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Research Note

Raw and Processed Fish Show Identical Listeria monocytogenes Genotypes with Pulsed-Field Gel Electrophoresis

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ABSTRACT

A total of 257 raw fish samples at two different sites were examined for the presence of Listeria monocytogenes. The prevalence of L. monocytogenes was 4%. From 11 positive samples, nine different L. monocytogenes pulsed-field gel electrophoresis genotypes were recovered. From nine pulsortypes recovered from raw fish and 32 pulsortypes shown by 101 fish product isolates, two raw fish and fish product pulsortypes were indistinguishable from each other. Although the prevalence of L. monocytogenes in raw fish is low, the range of L. monocytogenes strains entering the processing plant in large amounts of raw material is wide. This indicates that the raw material is an important initial contamination source of L. monocytogenes in fish processing plants. This postulation is supported by the identical pulsortypes recovered from both raw and processed fish. Some L. monocytogenes strains entering a plant may thus contaminate and persist in the processing environment, causing recurrent contamination of the final products via processing machines.

Ready-to-eat fish products, among many other food items, are vehicles of listeriosis, a life-threatening disease caused by Listeria monocytogenes. Gravad and cold-smoked rainbow trout have been linked to the listeriosis epidemics in 1994 and 1999, respectively (9, 22). Although the vehicles of most sporadic listeriosis cases remain unknown, fish products have occasionally been linked to an illness of one or two persons (10, 23). Reported human listeriosis cases are not the only evidence of fish being an important source of listeriosis. In a Finnish retrospective study, a L. monocytogenes type recovered from several sporadic listeriosis cases during an 8-year follow-up (19) turned out to be identical to an epidemic strain that originated in fish (22).

L. monocytogenes has been isolated from several fish products (5, 7, 11, 17, 18, 20, 27). The contamination rate of fish rises sharply during processing, particularly during brining and slicing (2, 8, 27). The source of product contamination is thought to be the processing environment (2, 8, 12, 13, 27).

L. monocytogenes contamination is believed to enter the processing plant from multiple sources (2, 4, 13, 27). The roles of, for example, raw material, personnel, transport vehicles, and air-mediated contamination have been discussed. Even though Rörvik et al. (27), Auto et al. (2), and Hoffman et al. (13) concluded that raw material is not a main source of the contamination of final products, in several studies the possibility that the raw product is one of the sources of L. monocytogenes in a processing plant has not been excluded (2, 12, 13). The purpose of this study was to clarify the role of raw fish as an initial source of fish processing plant and product contamination by L. monocytogenes by showing identical genotypes in raw fish and fish product isolates using pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Sampling. A total of 257 raw rainbow trout (Oncorhynchus mykiss) samples were examined between 1998 and 2001. Fish were farmed in brackish water, open sea farms on the west coast of Finland. One sample contained slime, skin, gills, and fins from one to five fish heads. Heads were picked at two different sites (Table 1). Heads were examined fresh or after freezing at −20°C (Table 2). Fresh heads were stored in ice until the examination, which was conducted within 4 h of sampling. Frozen heads were examined immediately after melting to room temperature.

Bacterial strains. All 41 raw fish isolates of L. monocytogenes (19 isolates from fresh samples and 22 isolates from frozen samples) were recovered during this study. A total of 101 isolates recovered from the fish products (Table 3) of 21 monidenti-}

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TABLE 1. Prevalence of Listeria monocytogenes in raw fish sampled at two different sites

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>No. of samples</th>
<th>No. (%) of L. monocytogenes-positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughterhouse, during slaughtering</td>
<td>45</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Processing factory, before processing</td>
<td>212</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>257</td>
<td>11 (4)</td>
</tr>
</tbody>
</table>

International Organization for Standardization (1). Samples were spread from Fraser enrichment broth (Oxoid) to PALCAM (Oxoid) and Oxford (Oxoid) agars. In addition, L. monocytogenes blood agar (15) (Trypticase soy agar base [Difco, Becton Dickinson, Sparks, Md.], 10 g/liter of lithium chloride [Merk KgaA, Darmstadt, Germany], 10 mg/liter of polymyxine B sulfate [Sigma, St. Louis, Mo.], 20 mg/liter of cetazidime [Abtek Biologicals Ltd., Liverpool, UK]. and 5% sterile defibrinated sheep blood) was used. Five typical colonies from each selective plate were identified by hemolytic activity, Gram staining, catalase reaction, and an API Listeria kit (bioMérieux, Inc., Marcy l'Etoile, France).

