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SOURCES AND TRACKING OF *LISTERIA MONOCYTOGENES* IN A COLD-SMOKED PROCESSING PLANT

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KEY WORDS

Listeria monocytogenes; Smoked-salmon; PFGE;

ABSTRACT

The compliance of RTE foods with the safety criteria for *L. monocytogenes* in a cold-smoked salmon processing facility, as laid down in the Commission Regulation 2073/2005, was evaluated. The origin of *L. monocytogenes* was evaluated in the plant by tracing the bacterium along the production line by using the PFGE. The pathogen was isolated from in the raw materials, but none of the batches of semi-processed product was found positive. On the contrary, the pathogen was isolated from all the tested batches in the final product. The number of fish samples positive for *L. monocytogenes* clearly increased at the end of the manufacturing stage. The results of the enumeration of *L. monocytogenes* in the final product (0 days of storage), at 30 days of storage and at the end of the shelf-life, show that, none of the finished products were contaminated by the bacterium. Moreover, samples obtained from the environment revealed that the processing line was contaminated with the bacterium. PFGE with *AscI* and *ApaI* yielded respectively two and three restriction patterns. The same pulsotypes were isolated both from the fish and from the environment, suggesting that cross contamination occurred. An important factor in foodborne listeriosis is that the pathogen can grow to significant numbers at refrigeration temperatures when given sufficient time. The presence of *L. monocytogenes* in RTE foods is now regulated within the EU, which provides that *L. monocytogenes* should be legally below 100 cfu g−1 during the shelf life of such products (Regulation 2073/2005). The findings from this study indicate that there is need of a good control in the manufacture and retail of pre-packaged cold-smoked salmon as not all the samples examined complied with the legal food safety criteria for *L. monocytogenes*. Moreover, strict attention must be paid to cleaning and disinfection to control the level of *L. monocytogenes* and to avoid in-plant colonization by this pathogen.

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INTRODUCTION

Listeria monocytogenes is of major concern for public health authorities and the food industry, as the cold-tolerant organism is known to cause human infections and has been associated with a large number of foodborne outbreaks of disease (Farber and Peterkin, 1991, Jacquet et al., 1995, Dalton et al., 1997 and Miettinen et al., 1999). Implicated foods include milk products, vegetables, salads and meat products. Even though their implication in human listeriosis is often only suspected, a variety of seafoods have also been found contaminated with *L. monocytogenes*, particularly cold-smoked salmon and ready-to-eat seafoods (Ben Embarek et al., 1994).

Distribution, colonization and adaptation to various environments by *L. monocytogenes* is promoted not only by its tolerance of a wide range of temperatures (including refrigeration) and pH but also by its ability to form biofilms (Di Bonaventura et al., 2008; Lunden et al., 2000; Blackman and Frank, 1996). Moreover, the microorganism can survive the cold-smoking process and the smoked products are often consumed without further heating. Furthermore these products are mainly vacuum-packed, which ensures a long shelf life and potentially enables *L. monocytogenes* to grow during storage (Guyer and Jemmi, 1991).

All these properties together with the severity of human listeriosis make *L. monocytogenes* of particular concern for manufacturers of ready-to-eat (RTE) food products. The European Commission (EC) Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) concluded that the risk of listeriosis from foods containing less than 100 cfu g^{-1} is low (European Commission (EC), 1999). As a result, the EC Regulation on microbiological criteria for foodstuffs (Regulation (EC) 2073/2005), in force from January 2006, provides that *L. monocytogenes* should be below 100 cfu g^{-1} during the shelf life of RTE foods, and that processing areas and equipment used in the manufacture of RTE foods must also be monitored for *L. monocytogenes* (EC, 2005). However, for RTE foods intended for infants or special medical purposes, *L. monocytogenes* should not be present (absent in 25 g) throughout their shelf life (EC, 2005). Pre-packaged smoked-salmon have a high potential for contamination from *L. monocytogenes* especially due to cross contamination from the environment. The contamination of raw or processed food products by *L. monocytogenes* can have serious economic consequences for the food industry. In both in the EU and the USA, a rapid alert or refusal system is operating and *L. monocytogenes* has been the primary reason for rejection of chilled and frozen fish products. Contamination with the organism is of particular concern in cold-smoked fish because the heat applied during processing is not sufficient to inactivate the organism and the product is usually consumed without further cooking (Eklund et al., 1995).

