OCCURRENCE OF LISTERIAE AND SURVIVAL OF LISTERIA MONOCYTOGENES IN FISH IN SHARKIA GOVERNORATE

Hanan M. T. El-Lawendy

Animal Health Research Institute, Dokki, Giza (Zagazig branch)

Keywords Listeria monocytogenes, Listeriae, food safety, risk factor, fish, and seafood.

ABSTRACT

One hundred and eighty of fish samples were collected from different localities and fish markets at Sharkia governorate included 120 fresh fish samples 15 of each (Tilapia nilotica; Calarias lazera, Synodontis schall; Bagrus bayad; Schilbe mystus; Mugil cephalus; Carb; and Shrimp), and 45 frozen fish samples in addition to 15 smoked fish samples.

Listeria species could be recovered from 14 samples out of 180 (7.78 %), in fresh fish samples 3 out of 120 (2.5 %); frozen fish samples 7 out of 45 (15.56 %) and smoked fish samples 4 out of 15 (26.67 %). L. monocytogenes could be isolated from fresh fish samples (Calarias lazera) 2 out of 120 (1.67 %), frozen fish samples 5 out of 45 examined (11.11 %), and finally from 3 smoked fish samples out of 15 (20 %). The counts of L. monocytogenes isolated from fish samples ranged from $1.1X10^2$ to $2.2X10^2$. CFU/g The highest count recorded in smoked fish sample was $2.2X10^2$.

The effect of heat (cooking) on the survival of L. monocytogenes declared that the organism could not be recovered from 10 positive specimens either treated by frying or by dry heat. The public health importance of L. monocytogenes in sea foods under Egyptian condition was studied.

INTRODUCTION

Listeria monocytogenes is a facultative intracellular pathogen responsible for several out breaks and numerous sporadic cases of food borne Listeriosis **(Farber and Peterkin, 1991)**.

L. monocytogenes has been reported as a causative agent in several incidence of seafood borne Listeriosis in different localities **(Frederiksen, 1991, Dillon and Patel, 1993; Susan, 1997; Thimothe, et al., 2002 and Hoffman et al., 2003)**.

There is an increasing evidence that fish and seafood have been the cause of smaller outbreaks **(Farber et al., 2000)**. Most Listerial infections, however, occur sporadically in Switzerland.

L. monocytogenes has been isolated from several ready to eat seafoods including Schilbe mystus, cooked shrimp and crambmeat. Surimi based seafood, hot and cold smoked fish and smoked squid, eel, and mussels **(Jemmi 1993; Fletcher, et al., 1994; Fuchus and Nicolaides 1994; Rawles, et al., 1995; Susan, 1997; Gonzalez Rodriguez et al., 2002; and Neamatallah et al., 2003)**.

L. monocytogenes is difficult to be controlled in seafood and in foods in general, because it can grow at refrigeration temperatures, survived in brine solutions, and tolerate extremes in heat and PH **(Ryser and Marth 1991; Sumner and Ross, 2002)**. Regardless of the source, the presence of *L. monocytogenes* in seafoods primarily as a result of post processing contamination represents a significant problem, particularly for cooked ready to eat seafoods **(Oh, et al., 1992)**.

It was the intent of this study to acertain the prevalence of *L. monocytogenes* in fish at different localities in Sharkia Governorate, and to determine the effects of cooking (heat) on the survival of the organism.

MATERIALS AND METHODS

Samples:

A total of one hundred and eighty fish specimens were randomly purchased from different fish markets at different localities at Sharkia Governorate. Collected samples were classified into:

a- Fresh fish (120 specimens), 15 of each of the following: Tilapia nilotica; Calarias lazera, Synodontis schall; Bagrus bayed; Schilbe mystus; Mugil cephalus; Neptunus pelagicus (Carb); and Penaeus japonicus (Shrimp).

b- Frozen Fish (45 Specimens), 15 of each of the following: Sardinella maderensis; Scomber scombrus; and Saurus spp.

c- Smoked fish (15 Specimens).

Qualitative detection of L. monocytogenes:

Twenty five grams from each sample (Muscle) were blended with a sterile moulinex type blender equipped with metallic flask with 225 ml UVM_1, enrichment broth, then was incubated at 30°C for 24 hrs, after that 0.1 ml of the inoculated UVM_1, was transfer to 10 ml UVM_2 and

incubated at 30°C for 30 hrs. **(McClain and Lee 1988)** The technique recommended by **USDA FSIS, 1989** was adapted.

