Listeria monocytogenes in fish and fishery products
Review and proposal for research

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1. Introduction

Before 1980's *Listeria monocytogenes* (*L. monocytogenes*) bacteria were recognised as ubiquitous bacterium. The main concern about them is that they may cause serious illness in animals. During the last decade several serious outbreaks caused by *L. monocytogenes* were reported (Ryser 1991, Varnam 1991). In 1986, the Council of State and Territorial Epidemiologists recommended that Listeriosis ought to become a reportable disease in the United State. Currently, FDA requires that *L. monocytogenes* must be absent in ready-to-eat seafood products (Ryser 1991).
Listeriosis has been proved to be a foodborne epidemic infection, and fish and fishery products are considered as one of the main vehicle for transmission (Dillon 1992). Fish processing is an important industrial activity in the Netherlands and fishery products contribute considerably to the Dutch diet. However, as far as can be traced, little is known about the incidence of *L. monocytogenes* in fishery products on the Dutch market (Beckers 1988).

2. Characteristics

2.1 Biological features

The genus *Listeria* is composed of small, Grampositive, coccoid to rod-shaped, bacteria which exhibit a characteristic tumbling motility at room temperature. The organisms are catalase-positive, oxidase-negative and have a fermentative metabolism of glucose, producing acid but not gas. Endospores are not formed (Varnam 1991).

2.2 Classification

Seven species of *Listeria* are currently recognised, but only *L. monocytogenes* is associated consistently with disease in man, and two other haemolytic species, *L. ivanovii, L. seelegeri,* have been implicated on rare occasions. *L. monocytogenes* can be subdivided into 13 serovars on the basis of somatic and flagellar antigens (Varnam 1991).

2.3 Some influence factors on their growth and survival

The growth and survival of *L. monocytogenes* can be influenced by various factors (temperature, pH, organic acids, irradiation, water activity, composition of the atmosphere, salt, nitrite)

2.3.1 Temperature

As with all micro-organisms, temperature is of prime importance in the control and inactivation of *L. monocytogenes*. The maximum temperature for growth of *L. monocytogenes* is approximately 45°C, and the optimum in the range 30 to 37°C (Wilkins 1972). Behaviour at low temperatures is less easy to define, and while there is

agreement that *L. monocytogenes* is able to grow at low temperatures, a minimum of 1.1 ± 0.3°C being defined by Juntilla et al (1988), there is some disagreement as to how well it will grow (Griffiths 1989). The discrepancies over the behaviour of *L. monocytogenes* at low temperatures may be partly explained by strain variation, but other factors such as growth medium and incubation temperature of the inoculum may also be important. Harrison et al (1991, 1992) reported no growth of *L. monocytogenes* in the seafood inoculated during storage either at 4°C for 16 days, on ice for 21 days, or at -20°C for 3 months.

The ability of *L. monocytogenes* to survive heat treatments has been the subject of considerable controversy, which has not yet been fully resolved. Mackey (1989) concluded that while normal pasteurisation processes will inactivate *L. monocytogenes* in milk, the safety margin is considerably greater with a low-temperature long-time process (62.8°C/30 mins) than with the more commonly used high-temperature short-time process (71.7°C/15 seconds).

Results on the thermal resistance of *L. monocytogenes* during heat pasteurization of milk were reported by Bradshaw (1985, 1987). Other workers considered that *L. monocytogenes* could survive both HTST and LTLT pasteurisation, following the finding that cells may survive heating, but were not culturable on media commonly used (Knabel 1990).

The reported differences in heat resistance of *L. monocytogenes* could also be due to the nature of the heating menstruum. In most cases, it is likely that reports of unusual heat resistance result from uneven heating. Anon (1988) has reported that *L. monocytogenes* survives the boiling of shrimps, three of seven samples being positive after 3 minutes boiling and one of seven after 5 and 10 minutes boiling. A two-stage process involving boiling following by freezing to -20°C has been proposed to overcome the problem (Anon 1989).

2.3.2 pH value and organic acids

Generally, *L. monocytogenes* can't grow at pH below 4.5. The lowest pH at which growth has been recorded in a non-food system is 4.39 in trypticase soy broth adjusted with HCl and incubated at 30°C (George 1988). Lower limiting pH values for growth are also dependent on the acidulant, acetic acid being most effective and HCl the least (Farber 1989).

