

Use of Pulsed-Field Gel Electrophoresis To Link Sporadic Cases of Invasive Listeriosis with Recalled Chocolate Milk

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Pulsed-field gel electrophoresis established the linkage between recalled chocolate milk and a multistate invasive listeriosis outbreak during a four-product recall period. *Listeria monocytogenes* isolates from four hospitalized patients and an environmental dairy sample displayed *AscI* restriction endonuclease digestion profiles identical to that of the chocolate milk isolate.

Although outbreaks of invasive disease caused by *Listeria monocytogenes* have been associated with ingestion of a variety of contaminated foods (5), most listeriosis in the United States occurs as isolated cases (9). While routine monitoring for *L. monocytogenes* results in recalls of retail products and the de-

struction of large amounts of food, these products are only infrequently linked with human illness. Of the phenotype- and genotype-based *Listeria* typing methods (10), current literature substantiates that genomic fingerprinting using pulsed-field gel electrophoresis is probably the most reproducible and discrim-

TABLE 1. Epidemiologic and molecular characterization of *L. monocytogenes* isolates

Strain		Patient or product history ^a	Isolate source	<i>AscI</i> REDP ^b	Serotype ^c
JBL no. ^d	Study designation ^e				
Outbreak related					
2450	H1	56-yr-old M; immune disorder	Blood ^f		
	H2	72-yr-old M; bladder cancer	Brain biopsy sample	<i>m23k</i>	1/2b
	H3	53-yr-old M; diabetes	Blood ^f		
2451	H4	81-yr-old M	Blood	<i>m23k</i>	1/2b
	H5	6-yr-old M	Blood ^f		
2457	H6	2-yr-old F	Blood	<i>m23k</i>	1/2b
2519	H7	5-yr-old M	Blood	<i>m23k</i>	1/2b
Sporadic					
2452	S1	2-wk-old F	CSF ^g	<i>m21k</i>	1/2b,4c ^h
2447	S2	72-yr-old F; diabetes	Blood	<i>m13d</i> ⁱ	4b
2448	S3	60-yr-old M; posttransplant	Blood	<i>m12b</i>	4b
2449	S4	55-yr-old M; leukemia	Blood	<i>m23a</i> ⁱ	1/2b
Dairy					
2365	PrdA1	Dairy A; produced 6/24/94	Chocolate milk	<i>m23k</i>	1/2b
2369	PrdA2	Dairy A; produced 7/27/94	Whipping cream	<i>m23k</i>	1/2b
2367	EnvA	Dairy A	Floor drain	<i>m23k</i>	1/2b
2455	PrdB	Dairy B; produced 7/8/94	Butter	<i>m23b</i> ⁱ	1/2b
2456	EnvB	Dairy B	Floor drain	<i>m24c</i>	1/2b
2454	PrdC	Dairy C; produced 8/11/94	Ice cream	<i>m22k</i> ⁱ	4b
2453	EnvC	Dairy C	Floor drain	<i>m22k</i> ⁱ	4b

^a M, male; F, female. Dates are in the form month/day/year.

^b REDP designations (e.g., *m23k*) are based on the species name ("m" denotes *monocytogenes*), the number of bands within the first two arbitrary fields (e.g., "23" indicates the presence of two bands in field 1 [≤48.5 kb] and three bands in field 2 [48.5 to 97 kb]), and the designation for strains with equivalent bands in both arbitrary fields (e.g., "k" denotes a unique REDP displaying 2 bands in field 1 and 3 bands in field 2), as described previously (2).

^c Serotype predicted by REDP and independently established by the Centers for Disease Control and Prevention or World Health Organization reference center.

^d JBL, culture collection of J. B. Luchansky, Food Research Institute, Madison, Wis.

^e H1 to H5 were from Wisconsin residents; H6 and H7 were from Michigan and Illinois residents, respectively.

^f Culture negative.

^g CSF, cerebrospinal fluid.

^h Predicted by REDP to be serotype 4c, but agglutinates with flagellar factors A, B, and C and very weakly with somatic factor 1, suggestive of serotype 1/2b.

ⁱ REDP previously described (2).

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inating subtyping method (1–4, 6, 8). In this work, we report the statistical analysis of restriction endonuclease digestion profiles (REDP) on a time-space cluster of *Listeria* isolates to establish probabilistic linkage of sporadic invasive listeriosis cases with a recalled dairy product (1% low-fat chocolate milk) responsible for febrile gastroenteritis among picnickers in Illinois on 9 July 1994 (7).