For these reasons, prevention implies that the potential sources of contamination of smoked fish and the dissemination of *L. monocytogenes* in processing plants should be identified and traced. It may be impossible to control completely the presence of *L. monocytogenes* on the final product (Ben Embarek, 1994; Huss et al., 1995; Autio et al., 1999). However, as the contamination seems to take place during processing of salmon, it should be possible to reduce this contamination to a low

level by adherence to good manufacturing practices (Huss et al., 1995; Medrala et al., 2003).

To trace the transmission of *L. monocytogenes* in fish-processing environments, reliable typing systems should be employed. Classical typing based on phenotypic traits provides little information required for epidemiological and contamination studies and pulsed-field gel electrophoresis (PFGE) remains the current gold standard method for molecular subtyping of most foodborne bacteria, including *L. monocytogenes*.

The aims of this study were (i) to asses the compliance of RTE foods with the safety criteria for *L. monocytogenes* as laid down in the Commission Regulation 2073/2005 and (ii) to extend the knowledge about the origin of *Listeria monocytogenes* in a cold-smoked salmon processing facility by tracing the bacterium along the production line (PFGE).

MATERIALS AND METHODS

Product manufacture and the processing plant

The processing plant used ready-filleted salmon as raw material for production of cold-smoked salmon. Whole salmon were filleted, and all fillets were salted with dry salt, smoked (maximum temperature 25°C), skinned, sliced and vacuum-packed. All plants produced smoked herring as well. After each day's production, a cleanup crew carried out complete cleaning and sanitation procedures.

Sampling and microbiological analysis

During this study, in a plant that processes smoked salmon located in Central Italy, 3 visits were undertaken in 2008.

In 2008 one batch of product was sampled during each visit to the plant. For each batch, samples from raw material, semiprocessed product (after salting and smoking) and final product (sliced and vacuum packed) were collected. Moreover, the finished products of the same batch was also analysed at 30 days after packing and at the end of the shelf-life (60 days). The sampling procedure applied in this study was as described in the Regulation EC 2073/2005 (EC, 2005). In the same period, during the visits, environmental samples from the processing line and surroundings were collected.

For fish products, samples were analysed according to the method by UNI EN ISO 11290-1. In particular, 25 g of the sample were used as pre-enrichment in Half-Fraser broth (Oxoid, Milan, Italy) and incubated at 30°C for 24-48 hours. A loopful from black Fraser broth was streaked onto ALOA medium (Biolife, Mila, Italy) and incubated at 37°C for 24-48 hours. Suspected colonies, surrounded by the typical halo, were seeded onto TSA (Tryptone Soya Agar) (Oxoid) and incubated at 37°C for 24 hours. Finally *Listeria* isolates were identified to the species level using the API-*Listeria* system (BioMérieux, Mercy-l'Etoile, France). Isolates of *Listeria innocua* and *L. monocytogenes* were used as controls. Environmental samples were collected by using the "sponge-bag" system, after re-hydrating the sponges with 10 ml of Buffered Peptone Water (BPW) (Oxoid). To these samples, 90 ml of BPW

were added and the samples were treated accordingly to the UNI EN ISO 11290-1, as described before.

Pulsed Field Gel Electrophoresis (PFGE)

A subset of 12 *Listeria monocytogenes* strains were characterized by DNA macrorestriction with *Apa* I and *Asc* I (New England Biolabs, Beverly, MA, USA). The separation of the restriction fragments was carried out by PFGE in a CHEF Mapper XA system (Bio-Rad, Hercules, CA, USA), using the Pulse-Net protocol (Graves and Swaminanthan, 2001). A reference strain of *Listeria monocytogenes* (NCTC 10525) was used as positive control, while Lambda ladder PFG marker (New England Biolabs, Beverly, MA, USA) was used for fragment size determination. Gel images were visualized and acquired using the Gel-Doc UV trans-illuminator (Bio-Rad, Hercules, CA, USA). The banding patterns for each enzyme were assigned by visual analysis of the restriction endonuclease digestion profiles.