Although growth may not occur, *L. monocytogenes* can survive under acidic conditions. In orange serum adjusted to pH 3.6 with HCl, numbers of viable cells had only decreased by approximately one order of magnitude after 2 days at 30°C (Parish 1989). Rapid death followed after 4 days under these conditions. Fuchs (1991) investigated the frequency of *L. monocytogenes* in an acidified fish dish-ceviche marinated in lemon juice for at least 1 h (pH 4.2-5.1) and found *L. monocytogenes* in 3 of 32 analyzed samples. The lowest pH of fish meat from which listeria was isolated was 4.4. They considered at such pH, *L. monocytogenes* is unlikely to grow, but may survive for a considerable length of time.

L. monocytogenes is relatively little affected by increases in pH value and is able to grow at pH 9.5 (Lovett 1988).

2.3.3 Irradiation

Preliminary studies indicated that *L. monocytogenes* is fairly resistant to irradiation treatment, with viable counts remaining after 200 Krad irradiation following inoculation

of crabmeat with 10^7 cells/ml (Juaneau 1989). *L. monocytogenes* is also susceptible to short wave ultraviolet energy at a wavelength of 254 nm (Yousef 1988).

2.3.4 Aw level

Minimum a_w level for *L. monocytogenes* is 0.932 with glycerol as humectant and 0.942 with sucrose or NaCl (Griffiths 1989).

2.3.5 Composition of atmosphere

Relatively little attention has been paid to the effect of atmosphere on growth of *L. monocytogenes* in foods. Work with cooked chicken loaf showed that neither 50 nor 80% carbon dioxide inhibited growth (Ingham 1990).

2.3.6 NaCl

L. monocytogenes has been reported to be relatively tolerant to high NaCl concentrations. The results of the work with gradient plates illustrated that *L. monocytogenes* is able to grow in 10% NaCl at 35°C (neutral pH), while at 20°C, 8.8% NaCl was required to limit growth between pH 6.5-7.6 (McClure 1989). It was observed that *L monocytogenes* can survive for 120 days in blue cheese containing 12% NaCl in waterphase and is able to grow in cheese containing 4.5% NaCl in waterphase (Peterson 1993). Peterson et al (1993) reported that in smoked salmon containing 3, 5, 6% NaCl in waterphase, the effect of NaCl concentration on the growth of *L monocytogenes* depended on the storage temperature. At 10°C, no inhibition was found, while at 5°C the growth rate of *L monocytogenes* was suppressed significantly (Peterson 1993).

2.3.7 Nitrite

Nitrite is not inhibitory to *L. monocytogenes* at permitted levels unless there is an interaction with other inhibitory agents or conditions (Shahamat 1980).

2.3.8 Sorbic and benzoic acids

L. monocytogenes is susceptible to both sorbic and benzoic acids. The inhibition is greatest at pH 5.0 and 4°C (El-Shenawy 1988a, b, Ryser 1988). Sodium benzoate is most effective when used in combination with organic acid (El-Shenawy 1989).

2.3.9 Lactic acid

Recognizing that *L. monocytogenes* is more likely to contaminate the surface rather than interior of most seafood, Noel (1989) investigated the possibility of using various lactic acid treatments to inactivate *L. monocytogenes* on the surface of seafood. In this study, samples of peeled and unpeeled shrimp were immersed in a broth culture of *L. monocytogenes* , removed, thoroughly drained, and immersed in an aqueous solution of 1.5, 3.0, or 6.0% lactic acid for 1, 10, or 120 minutes. Lactic acid treated and untreated shrimp were then frozen at -20°C and examined for numbers of listeria during 28 days of storage. With all lactic acid treatments decreased numbers of listeria

and natural contaminants on all shrimps was recorded and, exposure to 1.5% lactic acid for 10 minutes was deemed most appropriate since this treatment did not adversely affect the product's appearance or organoleptic quality.

2.3.10 Others

The lactoperoxidase system and hydrogen peroxide

A number of proposals have been made for stimulating the naturally occurring lactoperoxidase system (Reiter 1981) as a means of cold sterilisation. The effect of the lactoperoxidase system on this organism is similar to that on other Gram-positive bacteria, in that growth is delayed (Siragusa 1989). *L. monocytogenes* is relatively resistant to hydrogen peroxide due to the high levels of catalase and superoxide dismutase present in *L. monocytogenes* (Dominguez 1987, Dallmier 1988).

Lysozyme and EDTA

Egg white lysozyme is presently approved for use to control spoilage organism in food in some countries. The effect of lysozyme (3mg/ml), Na_2EDTA (5-25 mM), and lysozyme and EDTA combinations on the growth of L monocytogenes in fresh cod were studied by Wang et al (1992). They found the growth of L monocytogenes in cod fillets was inhibited significantly by pre-treatment of fish with either lysozyme or EDTA. These effects were enhanced by using the EDTA-lysozyme combination.

