The Incidence of Listeria spp., Salmonella spp., and Clostridium botulinum in Smoked Fish and Shellfish

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ABSTRACT

The frequency of occurrence of Listeria spp., Salmonella spp., and Clostridium botulinum in samples of smoked finfish and smoked shellfish was analyzed over a 5-year period. Listeria monocytogenes was isolated from 14% of 1,080 samples. For those samples where the smoke process was known, the incidence of L. monocytogenes was higher in cold-smoked than hot-smoked products (51 of 240 cold-smoked compared to 19 of 215 hot-smoked products). Listeria species other than L. monocytogenes were also detected (in 7.2% of cold-smoked and 3.8% of hot-smoked products). The time and temperature smoke processing guidelines are reviewed for a few state authorities. L. monocytogenes was isolated from 15.2% of the 559 samples of foreign origin. There were four countries for which more than 70 samples were analyzed: Canada, Norway, the Philippines, and the United Kingdom. The occurrence of L. monocytogenes in samples from these four countries was 14.3%, 23.7%, 0%, and 16.1%, respectively. The 521 samples originating in the United States were processed by 194 plants. Thirty-seven plants in 13 states produced contaminated product. Salmonella species were isolated from 5 (3.2%) of 156 samples tested for this organism. All positive samples were of foreign origin (4 from the Philippines and 1 from the United Kingdom). No C. botulinum spores were detected in any of the 201 vacuum-packed samples tested for this organism.

Smoked fish and shellfish products can be a source of microbial hazards including Listeria monocytogenes, Salmonella spp., and Clostridium botulinum. L. monocytogenes has been identified in several foodborne outbreaks, in which pasteurized milk (19), coleslaw (46), and soft cheese (29) were implicated. This organism has also been isolated from a variety of fish and shellfish products (5, 11, 13, 50). An epidemic of perinatal listeriosis in New Zealand suggested a link to the consumption of raw fish and shellfish (34). Some sporadic cases have been identified involving undercooked fish and mussels (4, 16). Often, however, the source of Listeria outbreaks is never established.

L. monocytogenes is ubiquitous in nature and able to grow at low temperatures and in high salt concentrations up to 10% (36). Studies have shown that L. monocytogenes can significantly increase in numbers on smoked salmon during storage at 4°C (22, 45). Enhanced virulence of L. monocytogenes has been associated with growth at 4°C (10). Smoked fish are often packed under vacuum and stored for 3 to 4 weeks under refrigeration. These ready-to-eat food items are potentially high-risk foods. Regulatory agencies in the United States have adopted a zero-tolerance policy toward this organism in ready-to-eat food products.

Foods commonly involved in transmission of Salmonella spp. include eggs, meat and meat products, and milk. Salmonella food poisoning is only occasionally associated with fish other than shellfish (49). Contamination of shellfish from sewage-polluted waters continues to be a problem. Processed seafood products are usually considered to present a lower risk for infections attributed to Salmonella species, but outbreaks have occurred. Salmonella paratyphi B infections were associated with consumption of smoked halibut in Germany (33) and a fish-and-chip shop in the United Kingdom (21). Salmon was implicated as the source of infection for Salmonella montevideo food poisoning involving two catered social functions in the United Kingdom (6).

Foodborne C. botulinum intoxication is caused by the ingestion of preformed neurotoxin produced by the organism. The species is divided into seven types, A through G, based on the antigenic specificity of their toxins. C. botulinum type E is the most prevalent type in marine and freshwater environments and has been responsible for most of the botulism outbreaks from fishery products (14, 48). Outbreaks have usually been due to ethnic preparations of fish involving fermentation or salting, often without removal of viscera (48). Kapchunak, an uneviscerated salted air-dried whitefish, was responsible for a type E botulism outbreak in New York City in 1985 and an international outbreak in 1987 in New York City and Israel (7, 8, 47). Fishborne botulism is common in Alaska and Canada in Native American communities, with a seasonal peak from May to October (23). In 1982, a can defect in Alaskan canned salmon was the cause of illness and one death in Belgium from C. botulinum type E toxin (24).

