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## **INCIDENCE OF LISTERIA MONOCYTOGENES IN PASTEURIZED MILK AND SOME PASTEURIZED MILK PRODUCTS AND EFFECT OF BOILING ON ITS VIABILITY**

(With 2 Tables)

By

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**مدى تواجد ميكروب الليستيريا مونوسيتوجينس في اللبن المبستر وبعض  
منتجاته وتأثير درجة الغليان على حيوية الميكروب**

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جمعت ٧٥ عينة عشوائية من اللبن والقشدة والرايب المبستر بواقع (٢٥ عينة من كل نوع) وذلك من محلات البقالة والسوبر ماركت المختلفة بمدينة أسيوط. وكذلك من معمل تصنيع الألبان بكلية الزراعة جامعة أسيوط وذلك لمعرفة مدى تلوثها بميكروب الليستيريا مونوسيتوجينس. كذلك تم دراسة مدى حيوية وبقاء الميكروب في اللبن المحقون بـ  $10 \times 37$  <sup>٧</sup> خلية / مللى من هذا الميكروب وذلك بغلى اللبن وعد الميكروب على فترات زمنية مختلفة في ثلاث محاولات. وقد أظهرت النتائج أن ميكروب الليستيريا مونوسيتوجينس قد تم عزله من اللبن المبستر بنسبة ٤% بينما لم يتم عزله من القشدة والرايب المبسترين. أما بالنسبة لدراسة تأثير ميكروب الليستيريا بدرجة حرارة الغليان فقد أسفرت النتائج عن تناقص العدد من  $10 \times 37$  <sup>٧</sup> إلى  $10 \times 274$  <sup>٥</sup> و  $10 \times 214$  <sup>٤</sup> و  $10 \times 120$  <sup>٢</sup> في المحاولات الثلاث فى الدقائق الأولى والثانية والثالثة على التوالي بينما لم يتم عزل الميكروب بعد الدقيقة الرابعة للثلاث محاولات. هذا وقد ناقش البحث الأهمية الصحية والإجراءات التى ينبغى اتباعها لمنع تلوث المنتجات سابقة الذكر بهذا الميكروب.

### **SUMMARY**

A total of 75 random samples of pasteurized: milk, cream and Rayeb (25 samples of each type) were collected from different localities in Assiut City and examined for the presence of *L. monocytogenes*. The obtained results pointed out that *L. monocytogenes* could be isolated from 4% of the examined pasteurized milk samples, while could not be detected from pasteurized samples of cream and Rayeb. Furthermore, three trials

on sterile milk which were inoculated with  $37 \times 10^7$  CFU/ml of *L. monocytogenes* as an initial count to detect the thermal resistance of the organisms when exposed to boiling temperature at different times. The obtained results of the three trials revealed that the number of the organisms decreased and reached to  $274 \times 10^5$ ,  $214 \times 10^4$  and  $120 \times 10^2$  at the first, second and the third minute, respectively, while at the fourth minute the organisms could not be detected. The public health importance and the sanitary measures for control of the organisms were mentioned.

**Key words:** *Listeria monocytogenes*, pasteurized: milk, cream and Rayeb.

## INTRODUCTION

*Listeria monocytogenes* is a Gram positive rod shaped bacterium which was discovered nearly 60 years ago and finally named *Listeria* according to Dr. Lister (Pirie, 1940). The organism has a psychrophillic and a mesophillic nature as it can grow at temperatures between 1 and 45 with an optimal growth occurring between 30-37°C. Several species of *Listeria* were recognised in the Approved Lists of Bacterial Names (Audurier *et al.*, 1984) including *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. dentrificans*, *L. murrayi* and *L. grayi*.

In recent years a gradual awareness of the occurrence of *Listeria* organisms in both human being and animal species spread throughout the world while a considerable interest in *L. monocytogenes* as an agent of foodborne disease has become increasingly apparant.

*L. monocytogenes* is widely distributed in man and animal species including sheep, goats and many other animals that eliminate the agent in faeces as well as in the environment. Also, it has been isolated from soil, plants, mud pasteurate and streams (Acha and Szyfers, 1991). In addition it can be isolated from the stools of a large proportion of healthy people and stools of 20-30% of pregnant women and from the female genital tracts. The infectious disease associated with *Listeria monocytogenes* called Listeriosis and the organism is recognised by public health authorities as it plays an important role in cases of abortion in both human and animals, absces in liver, artherities, peritonities, endocarditis, conjunctivitis, pneumonia, septicaemia and meningeonecephalitis. Many other clinical forms have been published by different workers including mastitis (Gray and Killinger, 1966; Gitter

*et al.*, 1980 and Seeliger, 1988). Subclinical acute or chronic cases of mastitis in cows resulting in economic losses. Moreover, serious mortality rates from Listeriosis occurred in sheep herds in Australia were observed (Dennis, 1975).

