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LISTERIA ORGANISMS AS BACTERIAL PATHOGENS IN MILK AND SOME DAIRY PRODUCTS IN FAYOUM GOVERNORATE

(With 3 Tables)

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ميكروبات الليستريا الممرضة فى الألبان و بعض منتجاتها فى محافظة الفيوم

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جمعت ١٠٠عينة عشوائية من اللبن الخام ومنتجات الألبان (الجبن الطرى قليل الملح، الجبن الجاف، الأيس كريم، الزبادى (٢٠عينة لكل نوع) من اماكن مختلفة بمدينة الفيوم، وذلك لمعرفة مدى تلويثها بميكروبات الليستريا المختلفة. فى هذه الدراسة تم عزل ميكروبات الليستريا بنسبة ٦% من العينات وكان أعلى تواجد لها بعينات الجبن الطرى قليل الملح والأيس كريم بنسبة ١٠% و٥% علىالتوالى ولم يتم عزلها من عينات الجبن الجاف والزبادى. تم عزل ٦ عترات من أنواع الليسريا المختلفة من ١٠٠ عينة كان من بينهم ٢ عترة من نوع الليستريا مونوسيتوجيتز من كل من اللبن الخام، والجبن الطرى قليل الملح بنسبة ٥% لكل منهما. والليستريا ويلشيميرى تم عزلها من اللبن الخام والجبن الطرى بنسبة ٥% لكل منهما. والليستريا ينوكيا عزلت من الجبن الطرى بنسبة ٥% والليستريا ميراى عزلت من الأيس كريم بنسبة ٥% ولم يتم عزل أى نوع من ميكروبات الليستريا من عينات كل من الجبن الجاف والزبادى. ولقد نوقشت الأهمية الصحية والطرق والإجراءات الواجب إتباعها لمنع تلوث منتجات الألبان بهذه الميكروبات.

SUMMARY

A total of 100 random samples of raw milk and some dairy products (low salt soft cheese, hard cheese, ice cream and yoghurt, 20 samples each) were collected from different localities in Fayoum city and examined for the presence of *Listeria* spp. In this investigation the recovery rate of *Listeria* from the examined samples was 6%. The highest incidence of *Listeria* species was recovered from low salt soft cheese 15% followed by raw milk and ice cream samples with incidence of 10% and 5%, respectively. A total of 6 *Listeria* species were isolated from the examined100 samples. From 6 *Listeria* isolates, 2 isolates were

identified as *L. monocytogenes* isolated from raw milk and low salt soft cheese. 2 isolates were identified as *L. welshimeri* from raw milk and low salt soft cheese. one isolate was identified as *L. innocua* from low salt soft cheese and one isolate was identified as *L. murrayi* from ice cream. The public health importance and the sanitary measures for control of *Listeria* spp. were mentioned.

Key words: *Dairy products, milk, Listeria*

INTRODUCTION

The importance of *Listeria* as a foodborne pathogen has become increasingly apparent since the outbreaks reported in North America. The epidemics, which have been associated with pasteurized milk (Fleming *et al.,* 1985) and Mexican-style cheese (James *et al.,* 1985) have prompted concern about the survival and growth characteristics of *Listeria* in contaminated food stuffs during cold storage.

Currently seven species are known in the genus *Listeria, L. monocytogenes, L. seeligeri, L. welshimeri, L. innocua, L. murrayi, L. grayi and L. ivanovi.* The most important human pathogen in the genus is *L. monocytogenes* which is a facultative intracellular Gram-positive bacterium responsible for listeriosis a sever foodborne illness which may result in meningitis, septicemia, spontaneous abortion and gastroenteritis (Anonymous, 2000).

In domestic animals *Listeria* organisms can cause circling disease, encephalitis, septicaemia, mastitis and abortion (Rosenow and Marth, 1987).

This association with food together with the unique ability of *Listeria* to grow under a wide variety of food processing conditions including: refrigeration temperature (Palumbo and Williams, 1991, Gougouli *et al.,* 2008), its tolerance of moderate to high sodium chloride levels (Feresu and Jones, 1988), sodium nitrate (Shahamat *et al.,* 1980) and a wide pH range (George *et al.,* 1988, Belessi *et al.,* 2008), its growth in the presence or absence of oxygen (Linton *et al.,* 1995) and a high heat resistance compared to most other non spore forming food borne pathogens (Gervilla *et al.,* 1997) have prompted to study the occurrence of the organism in milk and other dairy products.

Therefore, the objective of the present work was planned to:

- Study the distribution of *Listeria* organisms associated with dairy products by bacteriological examination of raw milk, cheese, ice cream and yoghurt samples.

MATERIALS and METHODS

1- Collection of samples:

A total of 100 samples of raw milk and some diary products (low salt soft cheese "Talaga"), hard cheese (Romy), Ice cream and yoghurt were randomly collected from different markets and dairy shops in Fayoum Governorate. The samples were collected in clean sterilized equipments and taken under aseptic conditions, and transferred to the lab in an ice box, where they were examined for the presence of *Listeria* microorganisms.