This study examines the frequency of Listeria spp., Salmonella spp., and C. botulinum isolation from smoked finfish and shellfish products. The analyses were conducted over a 5-year period, 1991 to 1995.

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MATERIALS AND METHODS

Smoked fish and shellfish samples. Samples of smoked finfish and shellfish harvested and processed in the United States were obtained from local fish processors or distributors. Smoked fish and shellfish of foreign manufacturers were collected at the consignee of the shipment. In most cases at least 10 units of product (packages or whole fish) were collected from each lot.

Listeria species. The FDA protocol for listeria isolation (26, 37) was used to analyze smoked fish products for Listeria species. Briefly, a 25-g portion was added to 225 ml of enrichment broth, tryptic soy broth-yeast extract with acriflavin, nalidixic acid, and cycloheximide. Broth was streaked onto Oxford agar and lithium chloride-phenylethanol-moxalactam agar after incubation for 24 and 48 hours at 30°C. Typical colonies were characterized biochemically and serotyped when necessary for botulinum toxin.

Salmonella species. The conventional FDA culture method for Salmonella spp. was used (1). A 25-g portion of product was blended with 225 ml lactose broth for preenrichment followed by selective enrichment in selenite cystine broth and tetrathionate broth. Broths were streaked onto bismuth sulfite agar, xylose lysine deoxycholate agar, and Hekton enteric agar. Typical colonies were screened biochemically and serotyped when necessary with commercial or CDC antisera (Difco Laboratories, Detroit, MI; Centers for Disease Control, Atlanta, GA).

Clostridium botulinum. Vacuum-packed smoked fish samples were tested for C. botulinum type E spores using the method of Kautter et al. (31, 32). A 50- to 100-g portion (depending on the size of the product units) was placed in a sterile Stomacher bag or sterile Whirl Pak bag. The product was covered with freshly steamed and cooled Trypticase-peptone-glucose-yeast extract broth. Bags were sealed after expelling air and incubated at 26 to 28°C for 7 days. Smears of broth were Gram stained and examined microscopically for typical rods and spores and a mouse bioassay was performed as necessary for botulinum toxin.

Nitrites and water phase salt. When nitrite was declared as an ingredient on the product label, samples were assayed for nitrite as sodium nitrite using the AOAC colorimetric method (3). Samples were analyzed gravimetrically for moisture (3) and volumetrically for chloride expressed as sodium chloride (3). Water phase salt was calculated by the formula

$$\% \text{NaCl} = \frac{[\text{g NaCl} \times 100]}{[\text{g NaCl} + \text{g H}_2\text{O}]}.$$

RESULTS AND DISCUSSION

A total of 1,080 smoked finfish and shellfish products were analyzed between 1991 and 1995. Two hundred one (18.6%) and 156 (14.4%) of the samples were analyzed for C. botulinum and Salmonella spp., respectively. All samples (more than 50% of which were smoked salmon) were analyzed for Listeria spp. Vacuum-packed samples were tested for C. botulinum type E spores and water phase salt. Nitrite content was quantified when the product label declared it as an ingredient.

Food and Drug Administration guidelines consider a smoked seafood product violative if L. monocytogenes, Salmonella species, or preformed C. botulinum toxin is detected (20). Guidelines also consider a product violative if C. botulinum type E spores are detected and water phase salt or nitrite levels are insufficient, whereby there is the potential for toxin production. Nitrites are permitted in smoked salmon, sablefish, and shad as long as the level does not exceed 200 ppm. In smoked chub the nitrite requirement is 100 to 200 ppm (9). Water phase salt guidelines vary depending on smoking process, packaging, and presence of nitrites. For vacuum-packaged hot- or cold-smoked fish, water phase salt must be at least 3.5% when no nitrites are used and at least 3.0% with at least 100 ppm nitrite. For air-packaged hot-smoked product, the water phase salt must be at least 3.0% with or without nitrites. For air-packaged cold-smoked product, water phase salt should be at least 3.5% without nitrites and 3.0% with a minimum of 100 ppm nitrite (20).

