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ISOLATION OF LISTERIA MONOCYTOGENES AND OTHER LISTERIA SPECIES FROM MILK AND SOME DAIRY PRODUCTS

(With 2 Tables)

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من اللبن وبعض منتجات الألبان

إيناس البرنس

يعتبر مرض الـ Listeriosis من الأمراض الخطيرة التي قد تصيب الانسان نتيجة لتناول اللبن أو بعض منتجاته الملوثة بميكروب الليستريا مونوسيتوجينيس في شكل وبياء ، لذلك أجريت هذه الدراسة على مائتي عينة من اللبن الخام والجبن الدمياطى والزبد الفلاحى والزبادى (٥٠ من كل منها) تم جمعها بطريقة عشوائية من أماكن متعددة بمدينة أسيوط لمعرفة مدى تواجد هذا الميكروب . وبفحص هذه العينات أمكن عزل ميكروب الليستريا مونوسيتوجينيس من عينة واحدة من كل من اللبن الخام والجبن الدمياطى بنسبة ٢% ، بينما كانت عينات الزبد الفلاحى والزبادى خالية تماماً من هذا الميكروب . بالإضافة الى عزل ميكروب الـ L. innocua من عينة واحدة من اللبن الخام ، وعينتين من الزبد الفلاحى بنسبة ٢% ، ٤% على التوالي . أما ميكروب L. welshimeri فكان متواجد بنسبة عالية نسبياً فى الزبد الفلاحى . هذا وقد تمت مناقشة النتائج وأهمية وخطورة تواجد هذا الميكروب على صحة الانسان وما يجب إتخاذه لمنع إنتشاره .

SUMMARY

Two-hundred random samples of raw milk, Domiati cheese, cooking butter and yoghurt (50 each) were collected from street vendors, different local supermarkets, dairy farms and dairy shops in Assiut city, Egypt. The samples were examined bacteriologically for the presence of L.

monocytogenes and other *Listeria* spp. The obtained results revealed that *L. monocytogenes* was found in one sample (2%) of both raw milk and Domiati cheese, while the organism failed detection in the examined cooking butter and yoghurt samples. However, *L. innocua* could be isolated from one (2%) and 2 (4%) of the examined raw milk and cooking butter samples, respectively. Also, 4 (8%) of cooking butter samples proved to harbour *L. welshimeri*. The public health importance of *Listeria* spp. in milk and dairy products and the suggestive measures were discussed.

Keywords: *Listeria monocytogenes, Listeria species, Milk, Dairy products.*

INTRODUCTION

L. monocytogenes is a foodborne pathogen which is now very well known by individuals concerned with food safety (Farber, 1993). This organism is ubiquitous in nature and is widely spread in the environment, where it may survive for long periods (Eley, 1996). It has been associated with serious disease "Listeriosis" in man. The typical symptoms of listeriosis are septicaemia, meningitis or abortion in pregnant women, which occur most commonly in neonates and immunosuppressed patients (Miller *et al.*, 1997 and Arizcun *et al.*, 1998). Before the 1980s, *L. monocytogenes* was of concern primarily as a cause of abortion and encephalitis in animals such as cattle, sheep, goats, buffaloes and chickens (Gitter *et al.*, 1980 and Ralovitch, 1984). Moreover, in dairy cattle *L. monocytogenes* can cause mastitis leading to excretion of the organism in milk, subsequently, transmitted to human from these infected animals or through consumption of *Listeria*-contaminated foods (Papageorgiou and Marth, 1989). Recently, milk and dairy products are the most often incriminated as responsible for listeriosis than other types of food (Franco Abuin *et al.*, 1996). It has been well documented that three foodborne outbreaks of listeriosis were associated with consumption of pasteurized milk in Massachusetts, USA in 1983 (Fleming *et al.*, 1985), soft Mexican-style cheese in California, USA in 1985 (James *et al.*, 1985) and soft cheese of the Vacherin Mont d'Or type in Switzerland in 1987 (Papageorgiou and Marth, 1989). Furthermore, many surveys have been conducted and have shown that *L. monocytogenes* or other *Listeria* spp.

