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Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in Smoked and "Gravad" Fish

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Loncarevic, S., W. Tham and M.-L. Danielsson-Tham: Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and "gravad" fish. Acta vet. scand. 1996, 37, 13-18. – Altogether 150 samples of vacuum-packed fish were examined for the presence of *Listeria* species *Listeria monocytogenes* was isolated from 12 of 58 "gravad" fish samples, 3 of 26 cold-smoked and one of 66 hot-smoked fish samples Ten of these 16 positive samples harboured more than 100 *L monocytogenes* cfu/g. The highest level (132 000) was found in a sample of hot-smoked rainbow trout *(Oncorhynchus mykiss)* Serogroup 1/2 was most frequently found, followed by 4 and 3. One sample of gravad rainbow trout harboured more than one serogroup of *L monocytogenes L innocua* and *L seeligeri* were isolated from 12 and 1 samples, respectively.

enumeration; serogroup.

Introduction

Listeria monocytogenes has been associated with several food-borne outbreaks in North America and Europe *(Schlech et al.* 1983, *Fleming et al.* 1985, *James et al.* 1985, *Bille & Glauser* 1988, *McLauchlin et al.* 1991, *Goulet et al.* 1993) involving a variety of foods such as coleslaw, milk, soft cheese, paté and pork tongue in aspic. Fish and seafood have rarely been linked to outbreaks of listeriosis. However, one possible outbreak of listeriosis due to consumption of raw fish and shellfish, and one case from fish ingestion have been reported *(Lennon et al.* 1984, *Facinelli et al.* 1989).

L. monocytogenes occurs in faecally contaminated environment and may enter the food-processing plants via utensils, persons, birds, naturally contaminated raw products, etc. The bacterium can grow both aerobically and anaerobically at temperatures as low as 1°C. It has no specific nutrient requirements and grows in NaCl concentrations up to 10%.

Special risk products for listeriosis are ready-to-eat food items with long shelf-life during refrigeration. If such a product is contaminated with one single cell of *L. monocytogenes* before packaging, this microorganism might multiply to hazardous levels during prolonged storage. Smoked and "gravad" fish, particularly salmon, are very popular dishes in Sweden. After preparation they are packed under vacuum in oxygen impermeable film and stored up to 42 days at 8°C. They are then consumed in thin slices without further cooking.

The purpose of this study was to investigate the prevalence and number of *L. monocytogenes* in smoked and gravad fish obtained from the retail market in Sweden.

Materials and methods

Preparation of gravad and smoked fish

The preparation is usually performed in special processing plants.

Gravad fish: Raw salmon fillets are rubbed with a mixture of sugar, salt and peppar, covered with dill, put into a plastic bag and placed in a refrigerator for 2 days. The plastic bag is then opened and the fillets are packaged sliced or whole under vacuum.

Cold-smoked fish: The fillets are rubbed with salt or the cure is injected with multiple needles into the fillets. After that, the fish is smoked at 25-30°C for 2-3 h and then packed sliced or as whole fillets under vacuum. The NaCl concentration in the fish after curing and smoking is ca 2.5-3.5%.

Hot-smoked fish: After curing, the fish is smoked at >60°C for 3-4 h and then packed under vacuum.

One hundred and fifty vacuum-packed fish items were collected from retail markets. The items comprised the following categories: I) gravad fish (rainbow trout – *Oncorhynchus mykiss,* and salmon – *Salmo salar,* cut pieces or sliced) II) cold-smoked fish (rainbow trout and salmon) and III) hot-smoked fish (herring – *Clupea harengus,* mackerel – *Scomber scombrus,* rainbow trout, salmon and white fish – *Coregonus lavaretus).* They were transferred to the laboratory together with ice packs, and kept under refrigeration (4°C). They were analyzed on the recommended best-before day.

Enrichment and cultural procedures for detection and isolation of *L. monocytogenes* were done according to the International Dairy Federation Standard *(Anon.* 1990) with slight modification. From each sample, 25 g was cut out and macerated with 225 ml *Listeria* Enrichment Broth (LEB) in a stomacher. LEB base consisted of 30 g Tryptone Soya broth (Oxoid CM129, Unipath Ltd., Basingstoke, Hampshire, England), 6 g Bacto Yeast Extract (Difco 0127-01-7, Difco Lab., Detroit, MI, USA) and 1000 ml destilled water. The medium was complemented by adding 3 selective agents (2.3 mg acriflavine HCl, 9.2 mg nalidixic acid and 11.5 mg cycloheximide) to 225 ml of LEB. The macerate was incubated at 30°C for 48 h, and then 0.1 ml was streaked onto *Listeria* Selective Medium (Oxford Formulation) plates (Oxoid: agar base CM856 and supplement SR140) which were incubated at 37°C for 48 h.