Product recalls. During a 6-week period in 1994, the Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP) announced voluntary recalls of four different Wisconsin dairy products contaminated with *L. monocytogenes*: dairy A chocolate milk (July 26) and whipping cream (August 5), both distributed throughout Wisconsin and the upper peninsula of Michigan; dairy B butter (September 2), sold throughout southern Wisconsin and to a distributor in Pennsylvania; and dairy C ice cream bars (September 2), distributed in the upper portion of Wisconsin and in the upper peninsula of Michigan.

Surveillance and case finding. On 29 July 1994, the Wisconsin Division of Health instituted enhanced passive surveillance among diagnostic laboratories and hospitals to identify human illness potentially associated with the recalled dairy products. Health departments in other states where these four products were distributed were also notified of the recalls and provided with a questionnaire for sporadic listeriosis cases potentially associated with these products. Only individuals who consumed one of the recalled products and who were hospitalized or who presented at an emergency room or physician's office with symptoms were included in the investigation.

Production dates for chocolate milk consumed by three hospitalized and four nonhospitalized individuals ranged from 6 to 20 July 1994; production dates for milk consumed by other ill individuals were not available. Illness onset occurred between 25 June and 28 July 1994 for seven hospitalized patients and between 14 and 27 July 1994 for 12 nonhospitalized individuals. The median interval between consumption of chocolate milk and onset of the first symptom was 2 days for both hospitalized (range, 1 to 22 days) and nonhospitalized (range, 1 to 4 days) individuals. Among hospitalized patients, the median interval between onset of first symptom and hospitalization was 14 days, with a range of 1 to 39 days. Three hospitalized patients reported underlying medical conditions.

Laboratory studies. For seven patients hospitalized between 25 June and 28 July 1994, all of whom had consumed recalled chocolate milk, *L. monocytogenes* was isolated from the blood of three (H4, H6, and H7) and from a brain biopsy sample from a fourth (H2) (Table 1). Four *L. monocytogenes* isolates (S1, S2, S3, and S4) from individuals hospitalized in Wisconsin between 1992 and 1994 but not exposed to any of the recalled products described herein were obtained from stock cultures at the University of Wisconsin—Madison Hospital and Clinics. *L. monocytogenes* isolates from the four recalled dairy products (PrdA1, PrdA2, PrdB, and PrdC) and three associated dairy environments (EnvA, EnvB, and EnvC) were obtained from the Food Safety Laboratory of the WDATCP. For 12 nonhospitalized individuals who also had consumed recalled chocolate milk, seven blood cultures were negative and nine stool samples were negative for enteric pathogens.

Genomic fingerprinting. Genomic DNA from listeriae were isolated, digested directly in agarose plugs, and resolved by the pulsed-field technique of clamped homogeneous electric field electrophoresis as previously described (2, 6). All patient (H2, H4, H6, and H7), product (PrdA1 and PrdA2), and environmental (EnvA) isolates associated with dairy A displayed identical REDP with *AscI* (Fig. 1A). The similarity among isolates was measured with Simpson's coefficient and visualized by clus-

