

CONCISE COMMUNICATION

An Outbreak of *Listeria Monocytogenes* Serotype 3a Infections from Butter in Finland

Outi Lyytikäinen,¹ Tiina Autio,³ Riitta Maijala,⁴
Petri Ruutu,¹ Tuula Honkanen-Buzalski,⁵
Maria Miettinen,³ Maija Hatakka,⁷ Janne Mikkola,^{1,a}
Veli-Jukka Anttila,⁸ Tuula Johansson,⁵ Leila Rantala,⁵
Tuula Aalto,⁶ Hannu Korkeala,³ and Anja Siitonen²

¹Department of Infectious Disease Epidemiology and ²Laboratory of Enteric Pathogens, National Public Health Institute; ³Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki; Departments of ⁴Risk Analysis, ⁵Food Microbiology, and ⁶Food Control, National Veterinary and Food Research Institute; ⁷National Food Administration; and ⁸Helsinki University Central Hospital, Helsinki, Finland

In February 1999, an outbreak of listeriosis caused by *Listeria monocytogenes* serotype 3a occurred in Finland. All isolates were identical. The outbreak strain was first isolated in 1997 in dairy butter. This dairy began delivery to a tertiary care hospital (TCH) in June 1998. From June 1998 to April 1999, 25 case patients were identified (20 with sepsis, 4 with meningitis, and 1 with abscess; 6 patients died). Patients with the outbreak strain were more likely to have been admitted to the TCH than were patients with other strains of *L. monocytogenes* (60% vs. 8%; odds ratio, 17.3; 95% confidence interval, 2.8–136.8). Case patients admitted to the TCH had been hospitalized longer before cultures tested positive than had matched controls (median, 31 vs. 10 days; $P = .008$). An investigation found the outbreak strain in packaged butter served at the TCH and at the source dairy. Recall of the product ended the outbreak.

Listeriosis is a foodborne illness that most often causes sepsis, meningitis, and miscarriage in susceptible hosts [1]. It is often difficult to implicate specific food vehicles because most cases do not occur during well-recognized outbreaks, infection results in a variable, but generally prolonged, incubation period (median, 3 weeks), and samples of food eaten by patients consequently are unavailable. Because it is found ubiquitously in nature, *Listeria monocytogenes* frequently is cultured from foods and the environment, requiring typing of isolates from patients and from suspected food items to recognize the similarity or dissimilarity of these isolates. The benefit of serotyping is limited; the majority of *L. monocytogenes* isolates belong to serotypes 1/2a, 1/2b, or 4b. A more discriminating subtyping method, pulsed-field gel electrophoresis (PFGE), has been used successfully in epidemiological investigations [2].

Sources of listeriosis outbreaks have included a number of foods, such as raw vegetables, dairy products, meat, and seafood products [3]. Among dairy products, soft cheeses have

been shown to be relatively high-risk foods for epidemic and sporadic listeriosis. Butter is an unusual vehicle for listeriosis. Only 1 report in the literature has implicated butter in an outbreak; however, no positive butter samples were obtained. *L. monocytogenes* has been found in butter during product sampling in the United States, leading to ≥ 5 class 1 recalls. According to a European Community directive, *L. monocytogenes* should not be detected in 1 g of butter [4].

In early 1999, a cluster of patients with *L. monocytogenes* infection due to an unusual serotype (3a) was identified; most of these patients had been treated at 1 tertiary care hospital (TCH). The isolates were indistinguishable by PFGE. This rare serotype with the same PFGE pattern previously had been isolated in 1997 from 1 in-house control sample of butter produced by a Finnish dairy. We report the results of epidemiological, laboratory, and environmental investigations of the outbreak.