Effective 18 December 1997 the Food and Drug Administration will initiate hazard analysis critical control point (HACCP) regulation of smoked seafood (18). The new regulations will have no specific requirements for each individual factor (nitrite, water phase salt, time, and temperature) for the control of enteric pathogens in the product. The regulations will state that a HACCP plan must control the safety hazard associated with the toxin of C. botulinum for at least as long as the shelf life of the product under normal conditions and conditions of moderate abuse.

L. monocytogenes was isolated from 14% of the 1,080 samples. Of those samples for which the smoke process was known, a higher incidence of L. monocytogenes was found in cold-smoked fish products (Table 1). There was an association with the smoke process and the presence of L. monocytogenes (chi-square = 9.931, P = 0.002). A higher occurrence of L. monocytogenes was found in cold-smoked fishery products. United States regulations do not include smoking time and temperature requirements. The time and temperature guidelines for the smoke processes of some individual state authorities and the Association of Food and Drug Officials are summarized in Table 2. Other studies also linked the prevalence of this pathogen to the smoke process (hot or cold). Loncarevic et al. (35) found L. monocytogenes in 11.5% of cold-smoked and only 1.5% of hot-smoked fish from the retail market in Sweden. Farber (17) isolated L. monocytogenes from 31.2% of cold-smoked salmon. Rørvik and Yndestad (44) found the prevalence of L. monocytogenes to be 9% in cold-smoked salmon from Norway. Ben Embarek’s review (5) also showed, except for hot-smoked New Zealand mussels (which represented only 14 samples), a higher incidence of L. monocytogenes from cold-smoked fish than hot-smoked fish. Jemmi and Keusch (30) demonstrated that L. monocytogenes does not survive the hot-

### TABLE 1. Incidence of L. monocytogenes correlated with smoke process

<table>
<thead>
<tr>
<th>Smoke process</th>
<th>Number of samples</th>
<th>L. monocytogenes results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Cold</td>
<td>240</td>
<td>51</td>
</tr>
<tr>
<td>Hot</td>
<td>215</td>
<td>19</td>
</tr>
</tbody>
</table>

TABLE 2. Time and temperature guidelines for preparation of smoked fish

<table>
<thead>
<tr>
<th>State or organization</th>
<th>Reference</th>
<th>Cold smoking</th>
<th>Hot smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wisconsin</td>
<td>51</td>
<td>≤32.2°, ≤20 h ≥62.8°, ≥30 min</td>
<td>≤10°, ≤24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤48.9°, ≤6 h</td>
<td></td>
</tr>
<tr>
<td>Maine</td>
<td>38</td>
<td>≤32.2°, ≤20 h ≥62.8°, ≥30 min</td>
<td>≤10°, ≤24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤48.9°, ≤6 h</td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>40</td>
<td></td>
<td></td>
</tr>
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<td></td>
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</tbody>
</table>

a Degrees Celsius.
b Minnesota regulations allow only one smoke process.

smoke process (internal temperature 65°C, 20 min, followed by 60°C, 45 min). Jemmi and Keusch postulated the L. monocytogenes source to be postprocess contamination. During storage, growth of the organism may occur, even in a high-salt environment, and pose a potential health hazard (by 60°C, 45 min). Jemmi and Keusch postulated the source and route of L. monocytogenes contamination in fish processed by the cold-smoke method. They recommended elimination or reduction of the organism on the surface of the fish prior to smoking in order to control the hazard.