A loopful from UVM_2 was streaked on a Palcam agar plate and incubated at 30°C for 24-48 hrs. **(Van Netten et al., 1989)**, and another loopful was straked also onto oxford agar plate which was then incubated at 35°C for 24-48 hrs. Suspected colonies (Grey-green or black with a black halo and a sunken center) were picked up and streaked onto a trypticase soya agar plate supplemented with 0.6 yeast extract and the plate was incubated at 30°C for 24 hrs. till obtaining pure separate colonies. Pure colonies were incubated into tubes of TSA-YE which were incubated 35°C for 24 hrs. then identified according to **Donnelly 1992.**

Quantitative enumeration of L. monocytogenes:

25 g from samples were blended well in a sterile blender with 225 ml of 0.1% sterile peptone water solution then they were diluted decimally subsequently 0.1 ml of each dilution was streaked onto Palcam agar **(Ven Netten, et al 1989)**. Then plates were incubated microaerobically for 24 hrs. at 30°C. Presumptive listeria colonies were confirmed and counted.

The effect of heat treatment on the viability of L. monocytogenes:

The positive fish samples were subjected to cooking using dry heat treatments by usual way and frying using sufficient amounts of oil using sterile frying pan. After heat treatment each fish samples was transferred to sterile aluminum foil and examined for the presence of L. monocytogenes according to **USDA FSIS, 1989** technique.

RESULTS and DISCUSSION

Listeria spp. could be detected in 14 (7.78%) out of 180 examined specimens. L. monocytogenes could be isolated from 10 (5.56%) out of 180 examined specimens and the other 4 (2.22%) specimens could be identified as L. innocua, for L. monocytogenes the highest isolation rate prevalence was from smoked fish 3 (20%) out of 15 examined specimens followed by frozen 2 (13.33%) out of 15 examined Sardinella maderensis, and 2 (13.33%) out of 15 examined Saurus species, then 2 (13.33%) out of 15 examined Calarias lazera, while the lowest one was in Scomber scombrus one (6.67%) out of 15, while listeriae could not be isolated from other fresh water fish. Listeria spp. are wide spread in the environment and have been isolated from fresh and marine water and from sediments **Gonzalez- Rodriguez et al., 2002**

Our results confirmed previous findings that L. monocytogenes occurs widely in food with high prevalence in meat, fish and seafood products. The overall prevalence of 5.56 % is comparable to other studies **(Jemmi, 1993; Ben Embarek, 1994; Heinitz and Johnson, 1998)**.

A number of surveys have also shown that these organisms are frequently found in raw and processed meat fish at the retail level **(Jinneman et al. 1999)** on other hand Listeriae could be detected from 8 of 12 lots of trout fillets, but only one was contaminated with L. monocytogenes. As in other foods, fishery products more frequently contain L. innocua than L. monocytogenes. Since both species share ecological niches, so, the presence of L. innocua is considered as an indication of possible contamination with L. monocytogenes **(Jinneman et al., 1999)**. The Psychrotrophic nature of these bacteria could explain that they were only detected when storage progressed.

Contamination prevalence values for raw fish varied from 3.6% (sablefish) to 29.5% (US West Coast salmon), with an average overall prevalence of 14.6% **Hoffman et al. (2003)**. L. monoeytogenes could be detected in 40% of the intestinal contents of examined Schilbe mystus in Assiut **Hefnawy et al., (1989)**.

The prevalence of L. moncytogenes in cold smoked salmon and cooked fish products has been reported to range from 6-36% as high as 70% **(Eklund et al., 1995; Jorgensen and Huss, 1998)**. Nevertheless, cold smoked fish and other seafood are infrequently associated with human Listeriosis **(Ericsson et al., 1997)**.

It is believed that the problem of contamination of smoked fish is of great public health importance. Contamination of such products cannot be tolerated, because there is a great potential for the pathogen to develop at refrigeration temperatures, this represents an increasing health hazard for the consumers who store such products for a long time **(Jemmi et al., 2002)**. In cold smoked and marinated fish there is potential for growth of the pathogen under storage condition.

Listeriae other than L. monoeytogenes were isolated from three packages out of 54 pack cold smoked salmon and trout obtained at retail level at Spanish fish market **Gonzalez-Rodriguez et al., (2002)**.

The contamination rate for hot and cold smoked fish in Switzerland, Norway, and Canada is about 10% **(Jamml, 1993)**. L. monocytognes were recovered from 3.4% of cold smoked fish **(Fuchs and Nicolaides, 1994)**.