Methods

Surveillance. In Finland, physicians notify the National Infectious Disease Registry (NIDR) of culture-confirmed cases of listeriosis. Microbiology laboratories do the same for isolations of *L. monocytogenes* from blood, cerebrospinal fluid, genital tract, deep puncture, and surgical specimens, as well as from newborns. Strains of *L. monocytogenes* are sent to the Laboratory of Enteric Pathogens (LEP) at the National Public Health Institute for serotyping and PFGE. Food isolates, which are collected during routine surveillance, also are evaluated by serotyping and PFGE. Every dairy has a mandatory in-house control program, approved and supervised by the local food-control authorities, that includes sam-

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^a Present affiliation: Kantahämeen keskussairaala, Hämeenlinna, Finland.

Reprints or correspondence: Dr. Outi Lyytikäinen, Department of Infectious Disease Epidemiology, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland (outi.lyytikainen@ktl.fi).

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pling for *L. monocytogenes* from the plant's environment and products.

Laboratory methods. The human, food, and environmental *L. monocytogenes* isolates were serotyped for O and H antigens by using agglutination methods with commercially available antisera (Difco Laboratories, Detroit; Denka Seiken, Tokyo) in accordance with the manufacturers' instructions. In situ DNA isolation and macrorestriction analyses by using PFGE with restriction enzymes *AscI* and *ApaI* were done as described by Maslow et al. [5-7]. Detection and enumeration of *L. monocytogenes* from butter (1- and 25-g sample sizes) and from environmental samples, prepared according to International Dairy Federation (Brussels) Standard 122C, were done in accordance with the international standards modified as described by Johansson [8-11].

Case definition and finding. Outbreak-associated patients were defined as patients from whom the outbreak strain was isolated from a culture of a normally sterile site, from 1 June 1998 to 30 April 1999; patients with infection due to other *L. monocytogenes* strains during the same period were defined as non-outbreak-associated patients. Both were sought by review of the NIDR and the records of the LEP. Clinical information was collected from the attending physicians by using a standardized form.

Matched case-control study. Only patients admitted to the Helsinki University Central Hospital (a TCH) were included in the case-control study. This TCH, with 1,000 beds, serves as the only site for organ transplantations in Finland. For each case, 3 control subjects who were hospitalized within 1 month of the case patient and who were matched by age, underlying condition, and hospital ward were selected randomly from the hospital admission records. Case patients and their matched controls were interviewed by telephone about their consumption of butter at the hospital, as well as whether they had consumed the implicated butter brand at home between 1 June 1998 and 19 February 1999. Information about the use of antacids and H₂-blockers was retrieved from the patient's records.

Food and environmental investigations. At the dairy, the soured-cream butter was manufactured in a continuous butter maker using the NIZO process [12]. The products were 7-, 10-, and 500-g and 25-kg packages of butter as well as of fat blend. The products were sold in southern and western Finland to retail markets and hospital kitchens. Delivery of butter from this dairy to the TCH began on 1 June 1998, after which the 7-g butter packages were the only brand of butter served to the patients. Information about the amounts of butter delivered monthly to the hospital units and the number of patient days was acquired from the hospital administration. The butter packages with the earliest expiration dates were collected from the hospital kitchen for microbiological analysis. At the same time of the detection of the human outbreak, *L. monocytogenes* strain was isolated in the in-house control program of the dairy. A team of national and local food-control authorities inspected the dairy and reviewed the operation of the pasteurizer, including time and temperature records and peroxidase testing. The production line and dairy environment were sampled. A total of 139 butter samples from the dairy and from a wholesale store were analyzed for *L. monocytogenes*, including butter packages of 25 kg (*n* = 65), 500 g (*n* = 27), and 7 g and 10 g (as pooled samples of 10 packages; *n* = 10) and packages of fat blend (*n* = 37).

Statistical analysis. For categorical variables, proportions were

compared by using the χ^2 test with Yates correction or Fisher's exact test, as appropriate. Confidence intervals (CIs) for odds ratios (ORs) were calculated by using Epi Info software (version 6.04b; Centers for Disease Control and Prevention, Atlanta, GA). The means or medians of the continuous variables were compared by using the Student's *t* test or the Mann-Whitney *U* test, depending on the sample distribution.

