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Longitudinal studies on *Listeria monocytogenes* and other *Listeria* species in two salmon processing plants

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Abstract

Two plants processing salmon fillets and cold smoked salmon were investigated for occurrence of *Listeria* in products and the environment. Analyses were conducted for a period of 31 weeks. At plant A, 252 samples were examined of which 97 were from unprocessed fish and 155 from cold-smoked fish. At plant B, 189 samples of unprocessed fish were investigated. The first examination of unprocessed fish at plant A showed a presence of *L. monocytogenes* and *L.* spp. in 81% and 19% of the samples respectively. For cold-smoked fish the figures were 43% and 23%. At plant B, *L. monocytogenes* was isolated in 63% of the samples. During the test period, management at the processing plant initiated various hygiene precautions to improve the sanitary situation. The last batch of analyses of unprocessed fish at plant A showed a presence of *L. monocytogenes* and *L.* spp. in 42% and. 33% of the samples respectively. For cold-smoked fish, the figures were 6% and 11%. The isolation figures at plant B for *L. monocytogenes* and *L.* spp. were 50% and 17% respectively. The hygienic precautions did not have a significant effect on the presence of *L. monocytogenes* and *L.* spp. We suggest that *Listeria* bacteria are a part of the resident flora and are not eliminated by current cleaning and sanitation programmes. Cold-smoking, however, gave a significant reduction in the isolation of *L. monocytogenes* ($P = 0.0082$), while the isolation of *L.* spp. did not decrease after this process.

Keywords: *Listeria, salmon, processing plants, hygienic aspects*

Introduction

The interest in *Listeria monocytogenes* as a food-borne pathogen has increased in recent years. *L. monocytogenes* can cause listeriosis, a serious and often fatal disease to susceptible individuals (Meier and Lopez 2001) with a mortality rate of about 20% (Gellin and Broome 1989). Listeriosis has emerged as a food-borne illness in a series of outbreaks from contaminated milk, coleslaw and cheese (Ryser and Marth 1999). The psychrotropic nature of *L. monocytogenes* allows survival and growth during refrigerated storage. This is a special concern for products that do not receive heat treatment before consumption. *L. monocytogenes* and *L.* spp. have been isolated regularly from seafood since 1987 (Embarek 1994). Sporadic cases of listeriosis have occurred in seafood and the presence of the organism is of major concern in the fisheries sector (Ericsson et al. 1997). In Norway, 10–20 persons are infected

with *L. monocytogenes* yearly. Eighteen cases were reported in 2001 of which two of the patients died, while 17 cases were reported in 2002 and none of the patients died (MSIS 2001, 2002). The presence of *L. monocytogenes* in foods in Norway has previously been investigated. In smoked salmon, shrimps and ground fish the bacteria were isolated in 9%, 12%, and 18% of the samples respectively (Rørvik and Yndestad 1991). In a survey of smoked salmon, *L. monocytogenes* was found in 16% of the samples (Lunestad 1997). As this organism is ubiquitous and capable of growth at refrigeration temperatures, the zero-tolerance rules by FDA (Madden 1994) and the 100 cfu/g level tolerance in Europe (Commission of the European Communities 2000) issued in ready-to-eat foods presents a serious challenge to the food industry. Cold-smoked fish products, which are typically consumed without cooking, are foods of particular concern due to the lack of a heat inactivation step during processing. To prevent the occurrence of *L. monocytogenes* in smoked salmon it is important to identify the sources for the bacteria in the processing plants. The aim of this study was to determine the extent of *L. monocytogenes* in two salmon processing plants, to identify possible reservoirs, and contribute to the change of routines in the plants to achieve a higher level of food safety.

Material and methods

The sites for investigation chosen were locations where the risk of cross contamination and growth of *Listeria* was high. Six sites were chosen in plant A and five in plant B. A total of 441 samples were tested over a period of 31 weeks, 252 from plant A and 189 from plant B. Between 25 and 50 g were collected from each site twice daily. Some of the samples were small pieces of meat, while others were blended homogenous waste from several fishes.