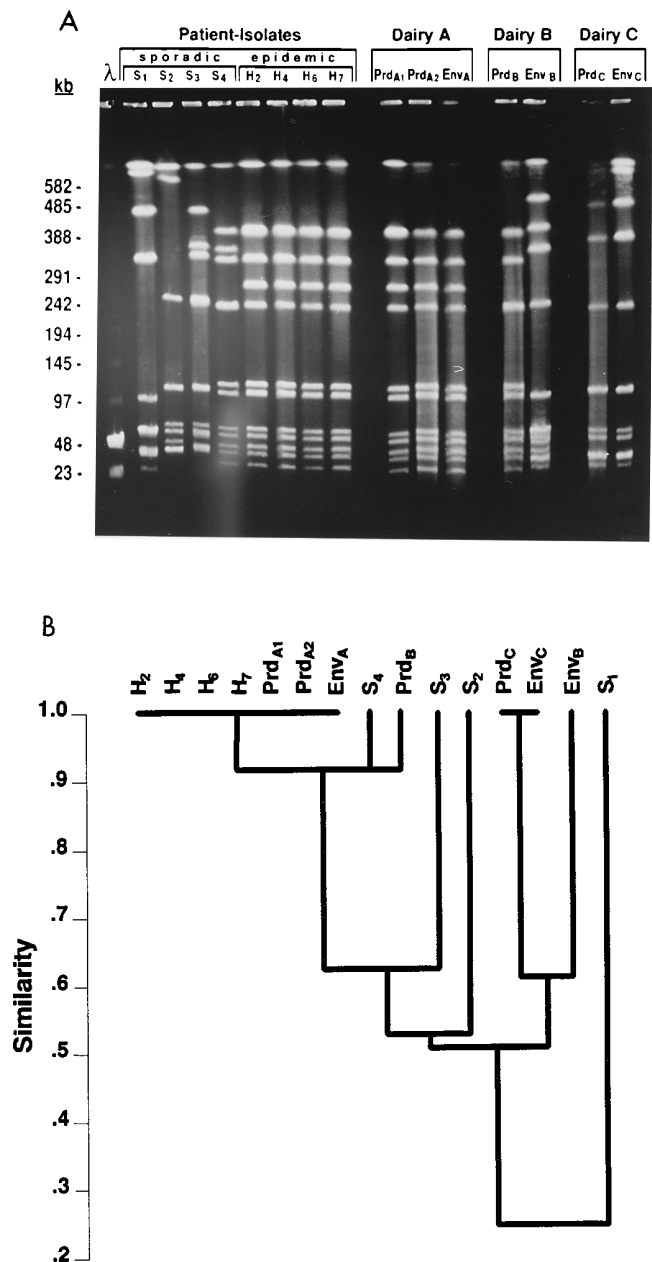


FIG. 1. (A) *AscI* REDP genomic relatedness of human, product, and environmental isolates of *L. monocytogenes*, determined as previously described (2). The electrophoretic conditions were as follows: 200 V ramped at 1 to 40 s over 23 h in 0.5× TBE buffer (2) while samples were maintained at 18°C. Bacteriophage lambda DNA concatemers and the low-range molecular size marker (New England BioLabs) were used as molecular size standards. (B) The similarity matrix of *AscI* REDP was subjected to cluster analyses as unweighted matched pair groups using averages (11) (using Simpson's coefficient [C/N , where C is the number of shared bands and N is the number of bands from the REDP with the largest number of bands between each of the matched pairs]). Horizontal lines represent the degree of similarity shared between the isolates or groups connected by the lines.

ter analysis (Fig. 1B). The REDP of sporadic isolates S1, S2, and S3 were distinct from each other and from the REDP of isolates associated with dairy A. With the exception of a difference in size of one *AscI* fragment, the REDP of the remaining sporadic isolate, strain S4, was very similar to the REDP of isolates associated with dairy A. As observed for dairy A,

product and environmental isolates for dairy C displayed identical REDP with *AseI*, although they were distinct from the REDP for dairy A isolates. In contrast, the REDP of the dairy B product isolate differed from the REDP of its corresponding environmental isolate and from the other dairy-related isolates.

Against a background average annual incidence of 0.9 case per population of 10^6 , it is unlikely that four cases of culture-confirmed invasive illness seen in the product distribution area would have been recognized as unusual or related. However, because isolates associated with dairy A (H2, H4, H6, H7, PrdA1, PrdA2, and EnvA) had identical REDP that were distinct from the other ca. 200 REDP in our database (2), it was possible to estimate the likelihood of co-occurrence of this pattern at 0.5 to 1.5%. Using the higher frequency of occurrence (1.5%), the probability of random joint occurrence of two independent isolates sharing this REDP is 0.000224, or 1 in 4,444. The probability of random joint occurrence of seven independent isolates sharing this pattern is 1.7×10^{-13} , which strongly argues that these isolates are a related, multistate (Wisconsin, Michigan, and Illinois) cluster that cause invasive disease.

In summary, the results of this study underscore the utility of pulsed-field gel electrophoresis for linking sporadic cases of illness with recalled products and illustrate the broad temporal and geographic bases required for investigating illness surrounding recalled commercial products. Only systematic molecular approaches such as this will uncover unrecognized outbreaks related to recalled products and provide an estimate of the etiologic fraction of invasive listeriosis due to these products.

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