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## ISOLATION OF *LISTERIA SPECIES* FROM RAW MILK AND SOME DAIRY PRODUCTS (With 3 Tables)

By

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عزل ميكروبات الليستيريا من اللبن الخام  
وبعض منتجات الألبان

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شملت تلك الدراسة جمع ١٨٠ عينة عشوائية من اللبن الخام وبعضاً من منتجات الألبان: الجبن القريش والثلوجات اللبنيّة وقد جمعت من مزارع الألبان وكذلك من الأسواق المختلفة بمدينة أسيوط وذلك لمعرفة مدى تواجد ميكروبات الليستيريا والتعرف عليها بكتريولوجيا بواسطة الاختبارات البيوكيميائية المختلفة. وقد أمكن عزل ميكروبات *L. monocytogenes* من ١ (٢٥٪)، ١ (٢٪)، ١ (٢٪) عينات اللبن الخام، الجبن القريش والاييس كريم كذا *L. innocua* من ٣ (٣٠٪)، ١ (٢٪)، ١ (٢٪) عينات اللبن الخام، الجبن القريش والاييس كريم بينما *L. welshimeri* تم عزلها من ١ (٢٥٪)، ١ (٢٪)، ٢ (٤٪) على التوالي من اللبن الخام، الجبن القريش والاييس كريم. ولقد استخدمت طريقتان للأغناء والاختصاص "Enrichment" في هذه الدراسة. وتم دراسة مدى ضراوة ميكروب الليستيريا مونوسيتوجين "*L. monocytogenes*" على الفئران البيضاء ولقد نوقشت الأهمية الصحية والطرق والاجراءات الواجب اتباعها لمنع تلوث اللبن ومنتجاته بهذا الميكروب.

### SUMMARY

A total of 180 random samples of milk and some milk products (Kareish cheese and Ice cream) were collected from different sources in Assiut and examined for the presence of *listeria species*. The obtained results pointed out that 1.25%, 2% and 2% of the examined milk, kareish cheese and ice cream were positive for *listeria monocytogenes*. While *L. innocua* could be isolated from 3.75%, 2%

and 2% of the examined samples respectively. Furthermore, 1.25%, 2% and 4% of milk, kareish cheese and ice cream contained *L.welshimeri*. Two methods for enrichment were performed. Pathogenicity of *L.monocytogenes* to mice were studied. The public health importance as well as recommended sanitary measures were discussed.

**Key words:** *Listeria* - Raw milk - Dairy Products.

## INTRODUCTION

The genus *Listeria* has clarified the picture of the heterogeneous group of bacteria (Seeliger and Jones, 1986). Seven species of *Listeria* are now recognized namely *L.monocytogenes*, *L.ivanovii*, *L.seeligeri*, *L.innocua*, *L.welshimeri*, *L.grayi* and *L.murrayi* according to biochemical properties Table (1). Only *Listeria monocytogenes* is presently considered the most recognized animal and human pathogen. Other occasional pathogens including *L.ivanovii*, *L.seeligeri*, *L.grayi* and *L.welshimeri*, have been documented recently to cause infections in animals and man (Rocourt *et al.*, 1986, Andre and Genico, 1987).

*Listeria monocytogenes* is a food borne pathogen which is lately of great concern not only for the food industry but also for regulatory agencies in different countries. (Farber and losos, 1988. and Lamont *et al.*, 1988).

Clinical manifestation of illness caused by *L. monocytogenes* comprise mainly, acute septicemia and abortion of pregnant women, meningitis, encephalitis, meningoencephalitis in immunocompromised men and women, lymphadenitis as well as endocarditis and abscess (Barza, 1985). The serious consequence of human listeriosis makes it of at most importance for public health significance and dairy industries (Al.Ashmawy, 1990).

The pathogen is wide spread in the environment, it has been isolated from food processing environments, finished food products and various dairy products such as ice cream, kareish cheese as well as raw milk (Hayes *et al.*, 1986, Lovett *et al.*, 1987; Farber *et al.*, 1989 Fenlon and Wilson 1989, El-Leboudy and Fayed 1992; Fathi and Saad 1992 Abdel Hakiem and Sabreen 1993 Hassanein 1994 and El Kholy and El-Leboudy 1995).

It was assumed that human acquired listeriosis due to direct contact with infected animals. Recently a number of outbreaks have confirmed that this disease can be indirectly transmitted from infected animals to human through consumption of contaminated dairy products (Hayes *et al.*, 1986; Fenlon and Wilson, 1989).

