

PREVALENCE OF LISTERIA SPECIES IN MEAT AND MEAT PRODUCTS

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SUMMARY

Four hundred and forty samples of meat and meat products , 40 each of (fresh meat, frozen beef, raw ground beef, frozen kofta, frozen ground beef, frozen beef sausage, frozen beef burger, hotdog, frankfurter, luncheon and basterma), were collected from different supermarkets and shops at Sharkia Governorate.

Listeria species could be isolated from all examined samples at different percentages ranged from 7.5 - 42.5 %, except luncheon and basterma samples could not be isolated.

Listeria monocytogenes could be isolated at variable percentage (2.5-10 %) of the examined frozen beef, frozen kofta, frozen ground beef, frozen

beef burger and frozen sausage.

From the nine isolates of Listeria monocytogenes isolated from examined samples, seven strains were belonged to *L. monocytogenes* type 1 and two strains were belonged to *L. monocytogenes* type 4.

The highest count of *L. monocytogenes* recorded as 3×10^4 /g in frozen beef sausage sample. The public health hazard as well as suggestive measures to reduce human Listeriosis has been discussed.

INTRODUCTION

Meat and meat products have frequently contaminated with Listeria monocytogenes and may serve as vehicles of this pathogenic bacterium. The frequent occurrence of *L. monocytogenes* in this

food may represent a potential risk for consumers, particularly for immunocompromised patients, because of its ability to survive and proliferate at refrigeration temperatures, *L. monocytogenes* may accumulate in meat and meat products during cold storage (Schillinger et al. 1991). Also *Listeria* spp. contaminate a variety of food products including red meats, seafood, and dairy products either at harvesting or during processing (Donnelly 1994).

Listeria is widely distributed in the environment. In the food industry it is usually found in cool damp environments on both food contact and non food contact surfaces, and has been isolated from floors, and drains (Slade, 1992). As a result of the ubiquitous character of *L. monocytogenes*, the organism easily enters the human food chain. In certain types of food the organism may multiply rapidly (Farber and Peterkin, 1991).

In recent years, Listeriosis has come to prominence as a leading cause of death from food borne illness. Although the responsible organism, *L. monocytogenes*, mainly infects immunocompromised and otherwise susceptible individuals, estimates indicate that in the United States alone, 1,092 cases and 248 deaths occurred in 1993 as a result of infection with this pathogen (Tappero et al. 1995).

So, the aim of the present study is to determine the incidence and initial level of *Listeria* species

especially *L. monocytogenes* in raw meat and meat products sold at the retail level.

MATERIAL AND METHODS

A total of 440 meat and meat products samples were collected from different supermarkets and shops at Sharkia Governorate to be examined for presence of *Listeria* species. The samples comprised 40 of each of the following: fresh meat, frozen meat, raw ground beef, frozen kofta, frozen ground beef, frozen beef sausage, frozen beef burger, hotdog, frankfurter, luncheon and basterma.

The technique recommended by USDA-FSIS (1989) was adapted using UVM1 enrichment broth at 30°C for 24 hours, after that 0.1 ml of the inoculated UVM1 was transferred to 10 ml UVM2 and incubated at 30°C for 24 hours (McClain and Lee 1988).

A loopful from UVM2 broth was streaked on a Palcam agar plate and incubated at 30°C for 24 hrs (Van Netten et al., 1989) and another loopful was streaked onto an Oxford agar plate which was incubated at 35°C for 24 hrs. Suspected colonies (bluish grey or black with a black halo and a sunken center) were picked up and streaked onto a trypticase soy agar plate supplemented with 0.6 yeast extract. Pure isolates were identified according to Donnelly (1992).

Serological identification of isolated *L. monocytogenes* : (Difco Laboratories Detroit Michigan USA).

The Rapid Slide Agglutination technique using Bacto-Listeria O Antisera types 1, 4 and poly

RESULTS

Table (1) : Incidence of *Listeria* species in meat and meat products.

