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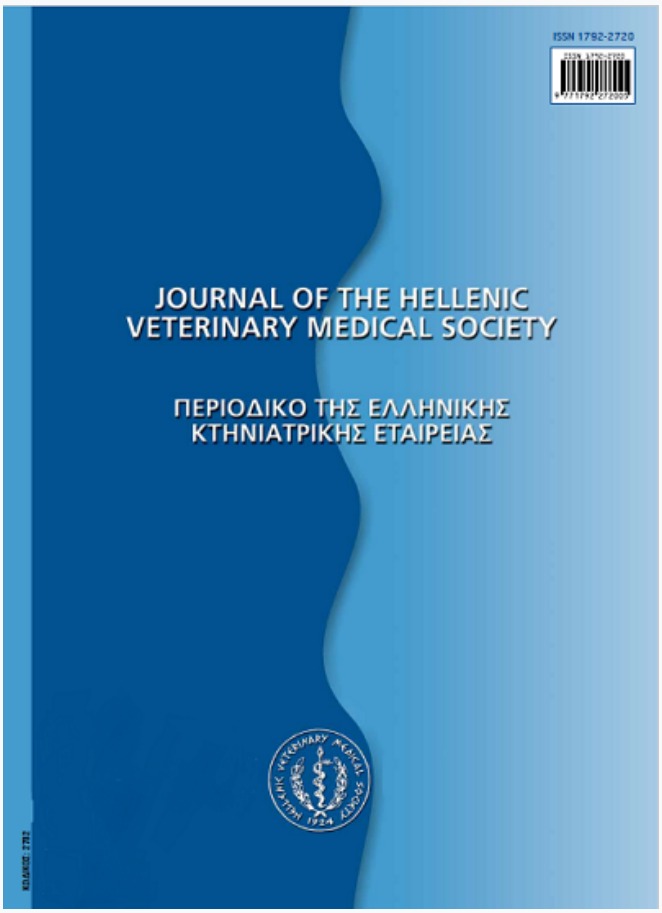

Prevalence of Listeria spp. in freshwater T\s\v(Oncorhynchus my kiss and Carassius gibelio) and the environment offish markets in Northern Greece

Th. PAPADOPOULOS (Θ. ΠΑΠΑΔΟΠΟΥΛΟΣ), A. ABRAHIM, D. SERGELIDIS (Δ. ΣΕΡΓΚΕΛΙΔΗΣ), I. KIRKOUDIS (I. ΚΥΡΚΟΥΔΗΣ), K. BITCHAVA (Κ. ΜΠΙΤΧΑΒΑ)

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Prevalence of *Listeria* spp. in freshwater fish (*Oncorhynchus mykiss* and *Carassius gibelio*) and the environment of fish markets in Northern Greece

Papadopoulos Th.[1], DVM, MSc, **Abrahim A.**[2], DVM, MSc, PhD, **Sergelidis D.**[2], DVM, PhD, **Kirkoudis I.**[3], DVM, PhD, **Bitchava K.**[4], DVM, PhD

[1]*Veterinary Centre of Amynteo, Prefecture of Florina*

[2]*Laboratory of Hygiene of Foods of Animal Origin, Department of Hygiene and Technology of Foods of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki*

[3]*Laboratory of Ecology-Wildlife Management, Department of Forestry and Environmental Management and Natural Resources, Democritus University of Thrace*

[4]*Laboratory of Ichthyology & Fish Pathology, Faculty of Veterinary Medicine, University of Thessaly*

Παρουσία των *Listeria* spp. σε ψάρια του γλυκού νερού (*Oncorhynchus mykiss* και *Carassius gibelio*) και στο περιβάλλον ιχθυοπωλείων της Βόρειας Ελλάδας

Θ. Παπαδόπουλος[1], DVM, MSc, **Α. Αμπραχίμ**[2], DVM, MSc, PhD, **Δ. Σεργκελίδης**[2], DVM, PhD, **Ι. Κιρκούδης**[3], DVM, PhD, **Κ. Μπιτχαβά**[4], DVM, PhD

[1]*Κτηνιατρικό Κέντρο Αμυνταίου, Νομαρχία Φλώρινας*

[2]*Εργαστήριο Υγιεινής Τροφίμων Ζωικής Προέλευσης, Τομέας Υγιεινής και Τεχνολογίας Τροφίμων Ζωικής Προέλευσης, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης*