Therefore, The aim of this work was to investigate the incidence of *Listeria* species in milk and some dairy products (Kareish cheese and ice cream) by two methods of enrichment prior to isolation, and study the pathogenicity of *L. monocytogenes* in mice.

## MATERIAL and METHODS

### Collection of samples:

A total of 180 random samples of raw milk (80) and milk products including kareish cheese (50) and ice cream (50) were collected from dairy farms, dairy shops, street peddlers, supermarkets and groceries in Assiut city.

All samples were dispatched in clean, dry sterile containers with a minimum of delay.

### Preparation of samples

Milk samples were tested for heat treatment (Richardson, 1985) and shaken thoroughly. Ice cream samples were melted in a thermostatically controlled water bath ( $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) for 15 minutes and well mixed. On the other hand, kareish cheese samples were grinded well in a sterile mortar (Richardson, 1985).

### Enrichment procedure

10 mL of milk, ice cream and 10 gm of prepared cheese were added to 90 mL of an enrichment broth. Tryptose broth containing 40ug/ml Nalidixic acid and 30 U/ml polymyxin B sulphate as described by Hofer, (1983), double enrichment broth media were prepared for each sample, one was incubated at  $37^{\circ}\text{C}$  for 24 hrs. and the second was held at  $4^{\circ}\text{C}$  (cold enrichment), for two weeks.

### Isolation and identification of listeria species:-

After incubation of enrichment broth 0.1 ml of each was streaked on the surface of tryptose agar supplemented by 40 ug/ml Nalidixic acid and 30 U/ml polymyxin B sulphate and incubated at  $37^{\circ}\text{C}$  for 48°C. Colonies resembling those of listeria were Gram

stained. Confirmatory tests were done on each isolate as described by Rocourt and Grimont, (1983) and Lovett(1988).

**Mice pathogenicity:**(Seeliger and Jones,1986).

Each of the cultural isolates which was confirmed biochemically as *L.monocytogenes* was grown two times in tryptose soya broth with 0.6% yeast extract at 35°C for 24 h. The broth was centrifuged and the sediment was washed by 0.01% phosphate buffer saline at pH 7.4 The bacterial culture was diluted to give 10<sup>4</sup>-10<sup>5</sup> colony forming unit (CFU)/1ml of broth. 0.1 ml containing 10<sup>4</sup> cell were inoculated I.P into white mice (20g). Mice were observed for five days. After death the listeria organisms were isolated from both brain and liver by smearing these specimens over an tryptose soya agar surface supplemented by 40 ug/ml Nalidixic acid and 30 U/ml polymyxin B sulphate and incubated at 37°C for 24-48 h.. The organisms were identified biochemically as *L.monocytogenes*.

## RESULTS

The results obtained were recorded in tables 2 and 3.

## DISCUSSION

In recent years, *L.monocytogenes* has become of considerable importance to food industry. (AL-Ashmawy, 1990 and Hassanein, 1994).

The findings reported in Table (2) declare that 3(1.67%) out of 180 examined samples were contaminated with *L.monocytogenes*, it could be detected in one (1.25%) of raw milk samples. Nearly similar results were reported by Fenlon and Wilson (1989), El-Leboudy and Fayed (1992), El-Kholy and El-Leboudy (1995), while higher figures (3.7-7%) were recorded by Hayes *et al.* (1986), Lovett *et al.* (1987), Twedt (1988) and Charlton *et al.* (1990). On the other hand, Hassanein (1994) recorded lower figure (0.4%).

These difference incidences may be due to differences in places or methods of isolation. The excretion of *L. monocytogenes* in cow's milk is well recognized. Cows with Listeria mastitis may produce normal appearing milk while containing large numbers of listeria organism. Moreover, the presence of *L.monocytogenes* in milk may be caused by exogenous contamination from farm environment, feed and/or faecal contamination (Fleming *et al* 1985).

*L.monocytogenes* could be detected in one (2%) of the examined ice-cream samples, this finding agrees with that reported (1.96%) by Farber et al. (1989). While higher incidence (10%) was reported by Ciflciglu et al (1992). On the other hand lower incidence (0.32%) was reported by Abdel-Hakiem and Sabreen (1993). The occurrence of *L.monocytogenes* in ice cream products in last few years has caused large economic losses for the ice cream industry due to partly to recalls of contaminated ice cream.

Concerning cheese samples the incidence of the *L.monocytogenes* was (2%), similar results were obtained by Fathi and Saad (1992) and Gul et al. (1995).