Type of examined sample	No. of examined samples	Positive samples	
		No.	(%)
Fresh beef	40	10	25
Frozen beef	40	17	42.5
Fresh minced beef	40	4	10
Frozen kofta	40	11	27.5
Frozen minced beef	40	13	32.5
Frozen beefburger	40	12	30
Forzen sausage	40	14	35
Hotdog	40	4	10
Frankfurter	40	3	7.5
Lancheon	40	0	0
Basteurma	40	0	0
Total	440	88	20

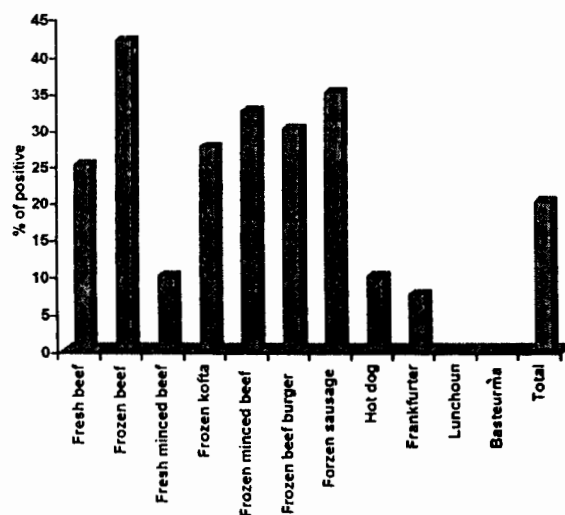


Fig. (1): Percentage of positive examined samples for *Listeria* species.

Table (2): Comparison of incidence of *Listeria* species from 440 samples of meat and meat products.

Type of examined sample	No. of examined samples	No & (%) of positive samples						
		<i>L. mono.</i>	<i>L. innocua</i>	<i>L. seeligeri</i>	<i>L. welshimeri</i>	<i>L. ivanovii</i>	<i>L. murrayi</i>	<i>L. grayi</i>
Fresh beef	40	0 (0.0%)	5 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (7.5%)	2 (5.0%)
Frozen beef	40	1 (2.5%)	8 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (12.5%)	3 (7.5%)
Fresh minced beef	40	0 (0.0%)	2 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.0%)	0 (0.0%)
Frozen kofta	40	1 (2.5%)	10 (25.0%)	4 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Frozen minced beef	40	2 (5.0%)	9 (22.5%)	4 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Frozen beef burger	40	4 (10%)	12 (30.0%)	0 (0.0%)	2 (5.0%)	0 (0.0%)	3 (7.5%)	0 (0.0%)
Forzen sausage	40	1 (2.5%)	6 (15.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (7.5%)	4 (10.0%)
Hotdog	40	0 (0.0%)	4 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.5%)	0 (0.0%)
Frankfurter	40	0 (0.0%)	2 (5.0%)	0 (0.0%)	1 (2.5%)	0 (0.0%)	0 (0.0%)	1 (2.5%)
Lancheon	40	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Basteurma	40	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	440	9 (2.05%)	58(13.18%)	8 (1.82%)	3 (0.68%)	0 (0.0%)	17 (3.86%)	10 (2.27%)

Table (3): Serotyping of confirmed isolates of *L. monocytogenes* from meat and meat products by using polyvalent and monovalent Bact-Listeria O Antisera (Difco).

Examined positive meat products	No. of examined samples	No. of isolates		Polyvalent type		Monovalent types			
		No.	(%)	No.	(%)	Type 1		Type 4	
						No.	(%)	No.	(%)
Frozen beef	40	1	2.5	1	2.5	1	2.5	0	0
Frozen kofta	40	1	2.5	1	2.5	1	2.5	0	0
Frozen minced beef	40	2	5	2	5	2	5	0	0
Frozen beef burger	40	4	10	4	10	3	7.5	1	2.5
Forzen beef sausage	40	1	2.5	1	2.5	0	0	1	2.50
Total	200	9	4.5	9	4.5	7	3.5	2	1

Table (4): Total viable counts of positive isolates of *Listeria monocytogenes*.