3. Source, prevalence and behaviour in fish and fishery-products

3.1 Source

L. monocytogenes has been isolated from a wide range of foods. This reflects the variety of means by which *L. monocytogenes* may enter food. A possible cycle of infection for *L. monocytogenes* was proposed by Dillon (1992).

Fish as one vehicle of infection has been suggested as a possible cause at an outbreak of listeriosis in Auckland, New Zealand (Lennon 1984). Further understanding of the source, prevalence and behaviour of *L. monocytogenes* in fish and fishery products is necessary.
The fact that high positive rates for L.monocytogenes in smoked, fermented fishery products suggest that the processing cannot be overlooked as a producing source of contamination. It was recorded in Canada that five factories of smoked fish have been closed down because of *L.monocytogenes* contamination (Dillon 1992). The primary source in processing plants is considered to be floors and floor drains, a larger survey isolated listeria in the decreasing order of frequency from drains, floors, standing water, residues and food-contact surfaces (Cox 1989).The ubiquitous nature of *L. monocytogenes*, together with specific properties such as its ability to attach to stainless steel under certain conditions (Herald 1987), mean that the organism is always likely to present a problem with respect to plant sanitation (Varnam 1991).

The prevalence of *L. monocytogenes* in fish and shellfish indicates survival in warm and freshwater environments. It was reported that *L. monocytogenes* is present in the water cycle and is frequently present in river waters in greater numbers than *Salmonella* (Watkins 1981). *L. monocytogenes* has been isolated from raw and treated sewage, effluent from abattoirs and poultry packing plants, lakes and canals. The pathogen has also been isolated from marine water and sediment samples (Colburn 1990). Therefore, there is a high risk of contamination or infection for fish and shellfish from the water environment. More work is needed to establish the major source of *Listeria* in seafood. Whether the organism is coming from the natural environment, pollution of aquatic environment, or from the workers and processing foodservices environment, remains to be answered for each different situation.

3.2 Prevalence

Many investigations have been carried out to detect the incidence of *L. monocytogenes* in fish and fishery products. The main results are summarized in Table 1. There are some characteristics in the prevalence of *L. monocytogenes* in fish and fishery products.

(1) Worldwide presence. The reports from different countries have proved the presence of *L. monocytogenes* in fish and fishery products in these countries. Weagant et al (1988) surveyed frozen seafood products from 12 countries for presence of *L. monocytogenes* and found 15 of 57 samples (26%) were positive, that came from 9 different countries.

(2) Presence in different fresh and frozen fish such as shrimp, lobster, crab, fin fish, cod, eel, herring, trout, salmon, and different fishery products including smoked, fermented, acidified, marinated, seafood analogs (surimi-based foods) and many ready-to-eat fishery products has been reported (Jemmi, 1993; Kradolfer, 1992; Farber, 1991).

(3) For smoked fish, the incidence of *L. monocytogenes* in cold smoked fish is higher than hot smoked fish.

(4) The presence of *L. monocytogenes* in cooked seafood indicates cross contamination. Cooking and prevention of recontamination are extremely important for controlling *Listeria*. Vacuum packaging of smoked fish does not prevent growth of the facultatively anaerobic Listeria. Sous vide fish products, by cooking fish in oiled parchment or sealed plastic bags to lock flavor, have an extended shelf-life when held under refrigeration. However, the safety of the products may be questioned as these products are only minimally processed and do not contain preservatives (Hackney, 1991)

Table 1. Frequency of *L. monocytogenes* in fish and fishery-products

References	fisheryproducts	analyzed samples	positive	
		n	n	(%)
Weagant (1988) (USA)	Frozen seafood	57	15	26
Jemmi (1993)	**Hot-smoked**	691	58	8.4
(Switzerland)	Cod	17	1	5.9
	Eel	35	0	
	Herring	186	17	9.1
	Mackerel	88	5	5.7
	Schillerlocken	32		0
	Sprat	26	1	3.9
	Trout	307	34	11.1
	Cold-smoked	434	49	11.3
	Haddock	6	1	16.7
	Halibut	40	9	22.5
	Salmon	388	39	10.0
Jemmi (1990)	Fish	909	111	12.2
(Switzerland)	hot-smoked	ND	ND	8.9
	cold-smoked	ND	ND	13.6
	fermented	ND	ND	25.8
Kradolfer (1992)	Hot-smoked fish	433	40	9.2
	Cold-smoked	210	15	7.1
Rorvik (1991)	Smoked-salmon	33	3	9
(Norway)	Minced fish	8	1	12
	Peeled Shrimp	16	8	18
Rorvik (1992)	Vacuum-packed			
(Norway)	smoked-salmon	ND	ND	11
Farber (1991)	**Ready-to-eat**	113	15	11.5
(Canada)	Shrimp	49	9	8.2
	smoked-Salmon	32	10	31.3
	Crab	7	1	14.3
	Surimi	25	0	