The external surfaces of frozen and fresh fish are the primary source of L. monocytogenes in cold smoked fish processing plants **(Eklund, et al., 1995)**. **Susan, 1997** reported that raw fish

were more frequently contaminated than the finished products, contamination of cold smoked fish can occur during or after processing, while contamination of hot smoked fish is probably due to postprocess contamination. The public health importance of contamination of smoked fish is the ability of L. monocytogines to grow during storage at certain refrigeration temperatures under aerobic and vacuum-packaged conditions **(Farber and Peterkin, 1991; Jemmi and Keusch, 1992)**.

The obtained results showed that L. monocytogenes counts in Calarias lazera, sardinella maderensis, Scomber scombrus, Saurus spp., and smoked fish ranged from 110-220 CFU/g. the highest count 220 CFU/g was detected in a sample of smoked fish from the public health point of view, a standard has been suggested enforcing the absence of L. monocytogenes in foods.

There have been recent outbreaks **(Farber et al., 2000)** in Switzerland introduced a quantitative limit of 100 L. monocytogenes.per gram.

From the results achieved in Table (3). it is evident that L. monocytogenes could not be recovered from both dry heated and fried fish. The fact that the organism failed to recovered from previously positive fish samples may be attributed to thermal inactivation of L. monocytogenes when the temperature greater than 68.9°C in the center of the products even in heavily contaminated products **(Zaika et al., 1990 and Kwiatek and wojton 1993)**.

Table (1): Isolation rate of *Listeria monocytogenes* and other Listeriae from fresh water, frozen and smoked fish at Sharkia governorate.

Type of examined sample	No. of examined Samples	Positive semples No.	%	L. mono. No.	%	Other listerae No.	%
Fresh water fish							
1- Tilapia nilotica	15	1	6.67	0	0	1	6.67
2- Calarias lazera	15	2	13.33	2	13.33	0	0
3- Synodontis schal	15	0	0	0	0	0	0
4- Schilbe mystus	15	0	0	0	0	0	0
5- Bagrus bayed	15	0	0	0	0	0	0
6- Mugil cephalus	15	0	0	0	0	0	0
7-Neptunus pelagicus (carb)	15	0	0	0	0	0	0
8- Penaeus Japoncus (shrimp)	15	0	0	0	0	0	0
Total of fresh fish	**120**	**3**	2.5	**2**	1.67	**1**	0.83
Frozen fish							
1- Sardinella maderensis	15	2	13.33	2	13.33	0	0
2- Scomber scombrus	15	2	13.33	1	6.67	1	6.67
3- Saurus spp	15	3	20.00	2	13.33	1	6.67
Total of frozen fish	**45**	**7**	15.56	**5**	11.11	**2**	4.44
Smoked fish	15	**4**	26.67	**3**	20.00	1	6.67
Total	**180**	**14**	**7.78**	**10**	**5.56**	**4**	**2.22**

L.mono= Listeria monocytogenes.

Table (2): Total viable counts of positive isolates of *Listeria monocytogens*.

Examined positive samples	No. of positive samples	Mean of Total viable counts
Calarias lazera	2	1.1×10^2 1.2×10^2
Sardinella maderensis	2	2×10^2 1.8×10^2
Scomber scombrus	1	1.4×10^2
Saurus spp	2	1.6×10^2 1.2×10^2
Smoked	3	1.4×10^2 1.8×10^2 2.2×10^2

Table (3): Effect of heat treatment on the viability of *Listeria monocytogens* in positive fish samples.

Heat treatment	**No. of Examined positive samples**	**Total viable counts range**	**After heat treatment**	
			+ve No.	**Reduction %**
Dry heated	5	$1.2\text{-}2.2X10^2$	0	100 %
Fried	5	$1.1\text{-}2X10^2$	0	100 %

REFERENCES

Ben Embarek, P. K. (1994) : Presence, detection and growth of Listeria monocytogenes is sea foods A review Int. J. Food Protection 23: 17-34.

Dillon, R. M. and Patel, T. R. (1993) : Effect of cold smoking and storage temperature on listeria monocytogenes in inoculated cod fillets (Gadus morhua). Food. Res, Int. 26:97-101

Donnelly, C. W. (1992) : Listeria. In: Compendium of methods for microbiological examination of foods. 3rd edition (Vanderzan, C. and Splittstoe sser, D.F. editors) pp. 637-663.

Eriesson, H.; Ekl, A.; Danielsson, M. L.; Lon carevic, T. S.; Mentzing, L. O.; Persson, I.; Unnersta L.; H. and Tham, W. (1997) : An outbreak of Listeriosis suspected to have been caused by rainbow trout. J. clin. Microbiol. 35: 2904-2907.