Examined positive meat products	No. of examined samples	Isolates of <i>L. monocytogenes</i>		Mean of Total viable counts
		No.	(%)	
Frozen beef	40	1	2.5	2.6×10^2
Frozen kofta	40	1	2.5	1.3×10^2
Frozen minced beef	40	2	5	- ve 2×10^3
Frozen beef burger	40	4	10	- ve 5×10^2 4.5×10^2 1.3×10^2
Forzen beef sausage	40	1	2.5	3×10^4
Total	200	9	4.5	--

DISCUSSION

Results given in Table (1) and Figure (1) revealed that *Listeria* organisms could be recovered from 88 (20 %) of 440 samples of meat and meat products. *Listeria* organisms could not be isolated from luncheon and basterma samples. The organisms could be isolated from fresh beef, frozen beef, raw ground beef, frozen kofta, frozen ground beef, frozen beef burger, frozen sausage, hotdog and frankfurter in the percentage of 25, 42.5, 10, 27.5, 32.5, 30, 35, 10, and 7.5 respectively.

Lower incidence of *Listeria* species in raw beef was recorded by Gohil et al. (1995), while higher ones was obtained by Hassouba (1997). At the same time higher incidence of *Listeria* in frozen ground beef and frozen sausage were obtained by Elgazzar and Sallam (1997).

It was clear that the incidence of *Listeria* spp. was greater in frozen meats than in fresh meats. This may be due to that frozen meat is more liable to be contaminated during their preparation and storage. While fresh meats were slaughtered in the slaughterhouse, and were quickly transported to the shops for sale, therefore, decreasing the possibility of contamination of *L. monocytogenes* (Wang et al., 1992).

Higher incidence of *Listeria* species in ground meat than carcasses surfaces or boneless meat

cuts can be attributed to contamination caused by cutting boards, knives, mincers, other work surfaces and human contact (Lowry and Tiong, 1985).

It is evident from the results given in table (2) that 9 samples out of the 440 examined samples were positive for *L. monocytogenes* (2.05 %). The positive samples revealed one from frozen beef and 8 were of frozen processed meat (one frozen kofta, 2 frozen ground beef, 4 from frozen beefburger and one from frozen sausage). *L. monocytogenes* failed to be isolated from fresh beef, fresh ground beef, hotdog, frankfurter, luncheon and basterma. Similar results were obtained by Fathi and Saad (1992). Higher incidence of *L. monocytogenes* in raw meat were reported by Noack and Joecket (1993). Also higher incidence of *L. monocytogenes* in minced meat were reported by McClain and Lee (1988) and Casolari et al. (1994).

In the present work *L. monocytogenes* failed to be isolated from frankfurter and hotdog samples. On the contrary, Marsden (1994) could isolate *L. monocytogenes* and *L. innocua* from examined hotdog samples. He also reported that under cooked hotdogs may constitute health hazard Chunhua and Muriana (1994) stated that *L. monocytogenes* was recorded at an incidence of 2.5 % in raitail frankfurter.

L. monocytogenes could not be isolated from luncheon samples. Similar results were obtained

by El gazzar and Sallam (1997) and Mohamed and Ali (1999).

On the contrary, Wilson (1989) and Furrer et al (1991) observed that the presence of such organisms in 4 and 6 % of the examined luncheon samples respectively. However, this lower incidence may be attributed the addition of spices and heat treatment during manufacture.

L. innocua, *L. seeligeri* and *L. murrayi* could be isolated at various percentages ranged from 2.5 to 25 % of the examined fresh and frozen meat and meat products.

L. ivanovii failed to be isolated from all examined meat and meat products.

Serological typing of isolated *L. monocytogenes* strains revealed that all isolated strains (9) proved to belong to *L. monocytogenes*. Further serotyping of the identified strains revealed that 1 (2.5 %), 1 (2.5 %), 2 (5 %) and 3 (7.5 %) proved to belong to *L. monocytogenes* type 1 in frozen beef, frozen kofta, frozen ground beef and frozen beef burger. While 1 (2.5 %) each from frozen beef burger and frozen beef sausage were belong to *L. monocytogenes* type 4 (Table 3). It was shown that the majority was serotype 1 with a smaller proportion of serotype 4. as recorded by Qvist and Liberski (1991) and Sharif and Tunail (1995). However, both serotype 1 and 4 proved to be pathogenic to man and animals (Donnelly, 1992).