RESULTS AND DISCUSSION

L. monocytogenes was isolated from one batch in the raw materials. None of the batches of semi-processed product was positive for *L. monocytogenes*. On the contrary, the pathogen was isolated from all the tested batches in the final product. The number of fish samples positive for *L. monocytogenes* clearly increased at the end of the manufacturing stage. The results of the enumeration of *L. monocytogenes* in the final product (0 days of storage), at 30 days of storage and at the end of the shelf-life, were reported in table 1. It can be noted that, by using the enumeration protocol, *L. monocytogenes* was not found in any of the finished products, but by using the enrichment protocol, the bacterium was isolated in all the finished products.

Samples obtained from the production plant revealed that the processing line was contaminated with *L. monocytogenes*. Of the environmental samples it was isolated in 4 sampling points out of 24 points sampled (16.6%). The most contaminated sites were the table (stainless steel and polytetrafluorethylene) and the slicing machine.

PFGE with *AscI* yielded two restriction patterns, and PFGE with *ApaI* yielded three restriction patterns, for the isolated *L. monocytogenes* strains. By using *AscI*, the pulsotype VIII and IX were isolated both from the fish and from the environment, whereas using *ApaI* restriction enzyme, pulsotype VII and IX were associated with both the product and the environment, but in the last the pulsotype VIII was also isolated. Pulsotype VIII appeared sporadically in the environment and were not detected later in the finished product. These strains may be well adapted to the processing environment but may be bad competitors and not able to persist on the product (Dauphin et al., 2001). Despite the presence of *L. monocytogenes* in the processing environment of the smokehouses, this bacterium was never isolated from intermediate products. This indicates the probable antibacterial effect of the cold-smoking process (Gudmundsdóttir et al., 2005), but the presence of the pathogen in the final product and on the environment suggests that cross contamination occurred.

Conventional cleaning procedures did not eliminate *Listeria monocytogenes*

completely. Moreover, the incoming raw material were contaminated by the pathogen. Hence cross-contamination could easily occur throughout the plant and the pathogen can and persist in the environment following cleaning. This was demonstrated, as the same pulsotype was isolated from raw material, found on the table and on the slicing machine after cleaning, as well as on the final products. This highlights the need to readdress the design and cleaning of processing plants as well as staff behaviour and equipment in transit between production areas. Strict rules are needed to control *L. monocytogenes* contamination through the entire plant.

In their studies, Vogel et al. (2001) and Autio et al. (1999) indicate that the slicing and brining processes in cold-smoked salmon processing may provide reservoirs for some *L. monocytogenes*. According to Johansson et al. (1999) the most critical steps of the production line were salting and slicing, mainly because of difficulties with cleaning the equipment thoroughly. Several other studies have concluded that the plant equipment and the processing environment (in-house flora) rather than the raw material is the source of *L. monocytogenes* in product (Ojeniyi et al., 1996, Autio et al., 1999). However this does not exclude the possibility that the raw material may be an important, initial source for contaminating the processing equipment and environment (Vogel et al., 2001).

Although listeriosis is a relatively rare disease, the severity of the disease and the frequent involvement of manufactured foods, especially during outbreaks, means that the social and economic impact of listeriosis is among the highest of the foodborne diseases (Adak et al., 2002). An important factor in foodborne listeriosis is that the pathogen can grow to significant numbers at refrigeration temperatures when given sufficient time. The presence of *L. monocytogenes* in RTE foods is now regulated within the EU, which provides that *L. monocytogenes* should be legally below 100 cfu g^{-1} during the shelf life of such products (Regulation (EC) No. 2073/2005). The aim therefore is to keep *L. monocytogenes* levels to a minimum and eliminate the organism wherever possible.

In conclusion, the findings from this study indicate that there is need of a good control in the manufacture and retail of pre-packaged cold-smoked salmon as not all the samples examined complied with the legal food safety criteria for *L. monocytogenes*. Moreover, strict attention must be paid to cleaning and disinfection to control the level of *L. monocytogenes* and to avoid in-plant colonization by this pathogen.

	Days of storage		
	0	30	60
Batch A	Neg	Neg	3.7×10^2
Batch B	Neg	1×10^2	8 x 10
Batch C	Neg	1×10^2	Neg

Table 1: Enumeration of L. monocytogenes in the final product, at 30 days of storage and at the end of the shelf-life.

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