In situ DNA isolation and PFGE. In situ DNA isolation and PFGE typing were performed as described by Autio et al. (3). Restriction enzymes Ascl (New England Biolabs, Beverly, Mass.) and Apal (Boehringer Mannheim, Mannheim, Germany) were used for the digestion of DNA.

PFGE pattern analysis. The numerical analysis of macro-restriction patterns (MRPs) and clustering was performed using commercial BioNumerics software version 2.5 (Applied Maths, Kortrijk, Belgium). For each isolate, fragments yielded by the Apal restriction enzyme that were bigger than 48.5 kb were included in the analysis. The similarity between restriction patterns, based on band position, was expressed with Dice coefficient correlation. Position tolerance was optimal when set at 1.0% for the total length of both Apal and Ascl patterns with no increase. The clustering and construction of dendrograms were performed by the unweighted pair-group method with arithmetic averages.

Serotyping. One to two representative isolates from each pulsortype were serotyped with the commercial Listeria antisera (Denka Seiken, Tokyo, Japan) as described by the manufacturer. All isolates representing an identical pulsortype were interpreted as belonging to the same serotype.

RESULTS

The prevalence of L. monocytogenes in raw fish was 4% (11 of 257). No statistically significant differences were present in the prevalence of L. monocytogenes-positive fish samples between the two sampling sites (Table 1) or between fresh and frozen samples (Table 2) (χ², P > 0.05).

Serotyping divided the 41 raw fish isolates and the 101 fish product isolates similarly into six serotypes, except for serotype 3a, which was more prevalent in raw fish (Table 4) (χ², P < 0.05). The most prevalent serotype, 1/2a, represented 73% of all of the L. monocytogenes isolates.

From 41 L. monocytogenes raw fish isolates of 11 positive samples, PFGE with restriction endonucleases Ascl and Apal yielded nine MRPs apiece, dividing the isolates into nine pulsortypes (Fig. 1). From one sample, two different pulsortypes were recovered (pulsotypes 85 and 86). The same pulsortype was recovered from four samples (pulsotype 81). From 101 fish product isolates, Ascl and Apal yielded 32 and 23 MRPs, respectively, resulting in 32 different pulsortypes (Fig. 1).

Two raw fish pulsortypes were indistinguishable from fish product pulsortypes (Fig. 1). Pulsotype 32 was recovered from gravad fish produced by two different producers in 1996 and 1998 and from raw fish in 2001. Pulsotype 77

TABLE 2. Prevalence of Listeria monocytogenes in fresh and frozen raw fish

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No. of samples</th>
<th>No. (%) of L. monocytogenes-positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>140</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Frozen</td>
<td>117</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>257</td>
<td>11 (4)</td>
</tr>
</tbody>
</table>

TABLE 3. Number of Listeria monocytogenes isolates originating from different fish products

<table>
<thead>
<tr>
<th>Fish product</th>
<th>No. of products</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow trout</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Oncorhynchus mykiss)</td>
<td>19 (23)</td>
<td>19 (23)</td>
</tr>
<tr>
<td>Cold smoked</td>
<td>14 (14)</td>
<td>14 (14)</td>
</tr>
<tr>
<td>Gravad</td>
<td>11 (11)</td>
<td>11 (11)</td>
</tr>
<tr>
<td>Gravad</td>
<td>25 (26)</td>
<td>26 (26)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (7)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Other fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravad</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Other</td>
<td>12 (16)</td>
<td>16 (16)</td>
</tr>
<tr>
<td>Roe product</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>92 (101)</td>
<td>101 (101)</td>
</tr>
</tbody>
</table>

a Other fish samples include seven whitefish (Coregonus albula), three salmon (Salmo salar), two squid (Colyoidea), one herring (Clupea harengus), and one coalfish (Pollachius virens).

