An Outbreak of Listeriosis Suspected To Have Been Caused by Rainbow Trout

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An outbreak of listeriosis in Sweden, consisting of nine cases, was investigated by means of molecular typing of strains from patients and strains isolated from suspected foodstuffs, together with interviews of the patients. Listeria monocytogenes was isolated from six of the patients, and all isolates were of the same clonal type. This clonal type was also isolated from a “gravad” rainbow trout, made by producer Y, found in the refrigerator of one of the patients. Unopened packages obtained from producer Y were also found to contain the same clonal type of L. monocytogenes. Based on the interview results and the molecular typing, we suspect that at least six of the nine cases were caused by gravad or cold-smoked rainbow trout made by producer Y. To our knowledge, this is the first rainbow trout-borne outbreak of listeriosis ever reported.

Cold-smoked and “gravad” rainbow trout (Oncorhynchus mykiss) and salmon (Salmo salar) have been focused on during recent years as potential sources of infection for Listeria monocytogenes. Investigations have shown that up to 10% of retail vacuum-packaged products contain L. monocytogenes (6, 8). However, as far as we know, such foods have never been connected with cases of human listeriosis. Vehicles often associated with sporadic cases and outbreaks of listeriosis have been soft and semisoft cheeses and other milk products (for a review, see reference 4).

Recently, there was a cluster of listeriosis cases in the province of Värmland in Sweden (2). The incidence of listeriosis in this region is usually about one case a year, whereas during the period August 1994 to June 1995, nine people fell ill with listeriosis. Gravad and cold-smoked rainbow trout and salmon are popular dishes in this province. Gravad rainbow trout and salmon are made from raw fillets that are rubbed with salt, or the cure is injected with multiple needles under vacuum in oxygen-impermeable film. Cold-smoked rainbow trout and salmon are made from raw fillets that are rubbed with a mixture of sugar, salt, and pepper, 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 in oxygen-impermeable film. Cold-smoked rainbow trout and salmon are made from raw fillets that are rubbed with salt, or the cure is injected with multiple needles into the fillets. Thereafter, the fish is smoked at 25 to 30°C for 2 to 3 h and then packaged sliced or whole under vacuum. The NaCl concentration in the fish after curing and smoking is approximately 2.5 to 3.5% (8). Cold-smoked and gravad rainbow trout and salmon are stored for 3 to 6 weeks after packing. Could such products have been vehicles for the listeria bacteria? We decided to try to identify the source or sources of infection for the nine cases by means of patient interviews, bacteriological investigations of suspected foods, and characterization of all L. monocytogenes strains isolated.

*MATERIALS AND METHODS

Patients. L. monocytogenes strains isolated from blood or cerebrospinal fluid of nine hospitalized patients were obtained from local hospitals. The strains were further characterized by means of serotyping, phage typing, and restriction enzyme analysis (REA) with pulsed-field gel electrophoresis. Data about the patients are presented in Table 1. Eight of the patients (one had died) were interviewed by the local health authorities using a standard questionnaire with special attention to their eating habits. Three of the patients still had various items of food from the period before their illness in their home refrigerators. All these foods were collected and analyzed for L. monocytogenes.

Food. A total of 29 food samples, 8 from the three patients’ refrigerators, 1 from a private water well, 16 bought in local shops, 3 obtained directly from producer Y (see Results), and 1 from a fish truck (Table 2), were included in the study. The methods of analysis used for detection of L. monocytogenes were an enrichment method and an enumeration procedure.

The enrichment was done according to the International Dairy Federation standard (5) with slight modification. Twenty-five grams of food was mixed with 225 ml of enrichment broth supplemented with antibiotics for the selective growth of L. monocytogenes. The mixture was incubated for 48 h at 30°C and 0.1 ml was streaked onto Listeria selective medium, Oxford formulation (agar base CM 856 and supplement SR 140; Oxoid) and incubated at 37°C for another 48 h. From each of the 11 food samples found to be positive, one to five presumptive Listeria colonies were saved for confirmation and typing.