to be commonly isolated from milk and dairy products worldwide (Farber *et al.*, 1988; Massa *et al.*, 1990; Loncarevic *et al.*, 1995; Hassan, 1996 and Aman and Ahmed, 1997). The incidence of *L. monocytogenes* in raw milk has been reported to be between 0 and 45.3% (Farber and Peterkin, 1991). Also, the number of this pathogen in soft cheese may be as high as 10^6 /g (Terplan *et al.*, 1986; Farber *et al.*, 1987 and Gilbert and Pini, 1988). Fortunately, many of the bacterium present in cream are removed with butter milk during butter manufacture and the *L. monocytogenes* population is reduced by 97.5% (Olsen *et al.*, 1988). Additionally, *L. seeligeri* and *L. welshimeri* have been documented to cause infections in human (Rocourt *et al.*, 1986 and Andre and Genicot, 1987). The use of *Listeria* spp. other than *L. monocytogenes* as indicators of the presence of that pathogen has been proposed (WHO, 1988).

Owing to the several outbreaks of listeriosis in the past few years, the hygienic relevance of the presence of *Listeria* spp. in food has moved to a fundamentally new level (Url *et al.*, 1993). This fact estimates us to determine the incidence of *L. monocytogenes* and other *Listeria* spp. in raw milk and in some dairy products.

MATERIAL and METHODS

Collection of samples:

Two-hundred random samples of raw milk, Domiati cheese, cooking butter and yoghurt (50 each) were collected from street vendors, different local supermarkets, dairy farms and dairy shops in Assiut city, Egypt. The samples were maintained at chill temperatures during transportation to the laboratory and tested for the presence of *L. monocytogenes* and other *Listeria* spp.

Isolation and identification of Listeria spp.:

The most widely used approaches are based upon (FDA) method (Lovett *et al.*, 1987). 25 ml or 25 g of each prepared sample were added to 225 ml *Listeria* Enrichment Broth (LEB) and incubated at 35° for 48 h, then loopfuls were streaked onto *Listeria* Selective Medium (LSM-Oxford Formulation). After incubation at 35°C for 48 h. The suspected colonies were picked up for purification. Identification and species differentiation were done according to Johnson *et al.* (1990) including carbohydrate fermentation, B-haemolysis on blood agar and CAMP test.

RESULTS

The obtained results were recorded in Tables (1 & 2).

Table 1. Isolation of *L. monocytogenes* from the examined samples.

Type of samples	No. of examined samples	Positive samples	
		No.	%
Raw milk	50	1	2%
Dommati cheese	50	1	2%
Cooking butter	50	0	-
Yoghurt	50	0	-

Table 2. Isolation of other *Listeria* species from the examined samples.

Type of samples	No. of positive samples					
	<i>L. monocytogenes</i>		<i>L. innocua</i>		<i>L. welshimeri</i>	
	No./50	%	No./50	%	No./50	%
Raw milk	1	2%	1	2%	0	-
Dommati cheese	1	2%	0	-	0	-
Cooking butter	0	-	2	4%	4	8%
Yoghurt	0	-	0	-	0	-

DISCUSSION

The results in Table 1 revealed that *L. monocytogenes* could be detected in 1 (2%) of each of raw milk and Dommati cheese samples, while the organism failed detection in the examined samples of cooking butter and yoghurt. Similar findings were obtained by Husu *et al.* (1990) and Rola *et al.* (1994). However, higher percentages were stated by Ahmed (1990), Saito *et al.* (1991), Banks (1994), Abdel-Hady *et al.* (1996) and Hassan (1996). On the other hand, *L. monocytogenes* was recovered from only 0.2% of raw milk samples examined by Hassanein (1994). The main source of this microorganism in milk is probably faecal contamination or diseased dairy animal which can excrete this pathogen in their milk (El-Gazzar and Marth, 1991). *L. monocytogenes* also is able

to grow and increase in numbers during storage in farm bulk milk tanks (Lovett *et al.*, 1987).