TABLE 4. Distribution of Listeria monocytogenes isolates recovered from raw and processed fish according to serotype

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Raw fish</th>
<th>Processed fish</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2a</td>
<td>28 (68)</td>
<td>16 (75)</td>
<td>44 (73)</td>
</tr>
<tr>
<td>4b</td>
<td>7 (17)</td>
<td>12 (12)</td>
<td>19 (13)</td>
</tr>
<tr>
<td>3a</td>
<td>6 (15)</td>
<td>3 (3)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>1/2c</td>
<td>0 (0)</td>
<td>5 (5)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>4c</td>
<td>0 (0)</td>
<td>4 (4)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>1/2b</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Total no. of isolates</td>
<td>41 (100)</td>
<td>101 (100)</td>
<td>142 (100)</td>
</tr>
</tbody>
</table>
was also recovered from raw fish in 2001. In the same year, an identical pulotype was found in one gravad fish sample.

**DISCUSSION**

The prevalence of *L. monocytogenes* in raw fish was 4%. In previous studies, the prevalence of *L. monocytogenes* has varied from 0 to 50% (2, 5, 8, 12, 13, 24, 25, 27, 28), with an average overall prevalence of 9% (190 of 2,073). The differences in the prevalence of *L. monocytogenes* in raw fish may at least be partly due to water quality (5). Moreover, slaughtered raw fish from certain slaughterhouses may frequently be contaminated with *L. monocytogenes* because of contamination during slaughtering (26).

The most prevalent serotype in both raw and processed fish was 1/2a. In a previous study, the most prevalent serotype in raw fish was 4b (14). Serotype 1/2a has predominated in fish products (14, 16) and in fish processing plants (6, 16).

A total of nine different pulatypes were recovered from 11 *L. monocytogenes*-positive raw fish samples, indicating high genetic diversity of the bacteria in raw fish. Although the prevalence of *L. monocytogenes* in raw fish is low, the range of *L. monocytogenes* strains entering the processing plant with large amounts of raw material is wide. This supports our hypothesis that the raw material is a source of *L. monocytogenes* contamination in fish processing plants. Further support is found in the same pulatypes being recovered from both raw fish and fish product isolates of *L. monocytogenes* (Fig. 1). An explanation for the identical pulatypes may be that the strain originating from raw fish has entered and persisted in a processing plant, contaminating the final products via the processing environment. Alternatively, the *L. monocytogenes* strain in raw fish may have survived a nonsterilical process, resulting in contamination of the final product. In both cases, the initial source of the contamination of the final product...
is the raw material. Our findings are in accord with those of Eklund et al. (8), Norton et al. (25), and Fonnesbech Vogel et al. (12). Eklund et al. (8) reported raw fish as the source of L. monocytogenes contamination in a processing plant. However, they based their conclusion solely on prevalence studies, with no further typing of L. monocytogenes isolates being done. Fonnesbech Vogel et al. (12) found the same L. monocytogenes random amplified polymorphic DNA type from raw fish, the processing environment, and final products sampled at a smokehouse on several occasions. Norton et al. (25) concluded that both raw fish and the processing environment serve as potential sources of final product contamination. The most probable source sometimes being raw material, and other times, the processing environment. Rørvik et al. (27) and Autio et al. (2) reported that raw fish is not a source of final product contamination, concluding the main source to be the processing environment. Autio et al. (2) did not, however, exclude the possibility that the initial source of plant contamination is the raw material. Strains recovered from samples during a single sampling period may not necessarily represent all of the strains present in the plant (21). Moreover, L. monocytogenes strains have been suggested to be able to persist in the fish processing environment for years (2, 9, 12, 13, 25). We therefore conclude that raw material is an important initial source of L. monocytogenes in fish processing plants. Certain L. monocytogenes raw fish strains entering plants may contaminate the processing environment and persist there, causing recurrent contamination of the final products via processing machines.

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REFERENCES