2- preparation of samples: according to Marth (1978).

a- Raw milk: The sample was well agitated before examination.

b- Cheese: The sample must be representative of the food's outer surface as well as its interior part.

c- Ice cream: A homogenate was prepared by holding the ice cream container in a water bath at 42°C-45°C for not more than 15 minutes.

d- Yoghurt: Samples were prepared following the technique described by APHA, (1992).

3- Isolation and identification of *Listeria* spp:

The technique recommended by Federal Register (1988) was adopted by selective enrichment in *Listeria* enrichment broth (LEB), followed by Fraser's secondary enrichment broth (F.B), after 24-48 hours were streaked onto Oxford agar Plates (Curtis *et al.*, 1989).

Suspected colonies of *Listeria* were picked up and purified before being identified according to Hitchins (1995).

RESULTS

Table 1: Prevalence of *Listeria* spp. in the examined raw milk and some milk products

Types of samples	Number of examined samples	Positive samples	
		No.	%
1- Raw milk	20	2	10
2- Low salt soft cheese (Talaga)	20	3	15
3- Hard cheese (Romy)	20	0	0
4- Ice cream	20	1	5
5- Yoghurt	20	0	0
Total	100	6	6

Table 2: Percentage distributions of *Listeria* species in the examined raw milk and some milk products.

Types of samples	No. of examined samples	*L. monocytogenes*		*L. welshimeri*		*L. innocua*		*L. grayi*		*L. murrayi*		*L. seeligeri*		*L. ivanovi*		Total No. of *Listeria* spp.	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Raw milk	20	1	5	1	5	0	0	0	0	0	0	0	0	0	0	2	10
Low salts soft cheese	20	1	5	1	5	1	5	0	0	0	0	0	0	0	0	3	15
Hard cheese	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ice cream	20	0	0	0	0	0	0	0	0	1	5	0	0	0	0	1	5
Yoghurt	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	100	2	2	2	2	1	1	0	0	1	1	0	0	0	0	6	6

Table 3: Prevalence rate of *Listeria* species in the examined raw milk and some milk products samples.

Lesteria spp.	Types of samples									
	Raw milk		Low salt soft cheese		Hard cheese		Ice cream		Yoghurt	
	No.	%	No.	%	No.	%	No.	%	No.	%
L. monocytogenes	1	50	1	33.3	0	0	0	0	0	0
L. welshimeri	1	50	1	33.3	0	0	0	0	0	0
L. innocua	0	0	1	33.3	0	0	0	0	0	0
L. murrayi	0	0	0	0	0	0	1	100	0	0
Total	2	100	3	100	0	0	1	100	0	0

DISCUSSION

Listeriosis is listed among the zoonotic diseases there is sufficient evidence to prove that listeriosis is primarily a foodborne disease. Dairy products currently have stronger association with listerosis than other types of food with both raw and pasteurized milk as well as certain cheese have been identified as causes of food poisoning outbreaks. (Fleming *et al.* 1985, James *et al.* 1985, Jayarao *et al.* 2006).

Among raw milk samples as shown in Table: 1 *Listeria* was isolated with an incidence 10% this is supported by the result of Slade and Collins-Thompson, (1988) and Farber *et al.* (1988) who found a total of 11-14 % *Listeria* species from raw milk. A lower incidence 2.2% of *Listeria* species in raw milk was recorded by Hamdallah and Ahmed (2007).

In Table: 2 *L. monocylogenes* was isolated from 5% of examined raw milk samples. This incidence was nearly similar to that reported in a study carried out by Slade and Collins-Thompson (1988) who found an incidence of 5.9% *L. monocytogenes* from raw milk. Liewen and plautz (1988), Laura and Elmer (1990) and Macrae *et al.* (1993) recorded that *L. momocylogenes* was present in about 4% of the examined raw milk samples. Meanwhile, Kovincie *et al.* (1979) and Hamdallah and Ahmed (2007) isolated *L. monocytogenes* respectively from 3.2% and 1.6% raw milk samples.

A higher incidence of *L. monocytogenes* in milk was recorded by Hayes *et al.* (1986) 15%, Garayzabal *et al.* (1987) 14.3% to 75% and Oliveira *et al.* (1998) 8%.

The results given in Table 2 revealed that *L. welshimeri* 5% was isolated from raw milk samples thus finding go hand in hand with the finding of Kamel (1996) who recovered *L. welshmeri* from raw milk samples. No other species could be isolated from raw milk samples. These results were compatible with that results obtained by Farber *et al.* (1988) and Lund *et al.* (1991), while Gaya *et al.* (1998) isolated them from milk samples.

The incidence of *Listeria* species appears to vary from survey to another. This may be dependent upon factors such as, the method used for isolation, the geographic location, seasonal variation and milk quality (Garayzabal *et al.* 1987, Farber *et al.* 1988, and Lund *et al.* (1991).

Milk is generally bacteria free when it comes from the udder of healthy cow (Frazier and Westhoff, 1998), but *Listeria* may enter the milk supply in several ways. The main source of *Listeria* in milk is probably fecal contamination (Macrae *et al.* 1993).