Table 1 continued

References	fisheryproducts	analyzed samples n	positive n	positive (%)
Hartemink (1991) (Iceland)	Total seafood	91	10	11
	Dried haddock	5	0	
	Shellfish	11	0	
	Ocean perch	11	5	45
	Salmon	12	1	8
	Minced fish	3	0	
	Raw fresh trout	2	0	
	Shrimps	11	1	9
	Smoked			
	Salmon	8	0	
	Minced salmon	5	0	
	Herring	10	1	10
	Trout	8	1	13
	Other	5	1	20
Adesiyun (1993) (West Indie)	Fish	61	9	14.8
	Shrimp	41	2	4.9
Ryu (1992) (Japan)	Raw minced tuna	37	3	8.1
	Raw prawn	38	1	2.6
	Salted raw seafood	10	0	
	Other raw seafood	18	3	16.7
	Cooked seafood	5	0	
Wong (1990) (Taiwan)	Seafood	57	6	10.5
Fuchs (1991) (Peru)	acidified ceviche (marinated in lemon juice pH 4.2-5.1)	32	3	9

ND: no data

In these surveys a contamination rate of about 10-20% was found. However, little data are available on the levels of the *L. monocytogenes*-contamination, i.e. quantitative aspects. Kradolfer (1992) analyzed cold- and hot-smoked fish, both qualitatively and quantitatively. They found that most of the samples had levels below 100 *L. monocytogenes*/g, although levels of up to 10^4/g were detected. These results are in accordance with the studies of Guyer and Jemmi (1990), in which the detected levels in the analyzed samples of cold-smoked salmon were commonly very low (1 *L. monocytogenes*/g).

3.3 Behaviour

Studies on the behaviour of *L. monocytogenes* in fish and fisheryproducts is of recent origin with preliminary results from relatively few studies. According to data gathered by Lovett (1988), *L. monocytogenes* grew readily (generation time=12 h) in inoculated samples of raw shrimp,crab, and whitefish, with the pathogen attaining maximum populations of > 10^8 CFU/g in all four products following 14 days of storage at 7°C Brackett (1990) also reported that *L. monocytogenes* grew and retained similar levels of pathogenicity on artificially contaminated crabmeat during 14 days of storage at 5 or 10°C. McCarthy (1990) even reported that *L. monocytogenes* was recovered from laboratory-contaminated shrimp after 90 days of storage at -20°C. Only Harrison (1990) found *L. monocytogenes* failed to grow in overwrapped/vacuum-packaged raw shrimp and fin fish, with numbers of listeria generally decreasing < 10-fold after 21 days of storage in ice.

Most work on the behaviour of *L. monocytogenes* in fishery products was focussed on smoked fish. Guyer and Jemmi (1991) reported in their study with artificially contaminated salmon that *L. monocytogenes* are able to survive salting and cold-smoking. No obvious influence on the numbers of *L. monocytogenes* could be demonstrated. However, during storage, a significant growth was measured within 10 days at 8-10°C, and within 20 days at 4°C. After 30 days an increase of 4.5 log10 at both storage temperatures was recorded. These results were confirmed by the studies of Rorvik (1991) and Farber (1991), where up to 4.8 log10 increases of the inoculated *L. monocytogenes* had been observed. In these cited works different strains of *L. monocytogenes* were used: on the strength of their results, Guyer (1991) presumed that wild-type isolates, i.e. isolated from smoked salmon, can grow better on this type of product than reference strains. In contrast, according to Farber (1991) the reference strain (Scott A) grew faster than the wild-type isolate used. However, the latter strain was isolated from shrimp and not from salmon.
Very few studies on the behaviour of *L. monocytogenes* in hot-smoked fish have been reported. Jemmi et al. (1992) performed some investigations during processing and storage of artificially contaminated hot-smoked trout (core temperature 65°C for 30 min). After salting and smoking, *L. monocytogenes* could no longer be detected. The two parameters, heat and smoke, led to a 6 log10 reduction in numbers of this pathogen. They considered that *L. monocytogenes* frequently found in hot-smoked fish is due to the recontamination or post-processing contamination at the filleting or packaging stage. This was confirmed by another study with trout, artificially contaminated after the smoking process: during storage at 4°C, the concentration of *L. monocytogenes* remained about the same, while after 20 days at 8-10°C a significant increase could be measured (Jemmi 1992) and the pathogen levels can be compared with those found in Vacherin Mont d'Or (a soft cheese implicated in the Swiss listeriosis outbreak) (Bille 1990).