The 11 food samples found to be positive for Listeria were submitted to an enumeration procedure according to the method of Danielsson-Tham et al. (1). Ten grams of each food sample was mixed with 90 ml of sterile peptone water. Tenfold serial dilutions were done, and 0.1 ml of each dilution of the mixture was streaked onto Listeria selective medium plates and incubated at 37°C for 48 h. From each of the three samples which yielded detectable levels (≥100 CFU/g) of presumptive Listeria, 9 to 12 colonies were saved for confirmation and typing.

All collected isolates from both procedures were subjected to confirmation according to the method of Seeliger and Jones (11). One L. monocytogenes isolate from each of the 11 food samples found to be positive on enrichment and one L. monocytogenes isolate from each of the 3 food samples also found to be positive on enumeration (altogether 14 isolates) were serotyped and phage typed according to reference methods (9, 10) and analyzed by REA with three different enzymes. When a food sample yielded more than one isolate, the remaining isolates (2 to 12) were characterized by REA with only the restriction enzyme Apal. This limited characterization was done as an economy measure. If the Apal profiles obtained differed from the first isolate, complete typing of all isolates from the food sample was performed, i.e., serotyping and phage typing as well as REA by use of the three enzymes Apal, SmaI, and AscI. However, the strains isolated from food sample 1 (Table 2) were all analyzed with both Apal and Smal, since Apal alone could not discriminate between groups A and E (see Results).

REA. REA with Apal and SmaI (Boehringer Mannheim) was performed as described by Ericsson et al. (3). In the present study we also used the restriction enzyme AscI (New England Biolabs) as recommended by the manufacturer, using 5.0 U for each half gel plug. The running conditions were the same as those for Apal (Fig. 1).
RESULTS

The investigated foodstuffs and the results of the Listeria analyses are listed in Table 2. L. monocytogenes was isolated from samples of gravad rainbow trout found in the refrigerators of two patients, 5 and 6. The numbers of L. monocytogenes were, 100 CFU/g in the fish from patient 5 (food sample 1) and 6,200 CFU/g in the fish from patient 6 (food sample 5). The gravad rainbow trout from patient 5 was brand X, and that from patient 6 was brand Y. Altogether, 57 isolates of L. monocytogenes from 11 food samples were included, 26 isolates from the enrichment procedure and 31 isolates from the enumeration procedure.

The strains serotyped from foods all belonged to serovar 4b. L. monocytogenes strains isolated from the patients also belonged to serovar 4b. Based on phage typing and REA, all strains could be divided into five clonal types, A to E (Tables 1, 2, and 3). The predominant clonal type among the patients was B, isolated from patients 2 to 7. Clonal type B was also shared by the L. monocytogenes strains isolated from the gravad rainbow trout found in the refrigerator of patient 6. To exclude the possibility that patient 6 himself had contaminated the rainbow trout, unopened packages of brand Y rainbow trout were purchased from local dealers. In order to get a representative sample of brand Y, the sampling period was extended to several months. Five unopened packages from producer Y (food samples 6, 18, 19, 20, and 25 [Table 2]) of gravad and cold-smoked rainbow trout harbored clonal type B strains. The levels were <100 CFU/g in three of the fish and 120 and 2.5 million CFU/g in the other two fish. The last sample had been sent by mail on a Thursday and did not reach the laboratory until the following Monday, thus making it difficult to know the initial level. The time taken for the other food samples to reach the laboratory was 1 to 2 days.

In addition, we obtained material from the production plant of producer Y: rainbow trout residues from the packing machine and one gravad and one cold-smoked rainbow trout (food samples 27, 28, and 29), all of which contained L. monocytogenes clonal type B organisms. A local health authority laboratory supplied us with a strain (SLU 2268) from a gravad rainbow trout processed at plant Y as early as February 1994. This strain was also of clonal type B.

