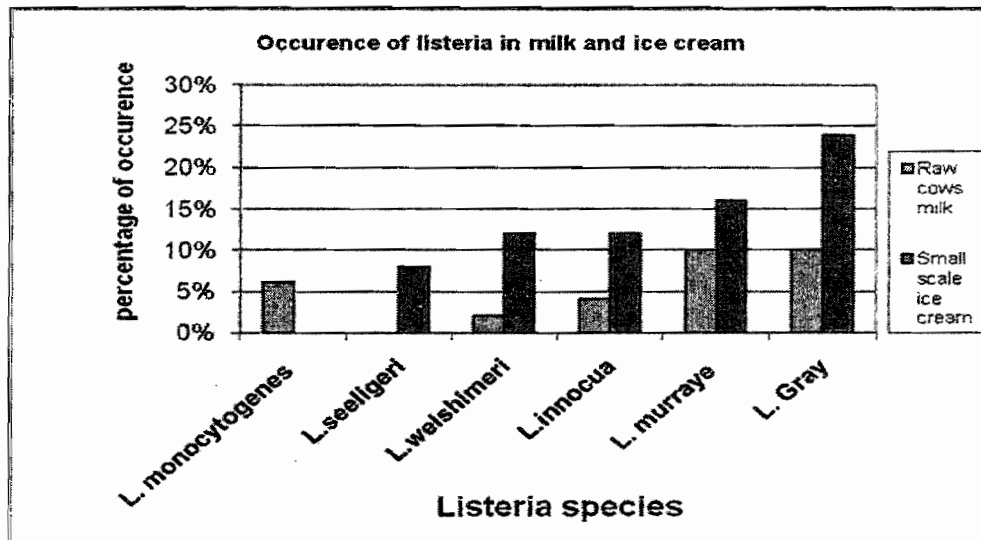


Fig. 2: Percentage of positive samples of *Listeria species* in the examined raw cows milk and small scale ice cream samples.



DISCUSSION

Listeria spp. could be detected in 15 (30 %) and 18 (72 %) of examined raw cow's milk and small scale ice cream samples respectively, while failed detection in large scale ice cream samples. *L. welshimeri* was identified in one sample (2%) in raw cows milk, and 6 samples (24%) in small scale ice cream, while *L. innocua* present in 2 samples (4%) of raw cow's milk, and in 6 samples (24%) of small scale ice cream. *L. murray* was isolated in 4 samples (8%) of raw cow's milk and 8 samples (32%) of small scale ice cream. *L. gray* was detected in 5 samples (10%) of raw milk and 12 samples (48%) of small scale ice cream. Concerning *L. monocytogenes*, it has been recovered from 3 samples (6%) of raw cow's milk and *L. seeligeri* was detected in 4 (16%) of small scale ice cream (Tables 1&2 and Figures 1&2)

Listeria species are found almost in every medium in the environment; soil, canal waters, plants, animal feeds, and food. So, contamination of milk is possible by various means from these sources (McLachlin, 1997). *Listeria monocytogenes* is the major wide spread human pathogens, the goal should be to exclude this organism from the food chain wherever possible and to maintain condition which will inhibit its multiplication in foods in which this bacteria can grow (WHO, 1988). The results in Table 1 & 2 and Figures 1&2 declared that the isolation rate of *Listeria* spp. in the examined raw cow's milk samples 15 positive samples represent 30%. This incidence was slightly high in relation to the findings of Saito *et al.* (1991) {5.3%}; Harvey & Gilmour (1992) {25%}; Morales *et al.* (1995) {7%}; Aman & Ahmed (1997) {3.33%}; Abdel Khalek & El Gamal (1998) {4%}; El-Prince (1999) {4%}; Salem (2000) {10%}.

In the present study, *Listeria species* isolated from raw cows milk was differentiated biochemically into 3 strains (6%) including *L. monocytogenes*, *L. innocua* (4%) and *L. welshimeri* (2%).

Non pathogenic *Listeria species* were detected including *L. murray* (5 strains, 10 %) and *L. gray* (5 strains, (10%). These results considered higher than that recorded by Davison *et al.* (1989) in raw milk in Canada. However, in USA Lovett *et al.* (1987); Rohrbach *et al.* (1992) and Jayarao and Hennig (2001) recorded 4.2, 4.4, and 4.6%, respectively. In the same time, El-Prince and Sayed (2004) found 2% incidence of *L. monocytogenes* in Assiut governorate. In the public health significance *L. monocytogenes* could be transmitted to the consumers by ingestion of contaminated milk (Low & Donachie, 1997).

The organism causes typical gastrointestinal illness (Dalton *et al.*, 1997). *L. welshmeri* pathogenesis to humans has been well documented (Cocolin *et al.*, 2002). The investigation revealed the presence of the non pathogenic *Listeria species*.

It is Apparent from the results (Tables 1 & 2 and Figures 1&2) that ice cream produced in a large scale failed to show growth of *Listeria species*. These L species were recovered from 19 samples (76 %) produced in a small scale.

High incidence of *Listeria species* in this study was higher in their incidence than that of Pednekar *et al.*, (1997) which recorded relatively lower incidence (28-42 %) and Akman *et al.*, (2004) found 41% incidence. Likewise lower incidence (7.3%) was recorded by Cotton & White (1992), while Choi *et al.* (2001) could not isolate *Listeria spp.* from any of examined ice cream samples.

Further identification showed that *L. monocytogenes* could not be isolated from small scale ice cream samples. This result is confirmed by the work done by Akman *et al.* (2004) and El Prince and Sayed (2004).

Further more there is high incidence of *L. seeligeri* (16%), *L. welshime* (24%) and *L. innocua* (24%). On the other hand *L. monocytogenes* was recovered from ice cream samples examined by Cotton & White (1992) and Baek *et al.* (2000)

Listeria spp. have been isolated from pasteurized milk and ice cream in which contamination occurred post production or recontaminated during further handling (Jeong and Frank, 1994 and El-Prince and Sayed, 2004.)

Listeriosis in humans are usually associated with haemolytic species of *L. monocytogenes*; *L. ivanovi* and *L. seeligeri*. However, *L. welshimeri* pathogenesis to humans has been well documented (Cocolin *et al.*, 2002). The presence of any *Listeria species* in food may be an indicator of poor hygiene during handling and preparation of ice cream (Jeong & Frank, 1994). Milk and dairy products under low temperature especially ice cream constitute a potential risk for Listeriosis (Maifreni *et al.*, 1993). The presence of any species of *Listeria* is indicative for the potential presence of *L. monocytogenes* because the physiology and habitat of the different species of *Listeria* are very similar (McLauchin *et al.*, 1990 and Fedio & Jackson, 1992).

The results in Table 3 showed that *L. monocytogenes* isolate from raw cow's milk samples belong to serotype 1, this result had been confirmed by Baek *et al.* (2000) who found that 90% of isolated *Listeria* belong to serotype 1.

Finally, the obtained results in this study prove the high incidence of *Listeria species* and specially *L. monocytogenes*. This result could be considered as a public health risk factor on the consumers. Pasteurization still remains the 1st line of defense against transmission of food born diseases through milk or dairy products.

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