4. Hazard effect on human health

After several caused outbreaks of listeriosis, more and more attention was paid to the pathogenesis of *L monocytogenes* in humans. During the past fifteen years, the role of food as a source for listeria infections in humans has been the subject of scientific and public concern.

Listeriosis is an infection with the intestines as the point of entry. Since *L. monocytogenes* is ubiquitous in nature, it was postulated that the quantity of *L. monocytogenes* in food is important in developing infection. However, the infective dose is still unknown. Incubation period of listeriosis may vary from one day to several weeks. Virulent strains are capable of multiplying in the macrophages and produce septicemia followed by infection of other organs such as the central nervous system, the heart, the eye, and may invade the fetus of pregnant women. The severity of the infection also depends on the condition of infected people. Listeriosis is of particular risk for foetuses, pregnant women, neonates, the elderly and persons with an impaired immune system (diabetics, cirrhotics) and is a most important bacterial cause of abortion, premature birth and neonatal infection (Varnam 1991). The lethality rates in previous outbreaks are high and range from 22% to 44% (Table 2) (Dillon 1992). While, in healthy adults, Listeriosis usually does not develop beyond the primary enteric phase, which may be symptom-free or having only mild 'flu-like' symptoms, in the case of cancer patients who are undergoing immunosuppresive therapy, listeriosis is a serious risk. The mortality for the latter group has been shown to be approximately 30%.

Contaminated food is increasingly recognized as an important vehicle of *L. monocytogenes.* Dairy products (milk, cheese, ice-cream, cream, butter) have all been implicated in outbreaks of listeriosis. Frequent isolation from fishery products (Weagant 1989, Rorvik 1991) and the demonstration of growth potential in chilled (+4°C), salted or smoked fish (Ben Embarek 1992, Guyer 1991, Rorvik 1991, Fuchs 1992) suggests that fish products are important in the transmission of *L. monocytogenes.* Seafood has also been associated with a listeriosis outbreak in New Zealand, although no conclusive evidence was supplied (Lennon 1984). Salads and vegetables may also one of the transmitters for listeriosis.

Table 2. The documented outbreaks of listeriosis

time	lethality	areas	suspected foods
1975	5/25 (25%)	Boston	Vegetables (lettuce,Celery, tomatoes)
1981	18/41 (44%)	Nova Scotia	Coleslaw
1983	14/49 (29%)	Massachusetts	Pasteurized milk
1985	48/142 (34%)	California	Mexican-style cheese
1988		Switzerland	Soft cheese
1980	5/22 (22%)	New zealand	Shellfish, fish

5. Conclusions

Of the seven recognised *Listeria* species, *L. monocytogenes* is that mainly associated with disease in man.
The characterisation of *Listeria* as well as the factors influencing the growth and survival is well documented
Optimal temperature for growth of *Listeria* is in the range of 30-37°C. *Listeria* will not grow at a pH lower than 4.5, but can survive under acidic conditions. *Listeria* is relatively tolerant to high NaCl concentrations. Organic acids may inhibit the growth of *Listeria*.
Some interesting gaps in knowledge on the behaviour of *Listeria* are : survival during boiling of shrimps, effect of changing the atmosphere.
Listeria may be present in a number of fishery products (fresh, frozen and after processing). The incidence of *Listeria* in cold smoked fishery products is higher than in hot-smoked.
Little quantitative data on the levels of *Listeria* in fishery products are available. The infective dose of listeriosis for human is also unclear.
Information on the incidence of *Listeria* in fishery products on the Dutch market is scarce.

6. Proposed research

It seems to be justified that attention is given to the incidence of *Listeria* in fishery products on the Dutch market.
Within the proposed survey priority may be given to smoked fishery products, shrimps (raw and cooked), sous vide and modified atmosphere packed fish products.
A sampling plan (number of samples, fishery products, locations) for the various fish species will be worked out in accordance with marketing data.
The implementation of available existing methods for the determination of *Listeria* should be established in the laboratory initially and if possible the sampling may take place in parallel.

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