Quantification of *L. monocytogenes* was made from all fishes positive for *L. monocytogenes* in the enrichment procedure. Ten g fish sample was blended with 90 ml sterile peptone water in a stomacher bag and macerated. Ten-fold serial dilutions of 1 ml of the macerate were made in peptone water. The macerate and each dilution were then surface-plated in 0.1 ml portions onto *Listeria* Selective Medium (Oxford Formulation) plates. Incubation was performed at 37°C for 48 h and presumptive *L. monocytogenes* colonies were counted.

Ten presumptive *Listeria* colonies, or all when fewer were present, were picked from each plate from the enrichment and quantification procedures, respectively, and streaked onto horse blood agar for control of purity, cell shape, Gram reaction, hemolytic reaction, tumbling motility (10h at 20°C), fermentation of rhamnose, xylose, hydrolysis of esculin and production of catalase *(Seeliger & Jones* 1986). All *L. monocytogenes* isolates were serotyped with *Listeria* O Antiserum 1 and 4 (Difco laboratories, Detroit, Michigan, USA). Strains not typable with Difco Antiserum were sent to Centre National de Référence des Listerias , Lausanne, Switzerland for serotyping according to reference methods *(Seeliger & Höhne* 1979).

Results

Altogether samples from 16 (10.7%) fishes representing 11 different plants were positive for *L.*

Table 1 Prevalence of *Listeria* spp after enrichment procedure.

Product	No of samples investigated	No (%) of samples positive for		
		L monocytogenes	*L innocua*	*L seeligeri*
Gravad fish				
Rainbow trout	34	8 (23 5)[a]	3 (8.8)[a]	0 (0)
Salmon	24	4 (16.7)[b,c]	5 (20 8)[b]	1 (4 2)[c]
Total	58	12 (20 7)	8 (13.8)	1 (1.7)
Cold-smoked fish				
Rainbow trout	13	1 (7.7)	4 (30.8)	0 (0)
Salmon	13	2 (15 4)	0 (0)	0 (0)
Total	26	3 (11.5)	4 (15 4)	0 (0)
Hot-smoked fish				
Herring	14	0 (0)	0 (0)	0 (0)
Mackerel	34	0 (0)	0 (0)	0 (0)
Rainbow trout	6	1 (16 7)	0 (0)	0 (0)
Salmon	4	0 (0)	0 (0)	0 (0)
White fish	8	0 (0)	0 (0)	0 (0)
Total	66	1 (1 5)	0 (0)	0 (0)
Grand total	150	16 (10 7)	12 (8 0)	1 (0 7)

a = 3 samples harboured both *L monocytogenes* and *L innocua*
b = 2 samples harboured both *L monocytogenes* and *L innocua*
c = 1 sample harboured both *L monocytogenes* and *L seeligeri*

monocytogenes after the enrichment procedure (Table 1). Ten of these 16 samples harboured more than 100 *L. monocytogenes* cfu per gram (Table 2). The level of *L. monocytogenes* in gravad fishes ranged from <100 to 3 400 cfu/g and in cold-smoked fishes from <100 to 25 400 cfu/g. The highest number was found in a sample of hot-smoked rainbow trout, 132 000 cfu/g. The *L. monocytogenes* isolates belonged to serogroups 1/2, 3 or 4 (Table 2). Serotyping of isolates from enrichment as well as from quantification procedure showed the same results for each of the sample at issue with one exception. In one sample of gravad rainbow trout the enrichment procedure yielded 9 isolates of serogroup 3, while the quantification procedure yielded 6 isolates of serogroup 3 and 2 of serogroup 1/2 (Table 2).

Discussion

Gravad fish was the category with highest prevalence (20.7%) of *L. monocytogenes* (Table 1). Also *Jemmi* (1990) found that such products were frequently contaminated with *L. monocytogenes.* He reported 26.9% (21 out of 78) of gravad salmon to be positive. This is not surprising since the procedure used is not bactericidal.

In our study, *L. monocytogenes* was isolated from 11.5% of the cold-smoked fish products. Prevalence of *L monocytogenes* in cold-smoked fish has also been reported by *Jemmi* (1990) and *Jemmi et al.* (1992). They found the organism in 13.6% of 324 and 11.3% of 434 samples, respectively. The temperature used in the cold-smoking process is not sufficient to kill the bacteria. The smoke itself has inhibitory