A two-step enrichment method according to the Nordic Committee on Food Analysis (NMKL nr. 136, 2. ed. 1999) was used. Each sample was incubated for 24 h in 225 ml UVM1-broth (Oxoid CM 863 + suppl. SR 142E) at 30°C after which 0.1 ml of this broth was transferred to 10 ml Fraser-broth (Oxoid CM 895 + suppl. SR 156E), and incubated for 48 h at 37°C. Broth from tubes that turned black were streaked on Oxford-agar (Oxoid CM 856 + suppl. SR 206) and Palcam-agar (Oxoid CM 877 + suppl. SR150). The plates were incubated at 37°C and read after 24 and 48 h. Confirmation of positive samples were carried out with CAMP test according to the NMKL standard 136, 1990, and the chromogenic media Rapid L'Mono (BioRad # 3563694). Micro-ID® Listeria System (Remel #38370) was used for biochemical testing.

Simple linear regression was used for analyses of development of the detection through the 31-week period of sampling. For the calculation of positive samples on smoked *L. monocytogenes* a second degree equation was used. The Mann-Whitney *U*-test for unmatched samples was used for comparison of the different series before and after smoking. *P*-values less than 0.05 were considered significant.

Results

L. monocytogenes and *L.* spp. were isolated in 66% and 13% of the samples analysed before cold smoking in plant A (Table I). After cold smoking they were isolated in 15% and 16% respectively. In series 1, 2, 3, 4, 5 and 6, *L. monocytogenes* were isolated from 81%, 81%, 56%, 44%, 86% and 42% of the samples respectively (Figure 1). Comparable figures for *L.* spp. were 19%, 19%, 0% (not detected), 11%, 0% (not detected), and 33%. The results from each series of analyses in plant A after cold smoking are shown in Figure 2. *L. monocytogenes* were isolated from 43%, 17%, 5%, 7%, 3% and 6% of the samples and *L.* spp. were isolated from 23%, 10%, 5%, 26%, 14%, and 11% respectively. There was a significant reduction in the

Table I. Isolation of *Listeria monocytogenes* and *Listeria* spp. in plant A.

Sampling location	*No. of samples*	*No. of isolates*	
		L. monocytogenes *(%)*	L. *spp.(%)*
Unprocessed salmon (before cold smoking):			
Salting area	4	2 (50)	–
Waste from skinning machine in prod. plant	47	27 (57)	7 (15)
Waste from portioning machine in prod. plant	46	35 (76)	6 (13)
Total	97	64 (66)	13 (13)
After cold smoking			
Waste of skinning machine in packing plant	52	7 (14)	9 (17)
Waste of slicer in packing plant	52	12 (23)	4 (8)
Filleting in packing plant	51	4 (8)	12 (24)
Total	155	23 (15)	25 (16)
Total no. of samples in plant A	252	87 (35)	38 (15)

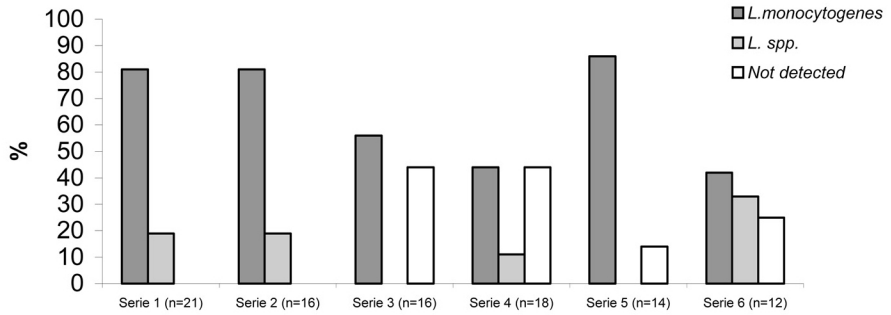

Figure 1. Analysis before cold smoking in plant A.

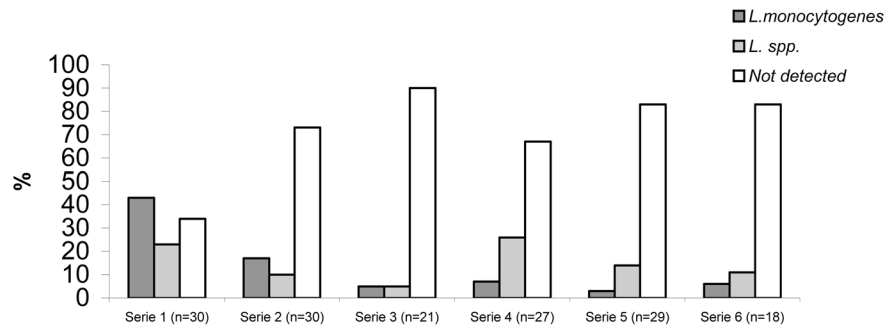

Figure 2. Analysis of salmon after cold smoking in plant A.