Concerning Domiati cheese samples, similar findings were obtained by D'Errico *et al.* (1990), Massa *et al.* (1990), Fathi and Saad (1992) and Banks (1994). While, Aman and Ahmed (1997) found that *L. monocytogenes* could not be isolated from the examined cheese samples. However, Bannerman and Bille (1988) stated that *L. monocytogenes* was recovered from 14.3% of cheese samples. Although, the increased amount of salt added to Domiati cheese retard *L. monocytogenes* growth (Ahmed *et al.*, 1989 and Marth, 1993), but many outbreaks of listeriosis were associated with different types of cheese (James *et al.* 1985 and Papageorgiou and Marth, 1989). Since, the contamination of soft cheese is often caused by insufficiently sanitized equipments (Terplan *et al.*, 1990).

It is obvious from the results presented in Table 2 that all *Listeria* isolates from the samples of milk and dairy products were identified as *L. monocytogenes*, *L. innocua* and *L. welshimeri*. It is clear that 1 (2%) of raw milk and 2 (4%) of cooking butter samples were positive for *L. innocua*. However, *L. welshimeri* could be isolated only from 4 (8%) samples of cooking butter. Nearly similar results were recorded by Saito *et al.* (1991), while D'Errico *et al.* (1990), El-Leboudy and Fayed (1992) and Aman and Ahmed (1997) stated higher isolation rate of *L. innocua* from raw milk samples. Milk may be easily contaminated from listeric animals as well as from the environmental surroundings and dirty equipments (WHO, 1992).

Regarding cooking butter samples, Adams and Moss (1995) reported that *L. welshimeri* was associated with human illness. Although, many of bacteria found in cream are removed with the butter milk during butter manufacturing, *Listeria* organism is able to grow during the initial stages of storage (Olsen *et al.*, 1988).

In case of yoghurt samples, *L. monocytogenes* or other *Listeria* spp. could not be detected. This finding go parallel with the results achieved by Rola *et al.* (1994). The failure of these microorganisms to grow could be attributed to lactic acid production and the resultant lower pH value (Ahmed, 1989).

It would appear from this investigation that contamination of milk and some dairy products by *L. monocytogenes* from the viewpoint of a

potential health hazard should not be ignored. The contamination may occur through insufficient heat treated raw milk or through contaminated equipments used for preparation and distribution of the dairy products. Therefore, stringent hygienic measures must be followed in dairy plants and farms.

REFERENCES

- Abdel-Hady, H.M.; Moawad, A.A. and Abouzeid, A.M. (1996):* Validation of simple method for rapid detection of *L. monocytogenes* in raw milk and kareish cheese. 4th Sci. Cong. Vet. Med. J. Giza, 44: 209-213.
- Adams, M.R. and Moss, M.O. (1995):* Food Microbiology. The Royal Soc. of Chem., Cambridge.
- Ahmed, A.A-H. (1989):* Behaviour of *L. monocytogenes* during preparation and storage of yoghurt. Assiut Vet. Med. J., 22: 76-80.
- Ahmed, A.A-H.; Ahmed, S.H.; Moustafa, M.K. and Saad, N.M. (1989):* Growth and survival of *L. monocytogenes* during manufacture and storage of Damietta cheese. Assiut Vet. Med. Med. J., 22: 88-94.
- Ahmed, M.E. (1990):* Isolation of *L. monocytogenes* from farm bulk milk cans. Egypt. J. Vet. Sci., 27: 109-115.
- Aman, I.M. and Ahmed, H.F. (1997):* Incidence and survival of some foodborne pathogens in milk and cheese. J. Egypt. Vet. Med. Assoc., 57: 151-163.
- Andre, P. and Genicot, A. (1987):* First isolation of *L. welshimeri* from human beings. Zentrabl. Bakteriologie. Parasitenkunde. Infektionskrankheiten. Hygiene. Abt. 1 Orig. Reihe A, 263: 605-606.
- Arizcun, C.; Vasseur, C. and Labadie, J.C. (1998):* Effect of several decontamination procedures on *L. monocytogenes* growing in biofilms. J. Food Prot., 61 (6): 731-734.
- Banks, W. (1994):* Microbiology of milk. Milk Industry-UK: Technical and Processing Suppl., 96: 18-20.
- Bannerman, E.S. and Bille, J.J. (1988):* A new selective medium for isolating *Listeria* spp. from heavily contaminated material. Appl. Environ. Microbiol., 54: 165-167.