[3]*Εργαστήριο Οικολογίας-Διαχείρισης Άγριας Ζωής, Τμήμα Δασολογίας και Διαχείρισης Περιβάλλοντος και Φυσικών Πόρων, Δημοκρίτειο Πανεπιστήμιο Θράκης*

[4]*Εργαστήριο Ιχθυολογίας και Ιχθυοπαθολογίας, Κτηνιατρική Σχολή, Πανεπιστήμιο Θεσσαλίας*

Correspondence: Sergelidis D.
Laboratory of Hygiene of Foods of Animal Origin, Department of Hygiene and Technology of Foods of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki
University Campus, 541 24 Thessaloniki
Tel: +30 2310 999970, Fax: +30 2310 999833, E-mail:dsergkel@vet.auth.gr

Αλληλογραφία: Δ. Σεργκελίδης
Εργαστήριο Υγιεινής Τροφίμων Ζωικής Προέλευσης, Τομέας Υγιεινής και Τεχνολογίας Τροφίμων Ζωικής Προέλευσης, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης,
541 24 Θεσσαλονίκη
Τηλ.: 2310 999970, Fax: 2310 999833, E-mail:dsergkel@vet.auth.gr

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ABSTRACT. In this study, a total of 405 samples from freshwater fish, personnel and environment were collected from retail fish markets in three cities in Northern Greece and they were examined for the presence of *Listeria* spp. They consisted of 136 samples from the skin and 136 from the flesh of 71 rainbow trout (*Oncorhynchus mykiss*) and 65 gibel carps (*Carassius gibelio*), 20 from workers' hands, 27 from workers' knives, 22 from working surfaces, 29 from wooden boxes, 15 from plastic boxes, 18 from floor surfaces and 2 from drainage lids. *Listeria* spp. was isolated from 10.62% of the samples, *L. monocytogenes* were 0.99%, 4.69% *L. seeligeri* and 4.94% *L. innocua*, respectively. *L. monocytogenes* was isolated from 3.54% of the environmental samples and none from fresh water fish and workers hands' samples. *Listeria* spp. was isolated from 1.54% of gibel carp flesh (1.54% *L. seeligeri*), from 18.46% of gibel carp skin (10.77% *L. seeligeri* and 7.69% *L. innocua*) and from 8.45% from rainbow trout skin samples (1.41% *L. seeligeri* and 7.04% *L. innocua*). The higher rate of isolation of *Listeria* spp. from the environmental samples emphasises the importance of sanitary conditions in order to reduce the risk of contamination by *L. monocytogenes* at the retail level.

Keywords: *Listeria* spp., fresh water fish, fish markets, environment

ΠΕΡΙΛΗΨΗ. Στην παρούσα μελέτη, 405 συνολικά δείγματα από ψάρια του γλυκού νερού, το προσωπικό και το περιβάλλον ιχθυοπωλείων τριών πόλεων της Β. Ελλάδας εξετάστηκαν για την παρουσία των *Listeria* spp. Τα δείγματα αυτά ελήφθησαν 136 από την επιδερμίδα και 136 από τη σάρκα 71 πεστρόφων (*Oncorhynchus mykiss*) και 65 κυπρίνων (*Carassius gibelio*), 20 από τα χέρια του προσωπικού, 27 από τα μαχαίρια, 22 από τις επιφάνειες εργασίας, 29 από ξύλινες κάσες, 15 από πλαστικές κάσες, 18 από τα δάπεδα και 2 από καπάκια αποχετεύσεων. *Listeria* spp. απομονώθηκαν σε ποσοστό 10,62% των δειγμάτων, εκ των οποίων 0,99% ήταν *L. monocytogenes*, 6,69% *L. seeligeri* και 4,943% *L. innocua*, αντιστοίχως. *L. monocytogenes* απομονώθηκε σε ποσοστό 3,54% των δειγμάτων του περιβάλλοντος και σε κανένα από τα δείγματα των ψαριών του γλυκού νερού και των χεριών του προσωπικού. *Listeria* spp. απομονώθηκε σε ποσοστό 1,54% των δειγμάτων από τη σάρκα των κυπρίνων (1,54% *L. seeligeri*), 18.46% των δειγμάτων από την επιδερμίδα των κυπρίνων (10,77% *L. seeligeri* και 7,69% *L. innocua*) και 8,45% των δειγμάτων από την επιδερμίδα των πεστρόφων (1,41% *L. seeligeri* και 7,04% *L. innocua*). Το υψηλότερο ποσοστό απομόνωσης των *Listeria* spp. από τα δείγματα του περιβάλλοντος τονίζουν τη σημασία των συνθηκών υγιεινής ώστε να ελαττωθεί ο κίνδυνος μόλυνσης με *L. monocytogenes* στο επίπεδο λιανικής πώλησης.
Λέξεις ευρετηρίασης: *Listeria* spp., ψάρια του γλυκού νερού, ιχθυοπωλεία, περιβάλλον

