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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)

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مدى تواجد ميكروب الليستيريا مونوسيتوجينس فى 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 فى اللبن المحقون بـ ١٠×٣٧ ^{٧} خلية / مللى من هذا الميكروب وذلك بغلى اللبن وعد الميكروب على فترات زمنية مختلفة فى 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 تأثر ميكروب الليستيريا بدرجة حرارة الغليان فقد أسفرت النتائج عن تناقص العدد من ١٠×٣٧ ^{٧} إلى ١٠×٢٧٤ ^{٥} و ١٠×٢١٤ ^{٤} و ١٠×١٢٠ ^{٢} فى المحاولات الثلاث فى الدقائق الأولى والثانية والثالثة على التوالى 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 $37x10^7$ 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 $274x10^5$, $214x10^4$ and $120x10^2$ 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 increasly 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 pasteure 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 meningeoenecephalitis. 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. monocytoenes have produced conflicting results. Early investigations indicated, however, that L. monocytogenes might be relatively heat resistant. Bearns 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 $37x10^7$ 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. monocyotgenes.

Time/min	Count/ml			
	Trial (1)	Trial (2)	Trial (3)	Average
0	$37x10^7$	$37x10^7$	$37x10^7$	$37x10^7$
1	$283x10^5$	$280x10^5$	$259x10^5$	$274x10^5$
2	$160x10^4$	$200x10^4$	$282x10^4$	$214x10^4$
3	$135x10^2$	$115x10^2$	$110x10^2$	$120x10^2$
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 (Bearns 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 explaination 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-Eleinin, 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 $37x10^7$ to $274x10^5$, $214x10^4$ and $120x10^2$ 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-Hakiem 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 Ikonomov 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.

REFERENCES

Abdel-Hakiem, E.H. and Sabreen, M.S. (1993): Heat resistance of some serotypes of Listeria monocytogenes using open tube technique. Symposium on Food Pollution, 15-16 Nov.: 81-87.

Abou-Eleinin, A.M. (1999): Studies on Listeria species in milk and milk products. Ph.D. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.

Acha, P.N. and Szyfers, B. (1991): Zoonoses and communicable diseases common to man and animals. 2nd Ed. Pan American Health Organization - Washington, D.C.

Ahmed, A.A.H. (1989): Behaviour of Listeria monocytogenes during preparation and storage of yoghurt. Assiut Vet. Med. J. 22: 76-80.

Aman, I.M. and Ahmed, H.F. (1997): Incidence and survival of some foodborne pathogenes in milk and cheese. J. Egypt. Vet. Med. Ass. 57: 151-163.

A.P.H.A. (American Public Health Association) (1992): Compendium of methods for the microbiological examination of foods. 3rd Ed. American Public Health Association, Washington, D.C., USA.

Audurier, A.; Taylor, A.G.; Carbonnelle, B. and MeLauchin, J. (1984): A phage typing system for Listeria monocytogenes and its use in epidemiological studies. Clin. Invest. Med. 7: 229-232.

Bearns, R.E. and Girard, K.F. (1958): The effect of pasteurization on Listeria monocytogenes. Can. J. Microbiol. 4: 5561.

Bradshaw, J.G.; Peeler, J.T.; Corwin, J.J.; Hunt, J.M. and Twedt, R.M. (1987): Thermal resistance of Listeria monocytogenes in dairy products. J. Food Prot. 50: 544-556.

Curtis, G.D.; Mitchell, R.G.; King, A.F. and Griffin, E.J. (1989): A selective differential medium for the isolation of Listeria monocytogenes. Lett. Appl. Microbiol. 8: 95-98.

Dedie, K. and D. Schulze (1957): Die Hitzeresistenz von Listeria monocytogenes in Milch. Berliner Münchener Tierärztl. Wsch. 70: 231-232.

Dennis, S.M. (1975): Perinatal lamb mortality in Western Australia. Listeric infections. Aust. Vet. J. 51: 75-79.

Donnelly, C.W.; Briggs, E.H. and Donnelly, L.S. (1987): Comparison of heat resistance of Listeria monocytogenes by neutrophils and macrophages of bovine origin. Ann. Mtg. Amer. Soc. Microbiol., Atlanta, GA, March 1-6, Abstr. p. -27.

Doyle, M.P. (1986): The thermal resistance of Listeria monocytogenes in milk. In Press. (Cited after Beckers, *et al.*, 1987).

El-Gazzar, F.E. and Marth, E.H. (1991): Listeria monocytogenes and Listeriosis related to milk, milk products and dairy ingredients: A review. I- Listeria monocytogenes, Listeriosis and responses of the pathogen to environmental conditions. Milrchwissenschaft. 46: 14-19.

El-Marrakchi, A.; Hamama, A. and El-Othmani, F. (1993): Occurrence of Listeria monocytogenes in milk and dairy products produced or imported into Morocco. J. Food Prot. 56: 256-259.

El-Prince, Enas, M. (2000): Search for some pathogenic microorganisms affecting raw milk quality in Kafr El-Sheikh Governorate. Ph.D. Thesis, Fac. Vet. Med., Tanta Univ., Egypt.

Farber, J.M.; Sanders, G.W. and Malcolm, S.A. (1988): The presence of Listeria species in raw milk in Ontario. Can. J. Microbiol. 34: 95-100.

Fedio, W.M. and Jackson, H. (1989): Effect of tempering on the heat resistance of Listeria monocytogenes. Appl. Microbiol. 9: 157-160.

Fernandez, J.F.; Dominguez, R.J.; Vazouez, J.L.; Blanco, C. and Fernandez, S.G. (1986): Listeria monocytogenes dans le lait pasteurize. Can. J. Microbiol. 32: 149-150.