- D'Errico, M.M.; Villari, P.; Grasso, G.M.; Romano, F. and Angelillo, I.F. (1990): Isolation of Listeria spp. from milk and cheese. Rivista della Societa Italiana di Scienza dell'a Alimentazione, 19: 47-52. Dairy Sci. Abst., 52: 929 (1990).*
- El-Gazzar, F.F. and Marth, E.H. (1991): L. monocytogenes and listeriosis related to milk, milk products and dairy ingredients. A review: Milchwissenschaft, 46 (2): 82-88.*
- El-Leboudy, A.A. and Fayed, M.A. (1992): Incidence of listeria in raw milk. Assiut Vet. Med. J., 27: 134-146.*
- Eley, A.R. (1996): Microbial Food Poisoning. 2nd (ed.) Chapman & Hall, London, UK.*
- Farber, J.M. (1993): Current research on L. monocytogenes in foods: an Overview. J. Food Prot., 56 (7): 640-643.*
- Farber, J.M. and Peterkin, P.I. (1991): L. monocytogenes, a food-borne pathogen. Microbiol. Rev., 55: 476-511.*
- Farber, J.M.; Johnston, M.A.; Purvis, U. and Loit, A. (1987): Surveillance of soft and semi-soft cheeses for the presence of Listeria spp. Intern. J. Food Microbiol., 5: 157-163.*
- Farber, J.M.; Sanders, G.W. and Malcolm, S.A. (1988): The presence of Listeria spp. in raw milk in Ontario. Can. J. Microbiol., 34: 95-100.*
- Fathi, Sh.M. and Saad, N.M. (1992): A survey of some selected food items for the presence of L. monocytogenes and other Listeria spp. Assiut Vet. Med. J., 27 (54): 114-120.*
- Fleming, D.W.; Cochi, S.L.; MacDonald, K.L.; Brondum, J.; Hayes, P.S.; Plikaytis, B.D.; Holmes, M.B.; Audurier, A.; Broome, C.V. and Reingold, A.L. (1985): Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med., 312: 404-407.*
- Franco Abuin, C.M.; Quinto Fernandez, E.J.; Fente Sampayo, C.; Rodriguez Otero, J.L.; Dominguez Rodriguez, L. and Cepeda Saez, A. (1996): Incidence of Listeria spp. in the environment of a cheese processing plant throughout one year. Arch. für Lebensmittelhygiene, 47: 25-27.*
- Gilbert, R.J. and Pini, P.N. (1988): Listeriosis and foodborne transmission. Lancet, II, 472-473.*

