

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 bacteriological 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 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 six 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 *ApaI*. This limited characterization was done as an economy measure. If the *ApaI* 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 *ApaI*, *SmaI*, and *AscI*. However, the strains isolated from food sample 1 (Table 2) were all analyzed with both *ApaI* and *SmaI*, since *ApaI* alone could not discriminate between groups A and E (see Results).

REA. REA with *ApaI* 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 *ApaI* (Fig. 1).

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TABLE 1. Results of typing *L. monocytogenes* strains isolated from nine patients with listeriosis

Patient	Disease	Predisposing condition	Outcome	Clonal type of isolated strain
1	Meningitis	Non-Hodgkin's lymphoma	Recovered	A
2	Mother Infant	Pregnancy	Recovered Recovered	B
3	Mother Infant	Pregnancy	Recovered Recovered	B
4	Septicemia	Chronic lymphatic leukemia, age 76	Recovered	B
5	Septicemia	Age 78	Recovered	B
6	Septic arthritis, septicemia	Age 89, rheumatoid arthritis	Recovered	B
7	Meningitis	Age 78	Died	B
8	Meningitis	Age 70, diabetes	Recovered	C
9	Mother Infant	Pregnancy	Recovered Died	D D

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.

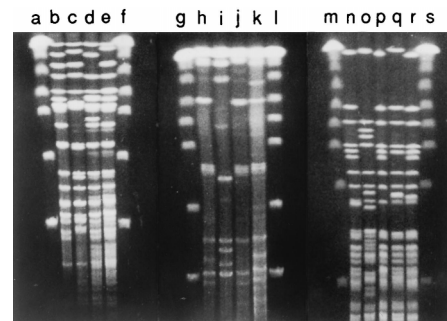


FIG. 1. REA profiles of *L. monocytogenes* produced by cleavage of DNA with *ApaI*, *AscI*, and *SmaI*. Lanes a, f, g, l, m, and s, lambda c1857 Sam7 concatemers; lanes b, h, and n, *ApaI* profile I, *AscI* profile I, and *SmaI* profile I, respectively, of clonal type A, strain SLU 2152; lanes c, i, and o, *ApaI* profile II, *AscI* profile II, and *SmaI* profile II, respectively, of clonal type B, strain SLU 2157; lanes d, j, and p, *ApaI* profile III, *AscI* profile III, and *SmaI* profile III, respectively, of clonal type C, strain SLU 2312; lanes e, k, and q, *ApaI* profile IV, *AscI* profile IV, and *SmaI* profile IV, respectively, of clonal type D, strain SLU 2330; and lane r, *SmaI* profile V of clonal type E, strain SLU 2173.

TABLE 2. Results of analyses of foodstuffs for *L. monocytogenes*

Food sample	Type of food ^a	Origin	Result (no.) for isolate from:		Clonal type
			Enrichment	Enumeration, CFU/g	
1	Cold-smoked rainbow trout, producer X	Refrigerator of patient 5	Positive (5)	<100	E ^b
2	Lamb chops, raw	Refrigerator of patient 5	Negative		
3	Water	Private well of patient 6	Negative		
4	Cognacwurst	Refrigerator of patient 6	Negative		
5	Gravad rainbow trout, producer Y	Refrigerator of patient 6	Positive (5)	6,200 (10)	B ^{b,c}
6	Gravad rainbow trout, producer Y	Local grocery store	Positive (6)	2,500,000 (9)	D ^b , B ^{b,c}
7	Smoked rainbow trout, producer X	Fish truck	Negative		
8	Cold-smoked rainbow trout, producer Y	Local grocery store	Negative		
9	Gravad rainbow trout, producer Y	Local grocery store	Negative		
10	Hot-smoked rainbow trout, producer Y	Local grocery store	Negative		
11	Slicing waste, cold smoked, producer Y	Local grocery store	Negative		
12	Hot-smoked fjordlax, producer Y	Local grocery store	Negative		
13	Cold-smoked rainbow trout, producer Z	Refrigerator of patient 8	Negative		
14	Fried herring	Refrigerator of patient 8	Negative		
15	Sliced smoke-cured loin of pork	Refrigerator of patient 8	Negative		
16	Sausage	Refrigerator of patient 8	Negative		
17	Gravad rainbow trout, producer Y	Local grocery store	Positive (1)	<100	C ^b
18	Gravad rainbow trout, producer Y	Local grocery store	Positive (1)	<100	B ^b
19	Gravad rainbow trout, producer Y	Local grocery store	Positive (1)	120 (12)	B ^{b,c}
20	Cold-smoked rainbow trout, producer Y	Local grocery store	Positive (1)	<100	B ^b
21	Cold-smoked rainbow trout, producer Y	Local grocery store	Negative		
22	Gravad rainbow trout, producer Y	Local grocery store	Negative		
23	Gravad rainbow trout, producer Y	Local grocery store	Negative		
24	Cold-smoked rainbow trout, producer Y	Local grocery store	Negative		
25	Cold-smoked rainbow trout, producer Y	Local grocery store	Positive (1)	<100	B ^b
26	Cold-smoked rainbow trout	Local grocery store	Negative		
27	Fish residues, packing machine	Producer Y	Positive (2)	<100	B ^b
28	Gravad rainbow trout	Producer Y	Positive (1)	<100	B ^b
29	Cold-smoked rainbow trout	Producer Y	Positive (2)	<100	B ^b

^a All rainbow trout had been packaged under vacuum.

^b Enrichment procedure.

^c Enumeration procedure.

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 gravad 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 *SmaI*. 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*

TABLE 3. Serovars, phagovars, and REA patterns defining clonal types of *L. monocytogenes* strains

Clonal type	Serovar	Phagovar	Profile		
			<i>ApaI</i>	<i>AscI</i>	<i>SmaI</i>
A	4b	2389:2425:3274:2671:47:108:340	I	I	I
B	4b	2389:3552:2425:1444:1317:3274:2671:52:107:108:340:312	II	II	II
C	4b	2389:2425:3274:2671:47:108:340	III	III	III
D	4b	47:52:340	IV	IV	IV
E	4b	2389:2425:3274:2671:47:108:340	I	I	V

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 *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. *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. *L. monocytogenes* clonal types C and D were also isolated from rainbow trout from producer Y.

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