The obtained results in Table (4) showed that the mean value of *L. monocytogenes* counts isolated from frozen beef, frozen kofta, frozen ground beef, frozen beef burger and frozen beef sausage sample ranged from 1.3×10^2 to 3×10^4 CFU/g.

The highest count recorded was 3×10^4 /g in frozen sausage samples. Nearly similar results were recorded by LeGuilloux et al. (1980) and Qvist and Liberski (1991). However, The ability of *L. monocytogenes* to multiply at refrigeration temperatures could be considered of a significance in foods intended for consumption without further cooking and in foods which have received cooking presumed sufficient to eliminate listeria, but nevertheless intended be received further cooking prior to consumption where the potential competitive microflora has been largely eliminate and thus even low numbers could pose a potential hazard if proper storage conditions are not adhered to. The high counts of Listeria in foods which have received minimal or no processing could be considered as a source for cross contamination occurring at the food chain (Schuchat et al., 1992).

In 1989, a human case of listeriosis which infected through consumption of a poultry product was dead. A cancer patient died after developing listerial meningitis and the source of *L. monocytogenes* was reported as turkey frankfurters (Wang-er et al. 1990).

In humans, the illness can range from a mild flu - like sickness (some times leading to a carrier state) to severe manifestations. The severe forms of human listeriosis present as meningoencephalitis followed by septic infections and occasionally isolated organ involvement. Groups at highest risk are pregnant women, neonates, adults with underlying disease (cancer, AIDS, diabetes, chronic hepatic disorder, transplant recipients), the elderly (> 65 years old) and other immunocompromised individuals. Death is rare in healthy adults but can occur at a rate as high as 30 % in persons at highest risk (Demetrios et al. 1996).

In order to minimize human listeriosis, foods should be cooked to an internal temperature of 70°C for more than 20 minutes to ensure destruction of *L. monocytogenes*. Reheat cooked food thoroughly (70°C), immediate aseptic packaging of the finished product to avoid post processing environmental contamination. Proper cold storage of meat and meat products (freezing - 18°C). Proper personal hygiene of food handlers

REFERENCES

- Casolari, C. ; Fabio, A. ; Menziani, G. ; Messi, P. and Quagliolo, P. (1994): Characterization of *Listeria monocytogenes* strains detected in meat and meat products. *Igiene-Moderna* 101 (2): 193-215.
- Chunhua, W. and Muriana, P. M. (1994): Incidence of *Listeria monocytogenes* in packages of retail franks. *Journal of Food-Protection* 57 (5): 382-386.
- Demetrios, K. P.; Mina bori and Antonios, M. (1996): Growth of *Listeria monocytogenes* in the whey cheeses, Myzithera, Anthotyros, and Manouri during storage at 5, 12, and 22 (C. *Journal of Food Protection* 59 (11): 1193-1199.
- Donnelly, C. W. (1992): *Listeria*. In *Compendium of methods for microbiological examination of foods*. Third edition (Editors: Vanderzant, C. and Splittstoesser, D. F.), pp 637-663.
- Donnelly, C. W. (1994): *Listeria monocytogenes*: In *foodborne disease handbook*, Vol. 1. Diseases caused by bacteria. (Edited by Hui, J. R.; Gorham, K. D.; Murrell, and Cliver, D.O.). Marcel Dekker Inc., New York, pp 215-252.
- Elgazzar, M. M. M. and Sallam, Kh. I. A. (1997): Occurrence of *Listeria monocytogenes* and other *Listeria* Spp. in meat products. *Alexandria Journal of Veterinary Science* 13 (4): 415 - 422.
- Farber, J. M. and Peterkin, P. I. (1991): *Listeria monocytogenes*, a food borne pathogen. *Microbiol. Rev.* 55: 476-511.
- Fathi, Sh. M. and Saad Nagah (1992): A survey of some selected food items for the presence of *Listeria monocytogenes*, and other *Listeria* species. *Assuit Vet. Med. J.* 27:54.
- Furrer, B.; Candrian, U.; Hoefelein, Ch. and Luethy, J. (1991): Detection and identification of *Listeria monocytogenes* in cooked sausage products and in milk by in vitro amplification of haemolysin gene fragments. *Journal of Applied Bacteriology* 70: 372-379.
- Gohil, V. S.; Ahmed, M. A.; Davies, R. and Robinson, R. K. (1995): Incidence of *Listeria* spp. in retail foods in the United Arab Emirates. *Journal of Food Protection*