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. Nord Eng. 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 species in the environment of a cheese processing plant throughout one year. Archiv für Lebensmittelhygiene. 47: 25-27.

Garayzabal, J.F.F.; Rodriquez, L.D.; Boland, J.A.V.; Cancelo, J.L.B. and Fernandez, G.S. (1986): Listeria monocytogenes dans le lait pasteurize. Canadian Journal of Microbiology 32, 149-150.

Gitter, M.; Braddely, R. and Blampied, P.M. (1980): Listeria monocytogenes in bovine mastitis. Vet. Rec. 107: 390-393.

Gohil, V.S.; Ahmed, M.A.; Davies, R. and Robinson, R.K. (1995): The direct enumeration of Listeria monocytogenes in a food with a low background microflora. Food Control. 6: 365-369.

Gray, M.L. and Killinger, A.M. (1966): Listeria monocytogenes in a food with a low background microflora. Food Control. 6: 365-369.

Greenwood, M.H.; Roberts, D. and Burden, P. (1991): The occurrence of Listeria species in milk and dairy products: a national survey in England and Wales. Int. J. Food Microbiol. 12: 197-206.

Hassan Nour, M. (1996): Incidence of Listeria monocytogenes in milk and some dairy products. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ., Egypt.

Hitchins, A.D. (1995): Listeria monocytogenes. In: 8th Ed. Food and Drug Administration. Bacteriological Analytical Manual. AOAC International Pub. Co., Gaithersburg, MD, USA.

Huang, J.; Lacroix, C.; Daba, H. and Simard, R.E. (1993): Inhibition of growth of Listeria strains by mesenterocin 5 and organic acids. Lait. 73: 357-370.

Ikonomov, L., and Todorov, D. (1957): Microbiological studies on the pasteurization of ewe's milk. III. Resistance of some pathogenic bacteria. Vet. Med. Nauki, Sof. 4: 99-108.

Jensen, A.; Frederiksen, W. and Gerner-Smidt, P. (1994): Risk factors for listeriosis in Denmark, 1989-1990. Scandinavian J. Infectious Diseases. 26: 171-178.

Kerr, K.G.; Rotowa, N.A. and Hawkey, P.M. (1992): Listeria spp. in yoghurt? J. Nutritional Med. 3: 27-29.

Linnan, M.J.; Mascola, L.; Lou, X.D.; Goulet, V.; May, S.; Salminen, C.; Hird, D.W.; Yonkura, M.L.; Hayes, P.; Weaver, R.; Audurier, A.; Plikaytis, B.D.; Fannin, S.L.; Kleks, A. and Broome, C.V. (1988): Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. 319: 823-828.

Loncarevic, S.; Danielsson-Tham, M.L. and Tham, W. (1995): Occurrence of Listeria monocytogenes in soft and semi-soft cheeses in retail outlets in Sweden. International J. Food Microbiol. 26: 245-250.

Lovette, J., Francis, D.W. and Hunt, J.M. (1987): Listeria monocytogenes in raw milk: Detection, incidence, and pathogenicity. J. Food Prot. 50: 188-192.

Massa, S.; Cesaroni, D., Poda, G. and Trovatelli, L.D. (1990): The incidence of Listeria species in soft cheeses, butter and raw milk in the province of Bologna. J. Appl. Bact. 68: 153-156.

Ozgen, H. (1952): Z. Tropenm. U. Paras. 4: 40.

Pirie, J.H. (1940): Listeria: change of name for a genus of bacteria. Nature. 145: 264.

Potel, J. (1951): The morphology, culture and pathogenicity of C. infantisepticum. Zbl. Bakteriol. Parasitol. 156: 490-496.

Rola, J.; Kwiatek, K.; Wojton, B. and Michalski, M. (1994): Incidence of Listeria monocytogenes in raw milk and milk products. Medycyna Weterynary Jna. 50: 323-325. Dairy Sci. Abst. 57: 925.

Roy, D. (1996): Listeria. The bacteria which came in from the cold. Producteur de Lait Queebecois. 16: 28-30-31. Dairy Sci. Abst. 58: 4866.

Ryser, E.T. and Marth, E.H. (1991): Listeria, Listeriosis and food safety. Marcel Dekker, Inc. Madison Avenue, New York.

Sabreen, M.S. and Korashy, E. (2001): Incidence and survival of Listeria monocytogenes in yoghurt in Assiut City. Assiut veterinary Medical Journal 45 No. 90, July, 2001.

Seeliger, H.P. (1988): Listeriosis-History and actual developments. Infection 16 (Suppl. 2): 80-84.

Sing, R.S. and Chander, H. (1990): Occurrence of Listeria monocytogenes in milk and milk products. Indian Dairyman. 3: 61-66.

Siragusal, G.R. and Johnson, M.G. (1988): Persistence of Listeria monocytogenes in yoghurt as determined by direct plating and enrichment methods. Int. J. Food Microbiol. 7: 147-160.

Stenberg, H., and T. Hammainen (1955): On determination in vitro of the resistance of Listeria monocytogenes to sodium chloride and heat and on experimental monocytosis in albino mice. Nord. Vet. Med. 7: 853-868.

Wang, L.L. and Johnson, E.A. (1992): Inhibition of Listeria monocytogenes by fatty acids and monoglycerides. Appl. Environ. Micro. 58: 624-629.

Zuniga Estrada, A.; Lopez Merino, A. and Mota De La Graza, L. (1995): Behaviour of Listeria monocytogenes in milk fermented with a yoghurt starter culture. Revista Latinoamericana de Microbiologia., 37: 257-265.