In experimentally infected mice, death occurred from 3-5 days after injection, the gross lesions found in mice which succumbed to the experimental infection revealed that the early macroscopic lesion appeared in the form of haemorrhagic foci in lung, liver and brain, and late lesions showed necrosis which started after the fourth day of inoculation and *L.monocytogenes* was isolated from infected mice. This result was compatible with that reported by El-Leboudy and Fayed (1992).

The frequency distribution of other listeria species were tabulated in Table (2), it revealed isolation of *L.innocua* (5/180) and *L.welshimeri* (4/180), no other species of listeria were isolated.

*L.innocua* was the most frequently isolated from raw milk in this study, the incidence was (3.75%), this is similar to results reported by Rodriguez et al. (1994), El-Kholy and El-Leboudy (1995). High incidence (9.7, 8.8%) was reported by Farber et al. (1988) and El-Leboudy and Fayed (1992). Low incidence (1.7%) was reported by Hassanein (1994).

Concerning ice cream samples *L.innocua* was isolated from 1(2%) of the examined samples, this is in agreement with Ciflciglu et al. (1992) and Abdel-Hakiem and Sabreen (1993). Also *L.innocua* could be isolated from (2%) Kareish cheese samples, nearly similar results reported by Fathi and saad (1992) and Gul et al. (1995).

*L.welshimeri* isolated from (1.25%) of milk samples examined, this agree with that reported by Hassanean (1994) and El-kholy and El-Leboudy (1995). The incidence in ice-cream was (2%) while in kareish cheese it was (4%), Fathi and Saad (1992) could isolate it from one sample (1%) of Kareish cheese.

*L.welshimeri* has been documented recently to cause infection for human (Andre and Genico, 1987). The use of any listeria species as an indicator for the presence of *L. monocytogenes* has been proposed (Johnston et al., 1990)

The data presented in table (3) shows that (12/180) of samples were contaminated with listeria. All the positive isolates were obtained after cold enrichment at 4°C for 2 weeks, while some positive (7/12) were obtained after enrichment for 24h. at 37°C.

These results substantiate what have been reported by Hayes *et al.*, (1986) and Doyle and Schonei (1987) they reported that the best method for detecting the organism was cold enrichment followed by surface plating on selective media.

Additional studies are needed to further evaluation of the cold enrichment procedure for isolating listeria from foods.

In conclusion, much attention should be given by specialists to study the presence of this pathogen in dairy products accustomed for isolation and identification of such organism. Finally and clearly, there is a definite need for information and continued educational programs to increase the awareness of farmers, food technologists and consumers on the safe handling of dairy products by medical authorities, veterinarians and food bacteriologists.

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Table (1): Differentiation of *Listeria* species: (Rocourt *et al* 1986)

Species	Beta-haemolysis	CAMP tests with <i>S. aureus</i> *	Fermentation of			Pathogenicity for mice
			Mannitol	L-Rhamnose	D-Xylose	
<i>L. monocytogenes</i>	+	+	-	+	-	+
<i>L. ivanovii</i>	++	-	-	-	+	+
<i>L. seeligeri</i>	+	+	-	-	+	-
<i>L. innocua</i>	-	-	-	v	-	-
<i>L. welshimeri</i>	-	-	-	v	+	-
<i>L. grayi</i>	-	-	+	v	-	-
<i>L. murrayi</i>	-	-	+	-	-	-

v= variable reactions; \* *Staphylococcus aureus*

Table (2): Incidence of *Listeria* species recovered from raw milk, ice cream and Kareish cheese samples.

Samples	No. of examined samples	Positive samples					
		<i>Listeria monocytogenes</i>		<i>L. innocua</i>		<i>L. Welshimeri</i>	
		No.	%	No.	%	No.	%
Raw milk	80	1	1.25	3	3.75	1	1.25
Ice cream	50	1	2	1	2	1	2
Kareish cheese	50	1	2	1	2	2	4
Total	180	3	1.67	5	2.78	4	2.22

Table (3): Comparison of two enrichment methods for isolation of *Listeria* species.

Samples	<i>Listeria monocytogenes</i>		<i>L. innocua</i>		<i>L. Welshimeri</i>	
	37°C/24 h	4°C/2 w	37°C/24 h	4°C/2 w	37°C/24 h	4°C/2 w
Raw milk	1	1	2	3	0	1
Ice-cream	1	1	1	1	1	1
Kareish cheese	0	1	0	1	1	2
Total	2	3	3	5	2	4