Eklund, M. W.; Poysky, F. T.; Paranjpye, R. N.; Lashbrook , L. C.; Peter Son, M. E. and pelroy, G. A. (1995) : Incidence and sources of listeria monocytogenes in cold smoked fishery products and pro coming plants. J. Food Prot. 58: 502-508.

Farber, J. M. and Peterkin, P. I. (1991) : Listeria monocyogenes, a food borne pathogen Microbiol. Rev. 55: 476-511.

Farber, J. M.; Daley, E. M.; Mack, C. M . T. and limerick, B. (2000) : A small outbreak of listriosis potentially linked to consumption of imitation carb meat. Lett. Appl. Microl. 31: 100-104.

Fletcher, G. C.; Rogers, M. L. and Wong, R. J. (1994) : Survey of Listeria monocytogenes in NewZaland seafood. J. Aquat Food Prod. Technol., 3: 13-24.

Frederiksen, W. (1991) : Listeria epidemiology in Denmark, 1981-1990 P. 84-49. In proceedings of the international Conference: Listeria and food safety aspect processing Association, Laval, France.

Fuchs, R. S. and Nicolaides, L. (1994) : Incidence of listeria in hot and cold smoked fish. Lett. Appl Microbiol 19: 394-396.

Gonzalez--Rodriguez, M. N.; Sanz, J. J.; Santos, J. A.; Otero, A. Garcia-Lopez, M. L. (2002) : Numbers and types of microorganisms in vacuum-packed cold smoked fresh water fish at the retail level. Int. J. Food Microbiol. 77 (1-2): 161-168.

Hefnawy, Y.; Sabah Moustafa, and Refai, R. S. (1989) : Occurrence Yersinia enterocolitica and Listeria monocytogenes in fresh water fish. Assiut Vet. Med. J. 21 (41): 135-140.

Heinitz, M. L. and Johnson, J. M. (1998) : The incidence of Listeria spp., Salmonella spp. and Clostridium botulinum in smoked fish and sheel fish. J. FoodProt. 61: 318-323.

Hoffman, A. D.; Gall, K. L.; Norton, D. M. and Wiedmann, M. (2003) : Listeria monocytogenese contamination patterns for the smoked fish processing environment and for raw fish. J. Food protection 66 (1): 52-60.

Jemmi, T. (1993) : listeria monocytogenes in smoked fish: an over view. Arch. Lebensmitt ehyg. 44: 10 13.

Jemmi, T.; and Keusch, A. (1992) : Behavior of listeria monocytogenes during processing and storage of experimentally contaminated hot smoked trout. Int. J. Food Microbiol. 15:339-346.

Jemmi, T.; Pak, S. and Salman, M. D. (2002) : Prevalence and risk factors for contamination with listeria monocytogenes of imported and exported meat and fish products in Swaziland 1992-2000. Preventive Vet. Med. 54: 25-36.

Jinneman, , K. C.; Wekell, M. M. and Ek lund, M. W. (1999) : Incidence and behavior of listeria monocytogene, in fish and seafood products in: listeria, listeriosis and food safety. Marcel Dekker, New York PP. 631-655.

Jorgensen, L. V. and Huss, II. H. (1998) : Prevalence and growth of Listeria monocytogenes in naturally contaminated seafood. Int. J. Food. Microbiol. 62: 127-131.

Kwiatek, K. and Wojton, B. (1993) : Listeria monocytogenes as a source of food borne disease. Gospodark Miesna 45 (5): 32-34.

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: 660-664.

Neamatallah, A. A.; Dewar, S. J. and Austin, B. (2003) : An improved selective isolation medium for the recovery of Listeria monocytogenes from smoked fish. Lett. Appl. Microbiol. 36 (4): 230-233.

Oh, D.; Marshall, D. L.; Moody, M. and Dankston, J. D. (1992) : Comparison of forced air cooling with static air cooling on the microbiological quality if cooked blue crabs. J. Food Prot. 55: 104-107.

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

Rawles, D.; Flick. G.; Pierson, M.; Diallo, A.; Wittman, R. and Groonenberghs, R. (1995) : Listeria monocytogenes occurrence and growth at refrigeration temperatures in fresh blue crab (Callinects sapidns) meat. J. Food Prot., 58: 1219-1221.

Sumner, J. and Ross, T. (2002) : A semi-quantitative seafood safety risk assessment. Int. J.

food Microbiol. 77 (1-2) 55-59.