Patient 5 had L. monocytogenes-contaminated cold-smoked salmon from producer X in his refrigerator. However, these strains were of clonal type E whereas the patient’s strain was of clonal type B. Patient 8 also had cold-smoked salmon (from producer Z) in his refrigerator, but no L. monocytogenes could be detected in the salmon.

The interviews performed by the local health authorities showed that all patients interviewed had been consuming gravad, cold-smoked, or hot-smoked rainbow trout or salmon during the previous 6 months. Two patients could confirm that the rainbow trout and salmon were manufactured by producer Y, three patients said it might have been producer Y, and three were unable to identify which brand they had consumed.

![REA profiles of L. monocytogenes produced by cleavage of DNA with ApaI, AscI, and Smal. Lanes a, f, g, l, m, and s, lambda cI857 Sam7 concatemers; lanes b, h, and n, ApaI profile I, AscI profile I, and Smal profile I, respectively, of clonal type A, strain SLU 2152; lanes c, i, and o, ApaI profile II, AscI profile II, and Smal profile II, respectively, of clonal type B, strain SLU 2157; lanes d, j, and p, ApaI profile III, AscI profile III, and Smal profile III, respectively, of clonal type C, strain SLU 2312; lanes e, k, and q, ApaI profile IV, AscI profile IV, and Smal profile IV, respectively, of clonal type D, strain SLU 2330; and lane r, Smal profile V of clonal type E, strain SLU 2173.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>Predisposing condition</th>
<th>Outcome</th>
<th>Clonal type of isolated strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Meningitis</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>Recovered</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>Mother Infant</td>
<td>Amniotitis</td>
<td>Pregnancy</td>
<td>Recovered</td>
</tr>
<tr>
<td>3</td>
<td>Mother Infant</td>
<td>Fever, premature labor</td>
<td>Pregnancy</td>
<td>Recovered</td>
</tr>
<tr>
<td>4</td>
<td>Septicemia</td>
<td>Chronic lymphatic leukemia, age 76</td>
<td>Recovered</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>Septicemia</td>
<td>Age 78</td>
<td>Recovered</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>Septic arthritis, septicemia</td>
<td>Age 89, rheumatoid arthritis</td>
<td>Recovered</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>Meningitis</td>
<td>Age 78</td>
<td>Died</td>
<td>B</td>
</tr>
<tr>
<td>8</td>
<td>Meningitis</td>
<td>Age 70, diabetes</td>
<td>Recovered</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>Mother Infant</td>
<td>Septicemia</td>
<td>Pregnancy</td>
<td>Recovered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died</td>
</tr>
</tbody>
</table>
DISCUSSION

All patients interviewed had been eating rainbow trout or salmon, but not all of them could remember the brand. This is not surprising, since in some cases the interviews were done several months after the onset of the disease. However, it is not unlikely that the rainbow trout were brand Y, since the production plant of producer Y is situated in the affected area and the products are sold in local shops and restaurants.

It could be hypothesized that the epidemic clonal type B strains have become established in the packing machine of producer Y and that this domestic flora might have intermittently contaminated the products packaged. This assumption is based on the following facts: (i) a clonal type B strain was isolated from residues found in the packing machine and (ii) it is difficult to efficiently clean such a machine (i.e., residues containing L. monocytogenes may be permanently present).

Strain SLU 2268, obtained from a local health authority laboratory 6 months before the outbreak started, was isolated from a gravid rainbow trout processed by producer Y. This strain shared the features of clonal type B, indicating that this strain has been part of the resident flora for a long time.

The rainbow trout that was left at the post office over the weekend (food sample 6), harboring 2.5 million CFU of L. monocytogenes/g, shows that such fish products are excellent growth media for L. monocytogenes and that it is important that the products be kept well refrigerated. Unfortunately, this is not always the case in retail stores.