occurrence of *L. monocytogenes* after the smoking process ($P = 0.0082$), while the occurrence of *L.* spp. did not decrease after this process. The cleaning procedures implemented by factory A had a significant effect ($P = 0.031$) on the level of *L. monocytogenes* in cold smoked salmon through the period of testing, but had no significant effect on the unsmoked fish. *L.* spp. however was not affected by the measures, neither before nor after smoking.

L. monocytogenes and *L.* spp. were isolated from 33% and 11% of the samples in plant B (Table II). In this series *L. monocytogenes* were isolated from 63%, 56%, 17%, 26%, 23% and 50% of the samples and *L.* spp. from 0% (not detected), 0% (not detected), 11%, 5%, 30%, and 17% respectively (Figure 3). The hygienic precautions had no significant effect on either the level of *L. monocytogenes* or the level of *L.* spp. in these unprocessed samples.

Discussion

During the test period the processing plants implemented various hygiene precautions to improve the sanitary situation. The most important measures have been to maintain strict hygiene enforcement of the zones of the production area, to change all the bands on the machinery, improve personal hygiene and remove waste from the floor. Outside traffic to the factory was also reduced to a minimum.

Both plants were thoroughly cleaned with chlorine and disinfecting foam every day. In addition to these daily cleansing routines, the machinery was disassembled and disinfected before the sampling started. Conveyor belts were treated with chlorine and walls and floors were treated with disinfecting foam. Despite these measures, the first samples taken at plant A revealed the highest levels of contamination throughout the entire analysis period. In the unprocessed fish, *L. monocytogenes* and *L.* spp. were present in 81% and 19% of the samples respectively (Figure 1). This indicates that cleaning alone could not stop the contamination.

The first step in fish processing is washing and removal of the head. Analysis of the waste from this process in plant B revealed *L. monocytogenes* in 26% of the samples. This indicates

Table II. Isolation of *Listeria monocytogenes* and *Listeria* spp. in plant B.

Sampling location	*No. of samples*	*No. of isolates* L. monocytogenes *(%)*	L. *spp.(%)*
Unprocessed salmon:			
Cleaning	42	11 (26)	5 (12)
Baader 2000 (filèting)	45	7 (16)	6 (13)
Deboning	20	9 (45)	1 (5)
Sorting	44	19 (43)	4 (9)
Fine trimming	38	17 (45)	4 (11)
Total no. of samples in plant B	189	63 (33)	20 (11)

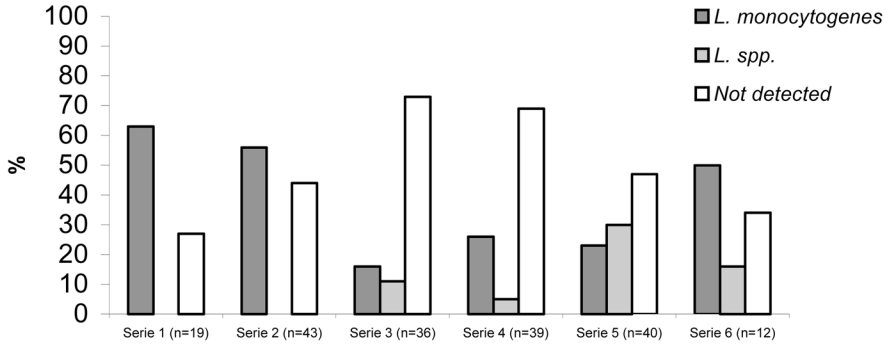

Figure 3. Analysis of salmon in plant B.

that *L. monocytogenes* is present in the fish before they enter the processing line. A study of salmon slaughterhouse plants has shown contamination on the surface of fresh fish (Rosef et al. 2002). As the fish is moved through the processing stages, *L. monocytogenes* is spread to the other production areas of the plant. This represents a secondary source of contamination (Eklund et al. 1995) – thus the fish are also most likely contaminated with the bacteria during slaughter or under transportation. The raw materials are therefore contaminated when entering the processing plants and recontaminated in the factory environment (Doyle 1988).