Results

The outbreak. Twenty-five outbreak-associated patients were identified (figure 1 and table 1). The patients with the outbreak strain were more likely to have had malignancy or organ transplantation and to have been admitted to the TCH than were the non-outbreak-associated patients. Nine of 10 outbreak-associated patients with possible nosocomial acquisition (i.e., isolation of *L. monocytogenes* >2 days after admission) were admitted to the TCH.

Matched case-control study and butter consumption. Of the 15 outbreak-associated patients admitted to the TCH, 7 were available for telephone interviews, 6 had died, and 2 were in terminal condition. Analysis of the 7 cases and 21 matched controls showed no association between illness and butter consumption at the hospital (5/6 vs. 16/21; OR, 1.7; 95% CI, 0.2-46.0) or at home (1/7 vs. 1/21; OR, 3.0; 95% CI, 0.1-117.0); the use of antacids or H₂-blockers was not associated with illness. The median length of hospital stay within 70 days (i.e., maximum incubation period for *L. monocytogenes*) before the

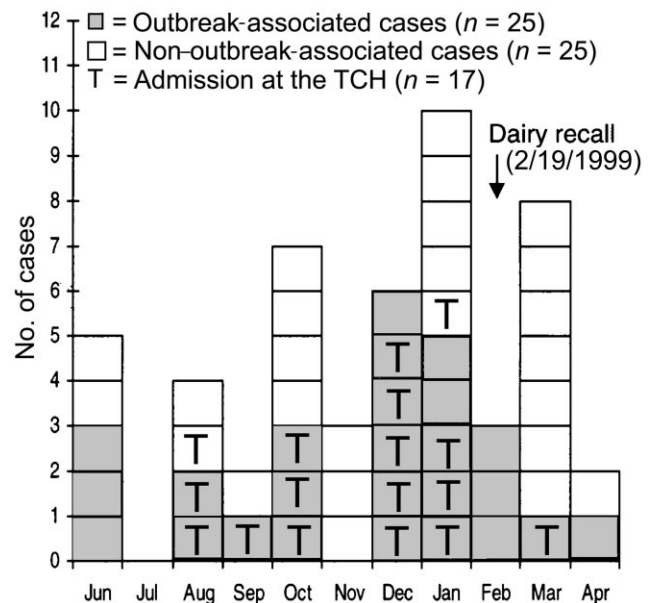


Figure 1. Case patients from whom the outbreak-associated and non-outbreak-associated strains were isolated, by month of culture yielding *Listeria monocytogenes* at the Helsinki University Central Hospital (a tertiary-care hospital [TCH]) and at other Finnish hospitals, from 1 June 1998 to 30 April 1999.

Table 1. Clinical and demographic characteristics of outbreak-associated and non-outbreak-associated cases of listeriosis in Finland from 1 June 1998 to 30 April 1999.

Characteristic	Outbreak-associated cases (n = 25)	Non-outbreak-associated cases (n = 25)	P
Source of positive culture			
Blood	20 (80)	20 (80)	.72
Cerebrospinal fluid	4 (16)	4 (16)	1.00
Other sterile site	1 (4)	3 (12)	.60
Median age, years (range)	53 (12–85)	69 (13–92)	.24
Male sex	10 (40)	14 (56)	.40
Underlying condition			
Hematologic malignancy	14 (56)	5 (20)	.02
Solid malignancy	4 (16)	1 (4)	.35
Solid-organ transplantation	2 (8)	0 (0)	.49
Nonmalignant underlying disease	4 (16)	15 (60)	.004
Pregnancy	0 (0)	3 (12)	.23
Newborn	0 (0)	0 (0)	1.00
No underlying condition (not pregnant)	1 (4)	1 (4)	1.00
Patient admitted to TCH	15 (60)	2 (8)	<.001
Nosocomial acquisition ^a	10 (40)	5 (20)	.22
Death	6 (24)	4 (16)	.72

NOTE. Data are no. (%) unless noted otherwise. TCH, tertiary care hospital (Helsinki University Central Hospital).