In one rainbow trout, food sample 6, two different clonal types of L. monocytogenes were identified: clonal type B and another corresponding to the clonal type isolated from patient 9 (clonal type D). This shows the need to analyze more than one isolate from a suspected foodstuff in order to eliminate the risk of false negatives. This observation is supported by a study by Loncarevic et al. (7), who found different clones when analyzing soft cheeses and rainbow trout and salmon. In that study, the quantification procedure revealed more clones than the enrichment procedure. The characterization methods used were serotyping and REA with the restriction enzymes ApaI and Smal. In the present study, however, the enrichment procedure produced two types while the enumeration procedure yielded only one type. In contrast, only one isolate of L. monocytogenes was investigated from each patient, since this is the common procedure when isolating L. monocytogenes in clinical cases.

It should be stressed that a human strain of L. monocytogenes may be permanently present. This is why it is important to isolate more than one isolate from each suspected foodstuff.

<table>
<thead>
<tr>
<th>Food sample</th>
<th>Type of food</th>
<th>Origin</th>
<th>Result (no.) for isolate from:</th>
<th>Clonal type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cold-smoked rainbow trout, producer X</td>
<td>Refrigerator of patient 5</td>
<td>Positive (5)</td>
<td>E&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Lamb chops, raw</td>
<td>Refrigerator of patient 5</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Water</td>
<td>Private well of patient 6</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cognacwürst</td>
<td>Refrigerator of patient 6</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Refrigerator of patient 6</td>
<td>Positive (5)</td>
<td>B&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Positive (6)</td>
<td>D&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Smoked rainbow trout, producer X</td>
<td>Fish truck</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Cold-smoked rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Hot-smoked rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Slicing waste, cold smoked, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Hot-smoked fjordlax, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Cold-smoked rainbow trout, producer Z</td>
<td>Refrigerator of patient 8</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Fried herring</td>
<td>Refrigerator of patient 8</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Sliced smoke-cured loin of pork</td>
<td>Refrigerator of patient 8</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Sausage</td>
<td>Refrigerator of patient 8</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Positive (1)</td>
<td>C&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Positive (1)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>19</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Positive (1)</td>
<td>100 (12)</td>
</tr>
<tr>
<td>20</td>
<td>Cold-smoked rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Positive (1)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>21</td>
<td>Cold-smoked rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Gravad rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Cold-smoked rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Cold-smoked rainbow trout, producer Y</td>
<td>Local grocery store</td>
<td>Positive (1)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>26</td>
<td>Cold-smoked rainbow trout</td>
<td>Local grocery store</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Fish residues, packing machine</td>
<td>Producer Y</td>
<td>Positive (2)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>28</td>
<td>Gravad rainbow trout</td>
<td>Producer Y</td>
<td>Positive (1)</td>
<td>&lt;100</td>
</tr>
<tr>
<td>29</td>
<td>Cold-smoked rainbow trout</td>
<td>Producer Y</td>
<td>Positive (2)</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

<sup>a</sup> All rainbow trout had been packaged under vacuum.

<sup>b</sup> Enrichment procedure.

<sup>c</sup> Enumeration procedure.
genes of clonal type B has only once previously been identified in Sweden by us. This strain was isolated in 1977 from a patient suffering from listeriosis. No food strain has been identified before as sharing the characteristics of clonal type B. Never before has \textit{L. monocytogenes} of clonal type D been recognized in Sweden by us, among either human or food strains. The clonal types A, C, and E, however, all belong to phagovar 2389:2425:3274:2671:47:108:340, which is a common feature in Swedish isolates from humans but rare in food isolates.

Based on the findings in this study, we suspect that at least six, and possibly eight, of nine cases of human listeriosis, all of which occurred during 1 year in a single province of Sweden, were caused by consumption of rainbow trout produced by plant Y. \textit{L. monocytogenes} clonal type B was isolated from six patients and was also the predominant clonal type found in products from the fish plant of producer Y. The three other patients were each infected by a different clonal type: A, C, or D. \textit{L. monocytogenes} clonal types C and D were also isolated from rainbow trout from producer Y.

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REFERENCES