Susan, M. Mccarthy, (1997) : Incidence and survival of Listeria monocytogenes in Ready—to-eat sea food Products. Journal of food protection, 60 (4): 372-376.

Thimothe, J.: Walker, J.; Suvanich, V.; Gall K. L.; Moody, M. W. and Wiedmann, M. (2002) : Detection of listeria in crawfish processing plants and in raw, whole crawfish and processed crawfish (procambarus spp). J. Food Prot. 65(11): 1735-1739.

USDA-FSIS "United state Department of Agriculture, Food Safty Inspection Service" (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 Moodijk, A.; Curtis, G. D. W. and Mossel, D. A. (1989) : Liquid and selective differential media for the detection and enumeration of listeria monocytogenes and other listeria species. Int. J. Food Microbiol. 8: 299-316.

Zalika, L. Palumbo, S. A.; smith, J. L.; Corral, F. del., Bhadari, S. Jones, C. O. and Kim, A. H. (1990) : Destruction of Listeria monocytogenes during frankfurter processing. Journal of Food protection 53 (1): 18-23.

الملخص العربى

مدى تواجد ميكروبات الليستيريا وبقاء الليستيريا مونوسيتوجينز فى الأسماك بمحافظة الشرقية

المشتركون فى البحث

د/ حنان مصطفى طه اللاوندى

قسم صحة الأغذية معمل بيطرى الزقازيق - معهد بحوث صحة الحيوان بالدقى القاهرة

أجريت هذه الدراسة على عدد ١٨٠ عينة من أنواع الأسماك المختلفة الطازجة والمجمدة والمدخنة منهم ١٢٠ من الأسماك الطازجة المعدة للبيع عبارة عن ١٥ عينة لكل من (البلطى، القرموط، شال، بياض، شلبه، البورى، كابوريا و الجمبرى) هذا بالإضافة إلى عدد ٤٥ عينة من الأسماك المجمدة المختلفة وعدد ١٥ عينة مختلفة من الأسماك المدخنة جمعت جميعها من الأسواق والأماكن المختلفة بمحافظة الشرقية وذلك لدراسة ومعرفة مدى وجود ميكروبات الليستيريا ومعرفة مدى إنتشا الليستيريا مونوسيتوجينز ومعرفة تأثير الحرارة (القلى والشى) على نمو وتكاثر الميكروب ودراسة مناقشة الأهمية الصحية لميكروب الليستيريا منوسيتوجينز فى الأسماك.

أسفرت النتائج عن عزل ميكروبات الليستيريا بأنواعها المختلفة من ١٤ عينة من ١٨٠ عينة تم فحصها (٧٫٧٨٪) منهم ١٠ عينات كانت إيجابية لعزل ميكروب الليستيريا منوسيتوجينز (٥٫٥٦٪) و٤ عينات كانت إيجابية لليستيريا انكوا (٢٫٢٢٪). تم عزل ميكروب الليستيريا منوسيتوجينز من عينتين من ١٥ عينة تم فحصها من القرموط بنسبة (١٣٫٣٣٪) وكذلك من ٥ عينات من ٤٥ عينة تم فحصها من الأنواع المختلفة من الأسماك المجمدة بنسبة (١١٫١١٪) ومن عدد ٣ عينات من ١٥ عينة تم فحصها من الأسماك المدخنة بنسبة (٢٠٪). أما الأنواع الأخرى من الليستيريا فقد تم عزل الليستيريا انكوا من عينة من البلطى بنسبة (٦٫٦٧٪) وعدد ٢ عينة من الأسماك المجمدة بنسبة (٥٫٥٦٪) وكذلك عدد ١ عينة من الأسماك المدخنة بنسبة (٦٫٦٧٪). تم إجراء العدد الكلى لميكروب الليستيريا منوسيتوجينز فى العينات الإيجابية حيث تراوح العدد من ١٫١ × ١٠^{٢} إلى ٢٫٢ × ١٠^{٢} وكان أعلى عدد كلى فى عينة من الأسماك المدخنة ٢٫٢ × ١٠^{٢}.

تم دراسة تأثير الحرارة على مدى مقاومة عترات الليستيريا مونوسيتوجينز حيث تم شى وقلى الأسماك الإيجابية لميكروب الليستيريا منوسيتوجينز ولم يتم عزل الميكروب بعد القلى أو الشى أو معاملة الأسماك المدخنة على الحرارة مباشرة، تم فى هذا البحث مناقشة الأهمية الصحية لميكروب الليستيريا منوسيتوجينز فى الأسماك بصفة عامة وخاصة المدخنة منها.