The persistence of *L. monocytogenes* in the processing plants during the test period suggests that these areas contain resident microflora that are not eliminated by current cleaning and sanitation programmes. Previous reports have shown that *L. monocytogenes* has the ability to adhere to surfaces and colonize the food processing environment (Krysinski et al. 1992; Herald and Zolotta 1998). Once these populations are established, they appear to become more resistant to cleaning and sanitation measures (Frank and Koffi 1990). The affected processing areas, therefore, can serve as primary sources of finished products contamination.

During the eight months of analyses the industry implemented a number of precautions aimed at reducing *L. monocytogenes* contamination. Both plants were investigated to identify areas of high risk where the bacteria might be established. The production procedures were improved to shield the product from both internal and external sources of contamination. Parts of the production equipment were changed or improved in such a way as to make disassembling and cleaning easier. These measures had a significant effect ($P = 0.014$) on the level of *L. monocytogenes* in cold smoked salmon throughout the period of testing (Figure 2), but had no significant effect on the unprocessed fish. *L.* spp. was not affected by the measures, neither before nor after smoking (Figures 1, 2 and 3).

Out of 58 *L.* spp. isolates found 15 were tested biochemically, and found to be *L. innocua*. This species has shown overgrowth in competition with *L. monocytogenes* in enrichment broths designed for the isolation of *L. monocytogenes*. This is believed to result from both a selective growth advantage of *L. innocua*, as well as the production of inhibitory compounds (Cornu et al. 2002). *Listeria* species have the ability to make biofilm and colonise the processing environment, and can survive this way for several years (Bremer et al. 2001; Djordjevic et al. 2002). If these interactions occur under the formation of biofilm, it is possible that after some time the biofilm will be dominated with *L. innocua*. If so, that may explain why the *L.* spp. isolates were more persistent during the various cleaning procedures done at the two plants. This, however, needs further investigation.

The reduction of *L. monocytogenes* that occurred during the cold-smoking process has been published earlier. One study concluded that this process does not generate enough heat to eliminate *Listeria*-organisms (Eklund et al. 1995). Another study reported that liquid smoke has an antimicrobial effect on *L. monocytogenes* (Messina et al. 1988). Guyer and Jemmi (1991) found that raw fish are more heavily contaminated with *L. monocytogenes* before smoking (28.6%) than on the finished product (6.3%). A temperature range of 17.1–21.1°C has been shown to eliminate *L. monocytogenes* in cold smoked salmon (Rørvik 2000). At a smoking temperature of 30.0°C, *L. monocytogenes* have a greater chance of surviving. The smoking process does not affect *L.* spp. in the same way (Rørvik 2000). The processing plants in this investigation utilize a smoking temperature of 18–19°C. During this study, samples were investigated before and after the smoking process. There was a significant reduction in the occurrence of *L. monocytogenes* after the smoking process ($P = 0.0082$), while the occurrence of *L.* spp. did not decrease after this process. From this study it appears that *L. innocua* is more resistant than *L. monocytogenes*. The zero-tolerance ruling issued by the US Food and Drug Administration (Madden 1994) and the level of 100 cfu/g. in Europe (Commission of the European Communities 2000) in ready-to-eat products present a major

challenge to the fish processing industry. It is necessary to develop and implement HACCP programmes to reduce the risk for contamination.

Conclusion

In spite of daily cleaning, the plants were regularly contaminated with *Listeria* bacteria. This indicates that cleaning alone cannot stop the spread of contamination. The plants implemented different measures to ensure that personnel in the production area maintained a high standard of cleanliness and utilized improved hygienic methods. These measures had no significant effect on the level of *L. monocytogenes* on unsmoked fish, but had a significant effect on the occurrence of *L. monocytogenes* in cold smoked salmon ($P = 0.014$). There was a difference in how *L. monocytogenes* and *L. spp.* reacted to the cold smoking process. *L. monocytogenes* was strongly affected ($P = 0.0082$), while the occurrence of *L. spp.* was confirmed before and after the process. This indicates that *L. monocytogenes* is more sensitive to the cold smoking process than other *Listeria* species. The persistence of *L. monocytogenes* in the processing plants during the test period suggests that it is part of the resident microflora and is not eliminated by current cleaning and sanitation programs. In that way the affected processing areas can serve as primary sources of finished product contamination. Research into factors promoting biofilm accumulation is needed to make further process development possible.

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