Milk and its products may be subjected to listeric infection from different sources such as infected and healthy animals and people, from silage, dust, waste water and others (Acha and Szyfers, 1991 and Franco Abuin *et al.*, 1996). Several outbreaks of Listeriosis occurred in Europe, USA and other countries due to consumption of raw and pasteurized milk, cheese, ice cream and other dairy products (Fleming *et al.*, 1985; Linnan *et al.*, 1988; Ryser and Marth, 1991 and Jensen *et al.*, 1994). Furthermore, several investigators could isolate *L. monocytogenes* from milk and milk products throughout the world (Farber *et al.*, 1988, Massa *et al.*, 1990, Loncarevic *et al.*, 1995, Hassan Nour, 1996 and Aman and Ahmed, 1997).

It has been reported that the studies done to evaluate the thermic resistance of *L. monocytogenes* have produced conflicting results. Early investigations indicated, however, that *L. monocytogenes* might be relatively heat resistant. Bearn and Girard (1958) isolated *Listeriae* from milk inoculated with over 50/000/ml after pasteurization at 61.7°C for 30 min. Doyle (1986) reported survival of *L. monocytogenes* in milk after pasteurization at 72°C for 16.4S while Fernandez *et al.*, (1986) recorded survival of *Listeria* spp. after pasteurization at 78°C for 15 seconds.

Because of the public health significance of *L. monocytogenes* as well as the economic losses caused by this organism, the present study was undertaken to study the prevalence of *L. monocytogenes* in pasteurized milk and some of its pasteurized products as cream and Rayeb, as well as to study the thermal resistance of the organism to boiling temperature at different times.

## **MATERIALS and METHODS**

A total of 75 random samples of pasteurized:milk; cream and Rayeb (25 samples of each) were collected from different supermarkets, groceries and the milk Technology Laboratory of Faculty of Agriculture, Assiut University. Samples were kept in refrigerator.

### **I-A- Preparation of samples**

Cartons or cans of collected samples were thoroughly cleaned from outside, then well mixed and aseptically opened.

## **B- Isolation and identification**

The technique recommended by FDA (Lovette *et al.*, 1987) was adopted by selective enrichment in Listeria enrichment broth (LEB) followed by selective plating onto Oxford agar plates (Curtis *et al.*, 1989). Suspected colonies of *L. monocytogenes* were picked up and purified before being identified according to Hitchins (1995).

## **II- Effect of milk boiling on the growth and survival of *L. monocytogenes***

### **- Culture preparation**

*L. monocytogenes* strain was obtained from the Institute of Milk Hygiene and Technology, Vet. Med. Univ., Vienna, Austria. The strain was inoculated into Listeria enrichment broth followed by plating 0.1 ml from decimal dilutions onto Oxford agar. The incubation was done at 37°C for 24h-48h for typical colonial morphology and purity.

### **- Experimental procedure:**

The previous strain was inoculated in sterile milk tested to ensure its freedom from *L. monocytogenes* to provide  $37 \times 10^7$  cells/ml., three trials were designed to confirm the effect of time of boiling on the growth and survival of *L. monocytogenes* in milk. Count of *L. monocytogenes* was achieved by direct plating of decimal dilutions of the boiled inoculated milk (A.P.H.A. 1992) onto Oxford agar plates, which incubated at 37°C for 24-24h and typical colonies presumed to be *L. monocytogenes* were counted.

## **RESULTS**

The obtained results were recorded in Tables 1 & 2.

**Table 1:** Incidence of *L. monocytogenes* in the examined samples of pasteurized: milk, cream and Rayeb.

Samples	No. of ex. samples	Positive samples	
		No.	%
Pasteurized milk	25	1	4
Pasteurized cream	25	-	-
Pasteurized Rayeb	25	-	-

**Table 2:** Effect of milk boiling on the growth and survival of *L. monocytogenes*.

Time/min	Count/ml			
	Trial (1)	Trial (2)	Trial (3)	Average
0	$37 \times 10^7$	$37 \times 10^7$	$37 \times 10^7$	$37 \times 10^7$
1	$283 \times 10^5$	$280 \times 10^5$	$259 \times 10^5$	$274 \times 10^5$
2	$160 \times 10^4$	$200 \times 10^4$	$282 \times 10^4$	$214 \times 10^4$
3	$135 \times 10^2$	$115 \times 10^2$	$110 \times 10^2$	$120 \times 10^2$
4	0	0	0	0

## DISCUSSION

Thermal processing is the most widely used method to preserve food in addition to destroy harmful microorganisms, thus rendering food safe for human consumption. Early studies dealing with possible resistance of *L. monocytogenes* to pasteurization (Bearn and Girard, 1958) but in 1983, interest in this topic was reviewed as a result of a Listeriosis outbreak in Massachusetts that was epidemiologically linked to consumption of pasteurized milk.