- Gitter, M.; Bradley, R. and Blampied, P.H. (1980):* L. monocytogenes infection in bovine mastitis. *Vet. Rec.*, 107: 390-393.
- Hassan, N.M. (1996):* Incidence of L. monocytogenes in milk and some dairy products. Ph. D. Thesis, Fac. Vet. Med., Cairo Univ., Egypt.
- Hassanein, R.A-H. (1994):* Epidemiological studies on the occurrence of listeria infection in animals and man. M.V.Sc. Thesis, Fac. Vet. Med., Assiut Univ., Egypt.
- Husu, J.R.; Seppanen, J.T.; Sivela, S.K. and Rauramaa, A.L. (1990):* Contamination of raw milk by L. monocytogenes on dairy farms. *J. Vet. Med. B.* 37: 268-275.
- James, S.M.; Fannin, L.; Agree, B.A.; Hall, B.; Parker, E.; Vogt, J.; Run, G.; Williams, J.; Lieb, L.; Salminen, C.; Prendergast, T.; Wemer, S.B. and Chin, J. (1985):* Listeriosis outbreak associated with Mexican-style cheese-California. *Morbidity and Mortality Weekly Rep.*, 34: 357-359.
- Johnson, J.L.; Doyle, M.P. and Cassens, R.G. (1990):* L. monocytogenes and other Listeria spp. in meat and meat products. A review. *J. Food Prot.* 53 (1): 81-91.
- Loncarevic, S.; Danielsson-Tham, M.L. and Tham, W. (1995):* Occurrence of L. monocytogenes in soft and semi-soft cheeses in retail outlets in Sweden. *Intern. J. Food Microbiol.*, 26: 245-250.
- Lovett, J.; Francis, D.W. and Hunt, J.M. (1987):* L. monocytogenes in raw milk: Detection, incidence, and pathogenicity. *J. Food Prot.*, 50 (3): 188-192.
- Marth, E.H. (1993):* Growth and survival of L. monocytogenes, *Salmonella* species and *S. aureus* in the presence of sodium chloride. *Dairy Food Environ. Sanit.*, 13: 14-18.
- Massa, S.; Cesaroni, D.; Poda, G. and Trovatelli, L.D. (1990):* The incidence of Listeria spp. in soft cheeses, butter and raw milk in the province of Bologna. *J. Appl. Bacteriol.*, 68: 153-156.
- Miller, A.J.; Whiting, R.C. and Smith, J.L. (1997):* Use of risk assessment to reduce listeriosis incidence. *Food Technol.*, 51: 100-103.

- Olsen, J.A.; Yousef, A.E. and Marth, E.H. (1988):* Growth and survival of *L. monocytogenes* during making and storage of butter. *Milchwissenschaft*, 43: 487-489.
- Papageorgiou, D.K. and Marth, E.H. (1989):* Fate of *L. monocytogenes* during the manufacture, ripening and storage of Feta cheese. *J. Food Prot.*, 52: 82-87.
- Ralovitch, B. (1984):* Listeriosis Research: present situation and perspective. *Akademiai kiado: Budapest*.
- Rola, J.; Kwiatek, K.; Wojton, B. and Michalski, M. (1994):* Incidence of *L. monocytogenes* in raw milk and milk products. *Medycyna Weterynaryjna.*, 50: 323-325. *Dairy Sci. Abst.*, 57: 925 (1994).
- Rocourt, J.; Hof, H.; Schnettenbrunner, A.; Malinverni, R. and Brille, J. (1986):* Meningite purulente aige a *L. seeligeri* chez un adulte immunocompetente. *Schweiz. Med. Wochenschr.*, 116: 248-251.
- Saito, A.; Tokumaru, Y.; Masak, M.; Taya, T. and Aoki, A. (1991):* Evaluation of enrichment and plating media for the isolation of *L. monocytogenes* from raw milk and contamination of raw milk by listeria. *J. Jpn. Vet. Med. Assoc.*, 44 (4): 378-383.
- Terplan, G.; Schoen, R.; Springmeyer, W.; Degle, I. and Becker, H. (1986):* Occurrence, behaviour and significance of *Listeria* in milk and dairy products. *Arch. für Lebensmittelhygiene*, 37: 129-156.
- Terplan, G.; Steinmeyer, S.; Becker, H. and Friedrich, K. (1990):* Nachweis von *Listeria* in Milch und Milchprodukten-ein Beitrag zum Stand 1990. *Arch. für Lebensmittelhygiene*, 41: 102-106.
- Url, B.; Heitzer, A. and Brandl, E. (1993):* Determination of *Listeria* in dairy and environmental samples: Comparison of a cultural method and a calorimetric nucleic acid hybridization assay. *J. Food Prot.*, 56 (7): 581-584, 592.
- World Health Organization (1988):* Foodborne Listeriosis. *Bull., WHO*, 66: 421-428.
- World Health Organization (1992):* WHO surveillance programme for control of foodborne infections and intoxications in Europe. *New Letter*, 35: 1-4