^a Defined as isolation of *Listeria monocytogenes* in samples taken >2 days after admission.

collection of the specimen that was positive for *L. monocytogenes* was longer among case patients than it was among control subjects (31 vs. 10 days; $P = .008$). During the same 70-day period, the median weight of butter consumed was 224 g (range, 0–1708 g) for case patients and 54 g (range, 0–644 g) for control subjects ($P = .205$ for case patients vs. control subjects). The median butter delivery per 100 patient days was higher to the hospital units in which the nosocomial cases occurred (hematology, pulmonology, and oncology) than to the units with no cases (1378 vs. 181 g; $P = .01$).

Food and environmental investigations. The outbreak strain was detected in all 13 butter samples obtained from the hospital kitchen. It also was detected in several lots of 7-, 10-, and 500-g butter packages from the dairy and the wholesale store, whereas samples from 25-kg packages of butter and of fat blend tested negative for the outbreak strain. In all butter samples that tested positive, the numbers of *L. monocytogenes* were <100 cfu/g (range, 5–60 cfu/g), except for 1 sample of 7-g packages that contained 11,000 cfu/g. The cream pasteurizer was found to operate properly. Small (7 and 10 g) butter packages had a separate packaging line from the other products: 1 whole lot from the butter silo usually was transferred to a separate butter wagon and packaged in small butter packages. However, if there was butter left after the packaging of small packages, it was occasionally pumped to the 500-g packaging machine. Of 430 environmental samples studied, the outbreak strain was detected in samples from the packing machines for both small and 500-g packages, from the screw conveyor of the butter wagon, and from 2 floor drains beneath the butter wagon of the small-packaging line.

Control measures. The production of 7-, 10-, and 500-g butter packages was stopped on 17 February 1999 and that of 25-kg packages on 4 March 1999. On 19 February 1999, the dairy recalled voluntarily their 7- and 10-g butter products, and the Finnish hospitals were requested not to serve butter from the implicated dairy to patients. The whole production facility, including the butter production line, machines, and pipes, was cleaned. Control samples from the process line and environment as well as from several test butter batches tested negative for *L. monocytogenes*. In April 1999, the dairy restarted the production of 25-kg and 500-g butter and fat blend packages. No further cases of listeriosis caused by the outbreak strain have been detected since 19 April 1999.

Discussion

We describe an outbreak of listeriosis transmitted by an unusual vehicle, pasteurized butter. The outbreak occurred mainly among persons with intense immunosuppression. To our knowledge, this is the first outbreak caused by *L. monocytogenes* serotype 3a, and its clonal origin was confirmed by PFGE.

Pasteurization is effective in removing *L. monocytogenes* from dairy products [3]. However, contamination of such products may take place after pasteurization and may be caused by sources in the processing-plant environment or by error in operation. The outbreak strain was isolated from the butter-producing equipment and the dairy environment. We could not confirm any error in operation. The source of *L. monocytogenes* may have been the screw conveyor in the butter wagon, which made contact while the butter from the wagon was packaged in the packing machines for small and, occasionally, 500-g packages. The outbreak strain was isolated in samples from both production lines and from both package sizes.

The outbreak strain was first isolated in samples of butter from the implicated dairy in 1997, which led to processing-line cleaning and increased monitoring of the products and environment. Despite intensified sampling, the dairy did not detect *Listeria* before February 1999. However, the process seems to have been contaminated for a longer period, because *L. monocytogenes* was detected in samples from several batches manufactured between September 1998 and February 1999. Long-term colonization of the food-production environment has been reported, and the high risk of cross-contamination to food during processing and packaging is well recognized [13].