INTRODUCTION

Listeriosis is a disease caused by bacteria of the genus *Listeria* and it usually affects immunosupressed persons, causing meningitis, septicaemia and perinatal disease (Newman et al. 1979, Lamont et al. 1988, Ahmed 1991, Koch and Stark 2006). *L. monocytogenes* is a pathogenic species in both animals and humans (McLauchlin and Jones 1999). However, in a few cases of human infections, *L. ivanovii* (Cummins et al. 1994, Lessing et al. 1994) and *L. seeligeri* (Rocourt et al. 1986) have been incriminated as causative agents.

Although an average of five to nine exposures to *L. monocytogenes* occur per person per year (Grif et al. 2003), listeriosis is a rather rare disease (Gerner-Smidt et al. 2005), but it is associated with a mortality rate of approximately 20% to 40% (Farber and Peterkin 1991, De Valk et al. 2005).

Elderly people, aged 65 years and older, pregnant women, unborn infants and neonates are the risk groups that are affected by the pathogen, due to which, during the last decade, a new form of the disease has been recognized with short incubation period, characterized by mild disorders of the gastrointestinal system (FAO 1999).

L. monocytogenes is ubiquitous in nature (Donnelly 1994) and it has been isolated from many different foods of animal and plant origin and ready-to-eat products in Greece and Europe (Abrahim et al. 1998, Angelidis and Koutsoumanis 2006, EFSA 2006). Decaying vegetation and soil, animal faeces, sewage, silage and water have been found to harbour this pathogen (Jay 2000). It has, also, been isolated from a range of fishery products, including frozen seafood, ready-to-eat shrimp, crabmeat and cold- and hot-smoked salmon, gravid salmon, surimi-based products, marinated fish, fermented fish and fish salads (Weagant et al. 1988, Jemmi 1990, Farber 1991, Ben Embarek 1994, Gudbjornsdottir et al. 2004).

Fish and fish products have been epidemiologically linked as vectors for outbreaks of listeriosis (Farber and Peterkin 1991, Madden 1994). Contaminated fishery products, such as cold-smoked salmon, smoked cod roe, shrimp, mussels and undercooked fish, have been assumed to be sources or sporadic cases of listeriosis (Ericson et al. 1997, Brett et al. 1998, Miettinen et al. 1999).

Lennon et al. (1984) observed a cluster of 22 perinatal listeriosis cases and a relationship between them and the consumption of contaminated raw fish and shell fish. Facinelli et al. (1989) reported a case of listeriosis due to consumption of undercooked fish, which was confirmed by phage typing and DNA fingerprinting. These cases of listeriosis emphasize the need for additional information on the behaviour and epidemiology of *L. monocytogenes* in fish and seafood and, moreover, about the routes of transmission to ready-to-eat fishery products, especially the minimum processed ones.

The prevalence of *Listeria* spp. has been reported in some marine fish in Greece (Soultos et al. 2006, Papadopoulou et al. 2006). However, scanty information is available on isolation from freshwater fish and the environment of the fish markets (Papadopoulou et al. 2006). Thus, the purpose of the present study was to investigate the presence of *Listeria* spp. in freshwater fish, the personnel and the environment of fish markets in Northern Greece.

MATERIALS AND METHODS

Sampling

Seventy one rainbow trout (*Oncorhynchus mykiss*) and 65 gibel carp (*Carassius gibelio*), with mean weight 340±10 g and 490±12 g respectively, were examined from fish markets from three cities in Northern Greece. Gibel carp originated from three nearby lakes and rainbow trout from fish farms located in streams near these lakes. The fish were brought to the markets covered with ice within the fishing day. The fish were collected in sterile stomacher bags and brought to the laboratory for examination in portable insulated boxes. In the laboratory, flesh and skin were sampled from each fish.