In Table 1 *Listeria* was recovered from 15% of low salt soft cheese. Terplan *et al.* (1986) isolated *Listeria* species from soft cheese with incidence of 10.2%, While Vitas and Garcia (2004) isolated them with incidence of 8.11%. Furthermore surveys in a number of countries have shown that *Listeria* may be a common contaminant of soft cheese (Farber and Peterkin, 1991; Zottola and Smith, 1991 and Eppert *et al.* 1997).

Table 2 illustrated that *L. monocytogenes, L. welshimeri* and *L. inncoua* were isolated from low salt soft cheese samples with an incidence of 5% from each of them. Silva *et al.* (1998) detected *L. monocytogenes* (10.6%), *L. innocua* (12.6%). Rota *et al.* (1992) isolated *L. innocua* from soft cheese.

No *Listeria* species could be isolated from hard cheese samples. Similar observations were recorded by El-Sukhon, (1993). On rare occasions, hard cheese have become contaminated with *Listeria* (Macrae *et al.* (1993). This may be attributed to the presence of high levels of lactic acid and low pH values (Ryser *et al.* 1985 and Marth, 1998).

One approach to reduce the prevalence of *Listeria* in cheese is the application of bacteriocin producing starter cultures that show antagonistic properties towards *Listeria* organisms (Joosten *et al.* 1995).

Junttila *et al.* (1988) and Macrae *et al.* (1993) demonstrated the resistance of *Listeria* to freezing. Among ice cream products *Listeria* was isolated with a percentage of 5%, also Marcae *et al.* (1993), Dean and Zottola (1996) isolated *Listeria* from ice cream samples in the same percentage.

In Table 2 the incidence of *L. murrayi* in ice cream was 5%. Coincides with Cox *et al.* (1989) who detected *Listeria* species in the environment of ice cream production.

Greenwood *et al.* (1991) reported that ice cream occasionally contains *Listeria* species. Rosenow and Marth, (1987) recorded that ice cream has been implicated but never directly involved in causing human listerosis. WHO working group (1988) who concluded that the incidences of *L. monocytogenes* in ice cream varied from 0 to 5.5% with very low levels of *Listeria* organisms (1-15 cfu/gm). Gougouli *et al.* (2008) found that under chilling conditions *L. monocytogenes* grew well at all temperature tested (4 to 16 °C) in ice cream samples. Under freezing conditions (-5 to -33°C) no significant changes in the inoculum levels tested (10^3 and 10^6 cfu/g) of *L. monocytogenes* in ice cream samples.

The results recorded in Table 1 proved that *Listeria* species could not be detected in any of 20 examined samples of yoghurt. Similar results were obtained by Kerr *et al.* (1992), Rola *et al.* 1994, Abou Eleinin (1999) and El-Prince (1999). The failure of these organisms to grow in yoghurt may be due to the presence of lactic acid production and the resultant lowering pH value of such products (Huang *et al.* 1993 and Marth, 1993).

On the other hand, GreenWood *et al.* (1991) isolated *L. monocytogenes* from one sample (2.13%) out of 47 yoghurt samples and the authors attributes the presence of *Listeria* species in yoghurt to the post processing contamination from the plant environment.

Although, milk used to produce the industrially fermented milk is pasteurized, contamination of the product with *L. monocytogenes* may occur after pasteurization if complex and less easily cleaned equipments are used in packaging, filling rooms or if bulk starter cultures are contaminated with organisms (Charlton *et al.* 1990).

Dairy products are involved in several outbreaks of listeriosis, so the identification of *Listeria* species in milk and dairy products could be very important for human health. *L. monocytogenes* has been involved in several outbreaks and sporadic cases of diseases associated with the consumption of pasteurized milk and other dairy products (Van Kassel *et al.* 2004, Makino *et al.* 2005).

There were 36 cases of listeriosis in France which were linked to Briede Meaux a soft cheese made from raw milk, in this case no deaths were recorded Leile *et al.* (2006).

It's estimated that 2500 *L. moncytogenes* infections occur in the USA each year. The *L. monocytogenes* detection rates observed in the cheese samples monitored after packing and at the end of the shelf life were 2.1% and 4.8%, respectively (Manfreda *et al.* 2005). The presence of *L. monocytogenes* in milk-based products can be related to raw milk contamination or to post-pasteurization contamination (Carminati *et al.* 2004).

In conclusion, from this work it is clear that contamination of dairy products by *Listeria* spp. especially *L. monocytpgenes* from the view point of a potential health hazard should not be ignored. *Listeria* could contaminate the dairy products through raw milk used without sufficient heat treatment or through contamination of equipments used for the preparation or distribution of the products. Therefore, application of good hygienic measures during production, storage and distribution of such products are essential to safe products quality, consequently prevent the risk of human hazards. It is important for food hygienists and employees working in the field of production of dairy products to understand the pattern of microbial growth specially those of public health concern such as *L. monocytogenes* in order to safeguard consumers.

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