From the results recorded in Table 1 it was obvious that *L. monocytogenes* were isolated from 4% of the examined pasteurized milk samples, the same results were obtained by Fleming *et al.*, 1985, and Garayzabal *et al.*, 1986 who noticed outbreaks of Listeriosis due to consumption of improper pasteurized and flavoured pasteurized milks in the United States 1995, also Ryser and Marth (1991) recorded 49 cases of Listeriosis due to consumption of pasteurized milk in USA (1983) and this may be attributed to post pasteurization contamination or due to the explanation of Roy (1996) who concluded that *Listeria* organisms secrete a sticky substance called glycocalyx to attach themselves to the surfaces to resist cleaning and disinfection so, it can multiply and contaminate pasteurized milk which comes into contact with surfaces, additionally Gitter *et al.*, (1980) suggested that pasteurization did not offer a guarantee of complete safety if the viable bacterial count is high before heat treatment. However, Bradshaw *et al.* (1987) and Fedio and Jackson (1989) revealed that preheating of raw milk before sterilization or pasteurization processes leads to increase heat resistance of the organisms as compared with milk control. On the other side, the

obtained results were in disagreement with Rola *et al.* (1994) who could not detect the organism from pasteurized milk. Concerning pasteurized cream samples, Table 1. Showed that *L. monocytogenes* could not be detected from the examined samples, and these results were in agreement with that of El-Marrakchi *et al.* (1993) and Rola *et al.* (1994) who failed to detect the organism from fresh and pasteurized cream. This may be due to the high acidity that affects the organisms. Huang *et al.* (1993) stated that the growth rate of *Listeria* decreased with the increase of Lactic acid and acetic acid concentrations in the medium.

Data recorded in Table 1. showed that *L. monocytogenes* could not be detected in the pasteurized Rayeb samples and this is completely in agreement with the results obtained by Sabreen and Korashy (2001) who failed to isolate *L. monocytogenes* from plain and fruit yoghurt samples. Other investigators obtained the same results when examined other fermented dairy products (Kerr *et al.*, 1992; Rola *et al.*, 1994; Abou-Elainin, 1999 and El-Prince, 2000) this may be attributed to the high content of lactic acid and the resultant lowering of its pH value and other inhibitory compounds produced by lactic acid bacteria. In addition to the free fatty acids released during storage period which aid in destruction of food borne pathogens (Wang and Johnson, 1992). In contrast to these results, El-Gazzar and Marth 1991, El-Marrakchi *et al.*, 1993 and Gohil *et al.*, 1995) documented that *Listeria* has the ability to grow or survive for extended period in fermented and non fermented dairy products at various temperatures. While, the lower incidence (2%) of *L. monocytogenes* obtained by Greenwood *et al.* (1991) attributed to the post processing contamination from the plant environment. Also growth and survival of *L. monocytogenes* in fermented products have been noted elsewhere (Siragusal and Johnson, 1988; Ahmed, 1989; Sing and Chander, 1990 and Zuniga Estrada *et al.*, 1995).

Results from the second part of the study indicated in Table 2 depicted the effect of boiling on the growth and survival of *L. monocytogenes*. From the tabulated data the cell count of *L. monocytogenes* declared to be significantly decreased by boiling from  $37 \times 10^7$  to  $274 \times 10^5$ ,  $214 \times 10^4$  and  $120 \times 10^2$  in the first, second and the third minute respectively. The organisms failed to be detected and completely disappeared after the fourth minute. These results were somewhat in agreement with that of Potel (1951) and Abdel-Hakim and Sabreen (1993) who demonstrated that *L. monocytogenes* died rapidly in milk heated at 80°C. White Stenberg and Hammainen (1955) and

Donnelly *et al.* (1987) stated that *L. monocytogenes* was rapidly inactivated in milk at 62°C. On the other hand, the obtained results were in disagreement with Ozgen (1952), Dedie and Schulze (1957) and Ikonov and Todorov (1957). The heat resistance of *L. monocytogenes* may be due to the intracellular state of the organism in naturally contaminated milk Fleming *et al.* (1985).

It is noteworthy from these trials that the reduction in viable cell number of *L. monocytogenes* and loss in its viability may be due to the thermal processing which is considered the most widely used method to preserve food. The high temperature short time (HTST) pasteurization supplemented by Food and Drug Administration is adequate for destruction of *L. monocytogenes* in milk.

In general from the public health point of view, application of good hygienic measures during production, handling and filling in final containers is essential to safe the quality of milk and its products. Consequently prevent the risk of human hazards. In addition, it is important for good hygienists and employees working in the field of dairy production to understand the pattern of microbial growth specially those of public health concern as *L. monocytogenes* to safeguard human health.

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