Moreover, 133 environmental and personnel swabbed samples were examined from the fish markets. Twenty of them were taken from workers' hands, 27 from workers' knives, 22 from work surfaces (wooden board), 29 from wooden boxes, 15 from plastic boxes, 18 from floor surfaces and 2 from drainage lids. For this purpose, about 100 cm^2 of plane surfaces, workers' hands and knives' surfaces were swabbed 2-5 times by the wet-dry double swab technique (Anonymous 2001), using sterile cotton swabs moistened with 0.1% peptone water containing 0.85% NaCl followed by a second swabbing using a dry swab. The 2-5 swabs were pooled as one sample. Swabbing was carried out in the middle of a working day. They were transported to the laboratory inside portable insulated boxes at 4°C and processed within 3 hours of collection.

The kind of retail markets investigated were super markets, fish markets and open air fish markets.

Isolation of *Listeria* spp.

Isolation of *Listeria* spp. was based on EN ISO 11290-1:1997 (Anonymous 1997). Immediately after the arrival of the fish in the laboratory, their skin surfaces were swabbed using the same technique that is described for environmental sampling. In this case, the sterile cotton swabs were moistened in tubes containing 10 ml of sterile half Frazier broth (4 swabs per sample). Gills were included in the swabbing area. In addition, 25-g samples of flesh of the fish were taken from the anterior-dorsal region, after aseptically skinned, and they were placed into sterile stomacher bags containing 225 ml of half Frazier broth; the samples were blended for 2 minutes in a stomacher (Lab Blender 400, A. J. Seward and Co. Ltd., London).

All fish, personnel and environmental swabbed samples were placed into half Fraser broth and incubated at 30°C for 24 hours. An aliquot of 0.1 ml of these primary enrichments were transferred into tubes containing 10 ml of full Fraser broth and incubated for secondary enrichment at 35°C for 24-48 hours. After incubation, a loopful of them was streaked onto Oxford agar (LAB M, Hal 10 UK) and ALOA agar (LAB M, Hal 10 UK) and it was incubated at 37°C for 24-48 h.

Identification of *Listeria* spp.

Three to four suspected colonies from ALOA and Oxford agar were subcultured to tryptone soy agar supplemented with 0.6% yeast extract (TSA-YE, Lab M) for purity and they were incubated at 37°C for 24 hours. All isolates were identified based on the criteria suggested by Seeliger and Jones (1986) and Lovett (Lovett 1988). In addition, the isolates of *L. monocytogenes* and *Listeria* spp. were identified using the Microgen™ *Listeria* ID MID-67 (Microgen Bioproducts Ltd., UK).

Statistical analysis

All statistical analysis was performed by a Student's t-test.

Table 1. Incidence of *Listeria* spp. in fresh water fish, personnel and environment of fish markets.

Origin of sample	Number of samples	Number and % of samples positive for			
		Listeria spp.	*L. monocytogenes*	*L. seeligeri*	*L. innocua*
Gibel carp flesh	65	1 (1.54%)	–	1 (1.54%)	–
Rainbow trout flesh	71	–	–	–	–
Gibel carp skin	65	12 (18.46%)	–	7 (10.77%)	5 (7.69%)
Rainbow trout skin	71	6 (8.45%)	–	1(1.41%)	5 (7.04%)
Workers' hands	20	3 (15%)	–	2 (10%)	1 (5%)
Environment	113	21 (18.58%)	4 (3.54%)	8 (7.08%)	9 (7.96%)
Total	405	43 (10.61%)	4 (0.99%)	19 (4.69%)	20 (4.94%)

RESULTS

Listeria spp. was isolated from 43 of the total 405 samples (10.62%). From these isolates, 4/405 (0.99%) were *L. monocytogenes*, 19/405 (4.69%) *L. seeligeri* and 20/405 (4.94%) *L. innocua,* respectively (Table 1).

Only *L. seeligeri* was isolated from 1/65 samples (1.54%) from gibel carp flesh (Table 1). *Listeria* spp. was isolated from 12/65 (18.46%) of the gibel carp skin and from 6/71 (8.45%) rainbow trout skin samples, respectively. *L. seeligeri* and *L. innocua* were isolated from 7/65 (10.77%) and 5/65 (7.69%) of the gibel carp and 1/71 (1.41%) and 5/71 (7.04%) rainbow trout samples. *L. monocytogenes* did not detected in any of the fish samples (Table 1).

As it is shown in table 1, *L. seeligeri* was isolated in 2/20 (10%) samples from workers' hands and *L. innocua* in 1/20 (5%).