Table 2. Serogroups and numbers of *L. monocytogenes*

Product	No of isolates serotyped and (serogroup)		Numbers (cfu/g)
	Enrichment	Quantification	
1. Gravad rainbow trout[1]	10 (4)	2 (4)	200
2 Gravad rainbow trout[1]	10 (4)	9 (4)	900
3. Gravad rainbow trout[1]	10 (4)	9 (4)	900
4. Gravad rainbow trout	10 (1/2)	n.d.	<100
5. Gravad rainbow trout[2]	10 (1/2)	n d.	<100
6. Gravad rainbow trout	7 (1/2)	10 (1/2)	1300
7. Gravad rainbow trout	9 (3)	6 (3), 2 (1/2)	3 400
8. Gravad rainbow trout	2 (3)	n.d.	<100
9. Gravad salmon[1]	10 (4)	8 (4)	500
10. Gravad salmon	5 (1/2)	n d.	<100
11 Gravad salmon	2 (1/2)	n.d	700
12. Gravad salmon	5 (1/2)	n.d	<100
13. Cold-smoked rainbow trout	9 (1/2)	n.d.	<100
14. Cold-smoked salmon	2 (1/2)	10 (1/2)	25 400
15. Cold-smoked salmon[2]	9 (1/2)	4 (1/2)	400
16. Hot-smoked salmon[1]	10 (4)	10 (4)	132 000

1 = producer A
2 = producer B
n d. = not determined

effects on microorganisms. However, the short time the fish is exposed to the smoke during the cold-smoking process is not enough to inactivate the *L. monocytogenes* bacteria *(Dillon & Patel* 1993). The highest level of *L. monocytogenes* in cold-smoked salmon in our investigation was 25 400 cfu/g. It is not probable that the fish harboured such large number of *L. monocytogenes* at the packaging time. It is reasonable to think that multiplication took place during storage. This theory is supported by *Embarek* (1991), who showed that the number of *L. monocytogenes* in vaccum-packed cold-smoked salmon increased from 100 000 to 100 million cfu/g during storage at 10°C, but was inhibited at 5°C.

In the present study, *L. monocytogenes* was isolated from 1.5% (one sample only) of the hot-smoked fish products. *Jemmi* (1990) and *Jemmi et al.* (1992) found a higher percentage of contaminated hot-smoked fishes, 8.9% and 8.4%, respectively. *Jemmi & Keusch* (1992) pointed out that low levels of *L. monocytogenes* in raw fish will be eliminated by hot-smoking (core temperature 65°C during 20 min). However, products coming out from the hot smoke will be very susceptible for contamination with *L. monocytogenes.* The fairly large prevalence found in hot-smoked salmon *(Jemmi* 1990; *Jemmi et al.* 1992) and the large number (132000 cfu/g) found in our single positive hot-smoked sample show that also this product may be of public health concern.

The majority of *L. monocytogenes* isolates from the fish products in the present study as well as the isolates from an earlier Swedish investigation dealing with soft and semi-soft cheeses *(Loncarevic et al.* 1995) belong to serogroup 1/2. Also *Jemmi* (1990) found that the majority (81%) of the *L. monocytogenes* isolates from

gravad salmon, cold-smoked, and hot-smoked fish belong to serogroup 1/2. According to the available literature, there is no proven large food-borne outbreak associated with *L. monocytogenes* serogroup 1/2. However, the number of sporadic human cases due to this serogroup (in Sweden 41% of human cases) shows that it may play an important role in the epidemiology of listeriosis. One must also remember that there are few of the outbreaks where the source is actually found. One sample in the present investigation was found to harbour more than one serogroup of *L. monocytogenes*. This shows that serotyping of several isolates from each food sample is important, especially if the food is involved in a human case of listeriosis.

The present investigation shows that gravad and smoked fish often contain *L monocytogenes*. After growth during storage, consumers might be exposed to considerable numbers of *L. monocytogenes*. As the products are consumed without further heating, they may constitute a potential health risk for susceptible individuals. Therefore, in a short-term perspective, it is essential to reduce the keeping-time and lower the storage temperature to 4°C or below, or alternatively, freezing. In a long-term perspective, the hazards and critical control points (HACCP) for production of gravad and smoked fish must be identified and processes that will improve the safety must be developed.

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Sammanfattning

Förekomst av Listeria monocytogenes och andra Listeria-arter i rökt och gravad lax

Etthundra femtio prover av rokt och gravad vacuumförpackad fisk undersöktes på förekomst av *Listeria*-species. *Listeria monocytogenes* isolerades från 12 av 58 prover av gravad fisk, 3 av 26 prover av kallrökt och ett av 66 prover av varmrökt fisk Tio av de 16 proven innehöll mer än 100 cfu/g av *L monocytogenes* Det hogsta antalet (132 000) hittades i ett prov av varmrökt regnbågslax. Serogrupp 1/2 var vanligast förekommande, följd av 4 och 3 Ett prov av gravad regnbågslax inneholl två olika serogrupper av *L monocytogenes L innocua* och *L seeligeri* isolerades från 12 respektive ett prov.

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