- Hassouba, M. M. (1997): *Listeria* species in meat, meat products and chicken giblets. M.V.Sc. Thesis, Fac. Vet. Med., Cairo University.
- Le Guilloux, M. ; Dollinger, C. and Freyburger, (1980): *Listeria monocytogenes* Sa frequence dans les produits de char cuterie. Bull. Soc. Vet Prat. defrance 64: 45-53.
- Lowry, P. D. and Tiong, I. (1985): The incidence of *Listeria monocytogenes* in meat and meat products factors affecting distribution. In Proc. 34 th Int. Congress Meat Sci. Technol. Part. B. PP 528-530.
- Marsden, J. L. (1994): Industry perspectives on *Listeria monocytogenes* in food: Raw meat and poultry. Dairy Food and Environmental Sanitation 14 (2): 83-86.
- McClain, D. and Lee, W. H. (1988): Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J. Assoc. Off. Anal. Chem. 71 (3): 660-664.
- Mohamed, Amal A. and Ali, M. M. (1999): Incidence of *Listeria monocytogenes* in some meat products and poultry. Assiut Vet. Med. J.40 (80):187-196.
- Noack, D. J. and Joecke, J. (1993): *Listeria monocytogenes*. Occurrence and significance in meat and meat products and experience with recommendations for its detection and assessment. Fleischwirtschaft 73 (5): 581-584.
- Qvist, S. and Liberski, D. (1991): *Listeria monocytogenes* in frankfurters and sliced meat products. Dansk-Veterinaertidsskrift 74 (20): 773-774, 776-778.
- Schillinger, U.; Kaya, M. and Lucke, F. K. (1991): Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin - producing strain of *Lactobacillus sake*. Journal of Applied Bacteriology 70: 473-478.
- Schuchat, A.; Deaver, K. A.; Wenger, J. D.; Plikaytis, B. D.; Rengold, A. L.; Broome, C. and the *Listeria* study Group(1992): Role of foods in sporadic listeriosis. J. Am. Med. Assoc. 267:2041-2045.
- Slade, P. J. (1992): Monitoring *Listeria* in the food production environment I. detection of *Listeria* in processing plants and isolation methodology. Food Res. Int. 25:45-46.
- Sharif, A. and Tunail N. (1995): Detection of *Listeria monocytogens* in Foods of animal origin. Turkish Journal of Veterinary and Animal Sciences, 19 (5) 329-334.
- Tappero, J. W.; Schuchat, A.; Deaver, K. A.; Mascola, L. and Wenger, J. D. (1995): Reduction in the incidence of human listeriosis in the United States effectiveness of prevention efforts. The listeriosis Study Group. JAVMA 273:1118-1122.
- USDA - FSIS "United State Department of Agriculture, Food Safety and Inspection Services" (1989): Methods for isolation and identification of *Listeria monocytogenes* from meat and poultry products. Laboratory communication No. 57. US Department of Agriculture, Washington, DC.
- Van Netten, P.; Perales, I.; Van de Moosdijk, A.; Curtis, G. D. W. and Mossel, D. A. A. (1989): Liquid and solid selective differential media for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* species. Int. J. Food Microbiol. 8: 299-316.
- Wang, G. H.; Yan, K. T.; Feng, X. M.; Che, S. M.; Lui, A. P. and Kokubo, Y. (1992): A comparative study of the FDA a USDA methods for the detection of *Listeria monocytogenes* in foods. Journal of Food Microbiol. 13: 105-118.

Wenger, J. D.; Swaminathan, B.; Hayes, P. S.; Green, S. S.;
Pratt, M. ; Pinner, R. W.; Schuchat, A. and Broome, C.
V. (1990): *Listeria monocytogenes* contamination of
turkey franks Evaluation of a production facility. *Journal
of Food protection* 53:1015-1019.

Wilson, G. D. (1989): PP. 11-13 in Proc. Reciprocal meat
Conference, Vol. 41. National livestock and meat board
chicago, II.