Listeria spp. was isolated from 21/113 (18.58%) of the environmental samples, from 4/27 (14.81%) of the knives' samples, from 6/29 (20.69%) of wooden boxes, from 2/15 (13.30%) of plastic boxes, from 4/22 (18.18%) of work surfaces (wooden board), from 3/18 (16.67%) of floor samples and from 2/2 (100%) of the drainage lids samples. *L. monocytogenes*, *L. seeligeri* and *L. innocua* were isolated from 4/113 (3.54%), 8/113 (7.08%) and 9/113 (7.96%) of the environmental samples, respectively. *L. monocytogenes* was detected in 2/29 (6.90%) samples taken from wooden boxes, from 1/18 (5.55%) of floor samples and 1/2 (50%) of the drainage lids samples (Table 2).

As it is shown in table 3, the incidence of *Listeria* spp. was higher ($P \leq 0.05$) in the environment and the personnel of fish markets and open air markets (20% and 19.51%, respectively) than that of super markets (9.52%).

DISCUSSION

In the present study *L. monocytogenes* was not isolated from any of the freshwater fish samples examined. *L. innocua* and *L. seeligeri* were the dominant strains isolated from fish skin. Similar results were reported by Papadopoulou et al. (2006). They isolated *Listeria innocua* from 1% of trout (*Salmo truta*) samples from retail markets in Greece. These two *Listeria* species were also detected in trout (*Oncorynhus mykiss*) in Great Britain and Portugal. In Great Britain, 1 out 30 samples from fresh water fish was positive to *L. monocytogenes* (Davies et al. 2001).

Gonzalez-Rodrigues et al. (2002) reported that listeriae were not detected in salmon slices. The same authors found on trout fillets, recovered from eight different lots, 11 strains of *L. innocua*, after 4 days storage at 3°C. One of the examined fillets was contaminated with *L. monocytogenes*.

Panda and Garg (2003) isolated *Listeria* spp. from 28 out of 120 (23.33%) samples in fresh water fish in India. These included *L. monocytogenes* from 2 (1.66%) and *L. innocua* from 26 (21.66%) samples. In another study, also in India, Jallewar et al. (2007) isolated *Listeria* spp. from 20% of freshwater fish samples (walking catfish). Of these samples, 67%, 21%, 8% and 2% were *L. monocytogenes*, *L. seeligeri*, *L. grayi* and *L. welshimeti*, respectively. 33 % of the

Table 2. Incidence of *Listeria* spp. in environmental samples.

Location	Number of samples	Number and % of samples positive for			
		Listeria spp.	*L. monocytogenes*	*L. seeligeri*	*L. innocua*
Workers' knives	27	4 (14.81%)	–	2 (7.41%)	2 (7.41%)
Containers (wooden boxes)	29	6 (20.69%)	2 (6.90%)	3 (10.34%)	1(3.45%)
Containers (plastic boxes)	15	2 (13.3%)	–	1 (6.67%)	1 (6.67%)
Working surfaces (wooden board)	22	4 (18.18%)	–	2 (9.09%)	2 (9.09%)
Floor surfaces	18	3 (16.67%)	1 (5.56%)	–	2 (11.11%)
Drainage lids	2	2 (100%)	1 (50%)		1 (50%)
Total	113	21 (18.58%)	4 (3.54%)	8 (7.08%)	9 (7.96%)

Table 3. Incidence of *Listeria* spp. in environmental and personnel samples by the kind of retail markets' origin.

Location	Number of samples	Number and % of samples positive for			
		Listeria spp.	*L. monocytogenes*	*L. seeligeri*	*L. innocua*
Super markets	21	2 (9.52%)	1 (4.76%)	–	1 (4.76%)
Fish markets	30	6 (20%)	1 (3.33%)	4 (13.33%)	2 (6.67%)
Open air markets	82	16 (19.51%)	2 (2.44%)	6 (7.32%)	7 (8.54%)
Total	133	24 (18.04)	4 (3.01%)	10 (7.52%)	10 (7.52%)

positive to *L. monocytogenes* samples were muscle fish samples. *L. monocytogenes* was not detected in any marine and freshwater fish samples, which were obtained from local retail market in India, although 33% of the samples harboured *Listeria* spp. (Fucks and Surendran 1989).

L. monocytogenes was detected in 2 out of 20 trout (*Oncoryhncus mykiss*) samples from Great Britain, but neither was detected in samples from France and Portugal (Davies et al. 2001).

According to Miettinen and Wirtanen (2005), the prevalence of *L. monocytogenes* varied greatly between different fish farms of rainbow trout in Finland, from 0 to 100% in pooled samples and from 0 to 75%, according to individually studied fish samples. The prevalence of *L. monocytogenes*, *L. innocua*, *L. seeligeri* and *L. welshimeri* was 27.8%, 33.3%, 22.2% and 5.6%, respectively. Only 4.4% of the *L. monocytogenes* positive samples were obtained from skin or viscera in this study.