Listeria species other than L. monocytogenes were also detected in our study with a prevalence of 7.2% in cold-smoked and 3.8% in hot-smoked product (overall 5%). Listeria innocua was the most common species isolated, followed by Listeria seeligeri. These findings are similar to those reported by other researchers. Dillon et al. (11) found the prevalence of other Listeria species in smoked fish products to be 11.3%, while Loncarevic et al. (35) isolated these organisms from 8.7% of the smoked products tested. A higher incidence of L. monocytogenes than L. innocua in samples of smoked fish and shellfish is the opposite of the incidence of these organisms in samples from commercially prepared sandwiches (25), in which L. innocua is more prevalent than L. monocytogenes. Processing environment, processing methods, sample matrices, and seasonal sampling may all contribute to these differences, which warrant further study. The incidence of L. monocytogenes in samples from products of foreign origin was 15% (Table 3). Although the present study does not have enough samples from any one country for statistical significance, there are countries for which 70 or more samples were analyzed. Of those countries, the highest percentage of L. monocytogenes was found in smoked fish from Norway, followed by the United Kingdom, and Canada. In comparison, none of the 74 samples analyzed from the Philippines were positive for L. monocytogenes. L. monocytogenes has routinely been isolated from smoked fishery products from a variety of countries (12, 17, 35, 44). Ben Embarek's report (5) cites papers indicating that the level of L. monocytogenes varied from 0% in samples from Iceland to 75% isolation in samples from New Zealand. Hudson et al. (27) reported a high incidence of L. monocytogenes in smoked finfish (in 8 of 25 samples) and smoked shellfish (in 5 of 25 samples). Mussel accounted for all five of the shellfish samples positive for L. monocytogenes in their study. In the present study L. monocytogenes was isolated from 1 sample (hot-smoked mussels) of the 28 samples from New Zealand. (In the present study half of the samples from New Zealand were smoked mussels.) We will not speculate about why certain countries have a higher than average incidence of L. monocytogenes because we do not have access to the details of processing and handling of the products.

The results of L. monocytogenes analysis results of smoked fish processed in the United States are summarized in Figure 1. The 521 samples tested were collected from 194 processing plants. The figure shows the states containing the 37 plants in which L. monocytogenes was detected (37 of 194 = 19.1%). The locations of 141 plants from which no L. monocytogenes was isolated are also shown. Sixteen of the 521 samples collected carried no indication of the city or
state in which they were produced. Each of those samples was considered, in this study, to originate from a separate processing plant.

None of the 16 plants in Minnesota produced L. monocytogenes-contaminated product whereas 10 of 16 plants in the state of New York produced product in which the pathogen was detected. The smoke process temperature requirement (Table 2) is higher in Minnesota than in New York. Jemmi and Keusch (30) found that a temperature of 65°C was adequate to destroy L. monocytogenes in hot-smoked fish. We are not prepared on the basis of available information to speculate whether the process temperature or some other critical control factors may contribute to this difference in incidence.

Failure to control pathogen contamination in food production can have serious health and economic consequences. Dillon et al. (11) expressed great concern about the potential economic impact on the entire industry unless the problem can be controlled. Five of the 37 U.S. firms producing smoked fish contaminated with L. monocytogenes went out of business. Six firms also went out of business of the 157 firms which did not have L. monocytogenes-contaminated product.

Salmonella species were isolated from 5 (3.2%) of the 156 smoked fish samples. Four of the five samples were from the Philippines (two were scad, the market name of the other two fish was not given). The fifth, salmon, was packaged in the United Kingdom. Two of the samples positive for Salmonella spp. were hot smoked; the smoke process for the other three was not provided. The names of the processors were not available. More than two-thirds of the 156 samples tested for Salmonella spp. were of foreign origin. The smoke process was known for one-third of the samples, equally divided between hot- and cold-smoke processes. The Salmonella serotypes isolated from the five samples were Salmonella groups B, E1, E2, and E4 (Senftenberg). We are not aware of published surveys of smoked fish for contamination with Salmonella species. We postulate the hot-smoke process (Table 2) was sufficient to destroy the organism and that the source of contamination was postprocess.

No C. botulinum spores were detected in any of the 201 vacuum-packed samples tested. Growth and toxin production of C. botulinum type E is inhibited in these products by a combination of salt or salt plus nitrite, smoke, and refrigerated storage below 38°F (3.3°C) (14). Twenty-five of the 83 samples in the present study, for which analytical details were available, had a mean water phase salt concentration less than 3.0% (mean = 3.61; standard deviation = 1.55). Only a few samples had nitrites declared on the label. The potential for botulinic toxin production exists for such samples, especially over prolonged periods of refrigeration where temperature abuse may occur.

This study indicates foodborne pathogen contamination of smoked fish and seafood is a consumer health risk because these are ready-to-eat foods. Therefore these products should be routinely tested for presence of L. monocytogenes and Salmonella spp. and, if packaged by vacuum or modified atmosphere packaging, analyzed for C. botulinum.

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