The rarity of the causative serotype and the clustering of cases in a patient population spending long periods at a hospital facilitated the identification of the outbreak. The clustering in a particularly susceptible population might have been due to a low level of bacterial exposure, supported by a low concentration of *L. monocytogenes* in the butter samples. Another distinctive feature of the outbreak was the frequency of apparent nosocomial acquisition. Two hospital-associated outbreaks with a common source have been described [14, 15]. One outbreak involving 20 patients in 8 Boston-area hospitals was

suspected to be due to lettuce or raw vegetables. Another epidemic in a nursery was unique in that the index case occurred when a bottle of mineral oil became contaminated and was used for bathing other infants.

Although the outbreak strain could be detected, for most case patients, in samples of butter obtained from the kitchen of the hospital, our matched case-control study failed to confirm the association between butter consumption and illness. The matching according to the underlying condition probably led to matching by the level of exposure; this is supported by the finding that the units where the cases occurred used significantly more butter than did the units without cases. However, the implicated butter rarely was consumed at home. Furthermore, the case patients had more opportunities for consuming the implicated butter than did their matched controls, because they had stayed longer at the hospital before the onset of listeriosis.

This outbreak showed that, despite regular monitoring of dairy products and environment for *L. monocytogenes* and a good standard of plant sanitation guided by a hazard-analysis and critical-control-point program, comprehensive, laboratory-based, national surveillance with continuous typing of bacteria improves recognition of outbreaks.

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References

- Slutsker L, Schuchat A. Listeriosis in humans. In: Ryser ET, Marth EH, eds. *Listeria*, listeriosis, and food safety. 2d ed. New York: Marcel Dekker, 1999:75–95.
- Proctor ME, Brosch R, Mellen JW, Garrett LA, Kaspar CW, Luchansky JB. Use of pulsed-field gel electrophoresis to link sporadic cases of invasive listeriosis with recalled chocolate milk. *Appl Environ Microbiol* 1995;61:3177–9.
- Ryser ET. Foodborne listeriosis. In: Ryser ET, Marth EH, eds. *Listeria*, listeriosis, and food safety. 2d ed. New York: Marcel Dekker, 1999:299–358.
- Anonymous. Council directive 92/46/EC laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. *J Eur Communities* 1992.
- Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White TJ, eds. *Diagnostic molecular microbiology: principles and applications*. Washington, DC: American Society for Microbiology, 1993:563–72.
- Miettinen MK, Björkroth J, Korkeala HJ. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. *Int J Food Microbiol* 1999;46:187–92.
- Johansson T, Rantala L, Palmu L, Honkanen-Buzalski T. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *Int J Food Microbiol* 1999;47:111–9.
- International Organization for Standardization. Microbiology of food and animals feeding stuffs: horizontal method for the detection and enumeration of *Listeria monocytogenes*. I. Detection method. Geneva: International Organization for Standardization, 1996. International Standard ISO 11290-1.
- International Organization for Standardization. Microbiology of food and animals feeding stuffs: horizontal method for the detection and enumeration of *Listeria monocytogenes*. II. Enumeration method. Geneva: International Organization for Standardization, 1998. International Standard ISO/DIS 11290-2.
- Johansson T. Enhanced detection and enumeration of *Listeria monocytogenes* from foodstuffs and food-processing environments. *Int J Food Microbiol* 1998;40:77–85.
- International Dairy Federation. Milk and milk products: preparation of samples and dilutions for microbiological examination. Brussels: International Dairy Federation, 1996. IDF Standard 122C.
- Kimenai MP. Cream crystallization, section III—NIZO method. In: Continuous butter manufacture. Bulletin 204. Brussels: International Dairy Federation, 1986:11–5.
- Unnerstad H, Bannerman E, Bille J, Danielsson-Tham ML, Waak E, Tham W. Prolonged contamination of a dairy with *Listeria monocytogenes*. *Neth Milk Dairy J* 1996;50:493–9.
- Ho JL, Shands KN, Friedland G, Eckind P, Fraser DW. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Arch Intern Med* 1986;146:520–4.
- Schuchat A, Lizano C, Broome CV, Swaminathan B, Changmin K, Winn K. Outbreak of neonatal listeriosis associated with mineral oil. *Pediatr Infect Dis J* 1991;10:183–9.