Hansen et al. (2006) isolated *L. monocytogenes* from 8% of rainbow trout samples in Denmark and Vaz-Velho et al. (2000) isolated *L. monocytogenes* from one fresh salmon trout and one from swordfish out of 234 fish samples examined in Portugal.

The prevalence of the pathogen in raw salmon from Chile, Norway and the west coast of the US has been reported to be 8.0%, 21% and 29.5%, respectively (Hoffman et al. 2003). In other studies the prevalence of *L. monocytogenes* has been found to be 7.8% for salmon in the USA (Norton et al. 2001) and 7.4% in Denmark (Fonnesbech et al. 2001). *L. monocytogenes* was isolated in rainbow trout from retail markets in USA from 54% of the 74 samples examined (Draughon et al. 1999). In Turkey, *Listeria* spp. was isolated from 6.6% of the samples taken from

the intestinal content of fresh water fish (Ertas and Seker 2005).

According to the reported results in the literature, there is a broad range of *L. monocytogenes* prevalence in fresh water fish. Potential contamination sources may be polluted waters, soiled surfaces, ice, boxes, as well as human and avian sources (Parihar et al. 2008). Since *L. monocytogenes* is found in coastal waters and in surface waters of lakes, fish living or cultivated in these areas may possibly carry this pathogen (FAO 1999).

In the present study, the incidence of *Listeria* spp. and *L. monocytogenes* in the samples from personnel and the market environment was higher ($p \leq 0.05$) than that from fish samples. Hansen et al. (2006), in a study in Denmark on prevalence and survival of *L. monocytogenes* in Danish aquatic and fish processing environments, reported that the prevalence increased with the degree of human activity. It was not detected in a freshwater stream, it was 2% in the seawater fish farms, 10% in freshwater fish farms, 16% in fish slaughterhouses and 68% in fish smokehouses. During salmon filleting process, *L. monocytogenes* can be transferred from the flesh to cut surfaces, slicing machines and tables, contaminating 60% of these apparatuses, which become potential sources of contamination (Duffes 1999). The incidence of *L. monocytogenes* in boxes and floor surfaces may have serious impact on cross-contamination of fish and it may pose a health risk.

Vogel et al. (2001) reported that the RAPD (random amplified polymorphic DNA) profile of *L. monocytogenes* that was detected in cold-smoked salmon was identical to types that were isolated on the processing equipment and in the processing environment and not to the types isolated from raw fish. It seemed that contamination of the final product was due to contamination during processing rather than to contamination from raw fish. These findings do not minimize the importance of raw fish as a source of contamination to the processing environment and the final product.

The low incidence of listeriae in the fish skin compared to inorganic surfaces seems to be a consequence of the nature of the skin and mucus as part of the fish immune system containing anti-microbial substances (Greenlee et al. 1991, Fernandes and Smith 2002).

The presence of *L. monocytogenes* and other *Listeria* spp. in personnel and environmental samples from fish and open-air markets proves that contamination of boxes and floor samples is due to bad hygienic practices during processing of fresh fish. *Listeria* spp. was not detected in any of the super market personnel samples and, at a lower than the other markets' rate, from their environmental samples ($P \leq 0.05$), probably because super markets follow good hygienic practices and comply with food safety regulations (Table 3).

A common practice in retail fish markets is evisceration and scalding of fish before they are given to the consumers. This practice seems to contribute to the expansion of the contamination to fish, utensils, personnel and the environment of the retail market, if handling is not in accordance with the hygienic rules.

A very important prerequisite for control of *L. monocytogenes* is knowledge, concerning its niches during processing, especially for minimally processed products, such as cold-smoked fish, which have been a major delicatessen commodity. It is reported that *L. monocytogenes* was detected in 10-40% of samples from freshly produced cold-smoked fish (Autio et al. 1999, Jorgensen and Huss 1998).

Contaminated fish may be potential vectors for this psychrotrophic pathogen and they may cause contamination of the refrigerators' and kitchen's environment and thus cross-contamination of other foods, especially ready-to-eat foods that do not need additional thermal processing before consumption. Moreover, diligent enforcement of sanitary conditions of food contact surfaces and handling areas and personal hygiene practices may contribute to the reduction of potential contamination of fishery products by *Listeria monocytogenes* at the retail level. ■

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