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Restriction Enzyme Analysis of *Listeria monocytogenes* Strains Associated with Food-Borne Epidemics

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Listeria monocytogenes **(serotype 4b) has caused four major food-borne epidemics in North America. In this study, *L. monocytogenes* isolates from the Nova Scotia (Canada), Boston (Mass.), and Los Angeles (Calif.) outbreaks were examined by restriction enzyme analysis with the endonuclease *Hha*I. Human isolates ($n = 32$) from the 1981 Canadian outbreak were compared with a strain recovered from coleslaw, which was epidemiologically incriminated as the vehicle of infection. After *Hha*I digestion, 29 of 32 isolates exhibited the restriction enzyme pattern of the reference coleslaw isolate. The restriction enzyme patterns of the nine clinical isolates from the 1983 Massachusetts outbreak were identical to each other but differed from those of raw milk isolates recovered from sources supplying the pasteurizer. Isolates ($n = 48$) from the 1985 California outbreak were evaluated. The restriction enzyme patterns of the *L. monocytogenes* isolates from humans and from the suspect cheese samples were identical to those of four of five cheese factory environmental isolates. Isolates from each of these outbreaks exhibited a restriction enzyme pattern that was characteristic of that outbreak. The ease with which restriction enzyme analysis can be applied to all serotypes of *L. monocytogenes* argues for its use in the epidemiology of *L. monocytogenes*.**

Listeria monocytogenes has been the causative agent of major food-borne epidemics (1) in which dairy products (including milk [10] and cheese [5, 6, 14, 17, 20, 22]), vegetables (13), and coleslaw (27) have been incriminated as the contaminated foods.

From 1979 to 1985, four food-associated outbreaks, all involving *L. monocytogenes* serotype 4b, were reported in North America. Food preference surveys suggested that coleslaw was the vehicle of infection during the 1981 outbreak in Nova Scotia, Canada, which resulted in 41 cases of listeriosis and 14 deaths. *L. monocytogenes* was isolated from a patient's refrigerated coleslaw and from two unopened coleslaw packages obtained from the factory. Epidemiological investigations indicated that the cabbage used in the suspect coleslaw was grown in fields fertilized with manure obtained from a flock of sheep in which listeriosis had been reported (27).

The 1983 outbreak in Massachusetts involved 49 patients, including 7 perinatal cases and 42 immunosuppressed individuals over a 3-month period. *L. monocytogenes* was never cultured from the pasteurized milk product. The pasteurizer was found to be operating correctly at temperatures and times exceeding the current regulations of the Food and Drug Administration (7, 10). Yet, epidemiological surveys indicated that patients who had consumed pasteurized whole or 2% milk purchased from a particular supermarket chain were at greater risk than case controls matched for residence and predisposing conditions. Retrospective analysis suggested cases of listeriosis in dairy herds supplying the milk plant in question (10). Multiple serotypes of *L. monocytogenes*, including the 4b serotype associated with the outbreak, were recovered from milk tanks supplying the pasteurizer (12).

In 1985, the single largest listeriosis outbreak occurred in Los Angeles County, Calif. (14, 17, 22). In this epidemic unlike the others, a food source, Mexican-style soft cheese, was incriminated as the vehicle of infection during the outbreak. Of the 93 maternal-fetal cases that were reported, 87% of the cases involved Hispanic persons who recalled eating a particular brand of Mexican-style soft cheese. *L. monocytogenes* was cultured from a patient's refrigerated Mexican-style cheese and from unopened cheese packages recovered from the factory. It was speculated that raw milk, the probable source of *L. monocytogenes*, and pasteurized milk were mixed during cheese preparation (17, 22).

Because each of these epidemics involved *L. monocytogenes* serotype 4b, we wished to evaluate restriction enzyme analysis as a method of further differentiating these serologically indistinguishable isolates. Phage typing (2, 3, 11, 18, 23, 25, 26), multilocus enzyme analysis (4), and ribotyping (24) have been applied to characterizing serologically identical strains. Isolates of *L. monocytogenes* have been characterized by multilocus enzyme analysis employing a battery of enzymes with reactivity patterns constituting the definitive electromorph (4). However, bacteriophages are not available for all isolates (23), and the phage profile of an isolate may vary with time due to alteration in surface receptors. The World Health Organization expert Committee on Listeriosis proposed that alternatives to phage typing, including genetic analysis via restriction enzyme analysis, be explored (1). Restriction enzyme analysis, like ribotyping (24), requires digestion of chromosomal DNA with specific endonucleases; however, no further hybridization with specific probes is required. In endonuclease analysis, genetic changes that may not be detected by protein-based assays are visualized. Restriction enzyme analysis has been used to verify cross-infection with *L. monocytogenes* (9, 16, 23a). We previously reported that the restriction endonuclease *Hha*I offered the best discrimination of field isolates (28). In this study, restriction enzyme analysis with *Hha*I was used to differentiate *L. monocytogenes* strains of serotype 4b

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TABLE 1. Isolates of *L. monocytogenes* (serotype 4b) associated with the 1981 outbreak in Halifax, Nova Scotia

Isolate[a]	Source
81-498	Placenta
81-499	Endometrium
81-501	Placenta
81-502	Placenta
81-505	Cerebrospinal fluid
81-507	Cerebrospinal fluid
81-509	Throat
81-511	Vagina
81-512	Blood
81-515	Blood
81-516	Blood
81-558	Cerebrospinal fluid
81-590	Blood
81-591	Blood
81-592	Blood
81-618	Unknown
81-619	Stillborn baby
81-637	Throat
81-678	Unknown
81-679	Unknown
81-680	Baby
81-682	Baby
81-694	Throat
81-711	Umbilicus
81-712	Stool
81-739	Placenta
81-784	Unknown
81-859	Stool
81-861	Coleslaw
81-884	Vagina
81-886	Rectum
81-923	Baby
81-1087	Cucumber

[a] Provided by F. Ashton, Center for Health and Welfare, Ottawa, Canada.

incriminated in the Canadian, Massachusetts, and California epidemics.

(Portions of this work were presented at the annual meeting of the American Society for Microbiology, May 1990, Anaheim, Calif. [27a].)

MATERIALS AND METHODS

Isolates. Thirty-one clinical and two food isolates, including one recovered from a patient's refrigerated coleslaw (81-861) and cucumber (81-1087), associated with the 1981 outbreak in the Maritime Provinces of Canada were examined (Table 1).

The nine clinical isolates recovered from the 1983 Massachusetts epidemic were provided by Robert Weaver, Centers for Disease Control, Atlanta, Ga. The following clinical isolates were examined: F4636, F4637, F4638, F4639, F4640, F4641, F4642, F4644, and F4645. Raw milk isolates were obtained from the following sources: isolates V7 and V37CE (J. Heisick, Food and Drug Administration, Minneapolis, Minn.), F5069 (C. Donnelly, University of Vermont, Burlington), and F1057 and F1109 (Peggy Hayes, Centers for Disease Control). The isolates were of serotype 4b with the exception of the raw milk isolate V7, which was of serotype 1/2a.

L. monocytogenes isolates (n = 49) associated with the 1985 California outbreak were examined. The following clinical isolates (n = 18) were provided by Robert Weaver: F7149, F7150, F7257, F7206, F7207, F7213, F7214, F7215, F7223, F7224, F7225, F7226, F7231, F7243, F7244, F7245, F7248, and F7250. An additional human isolate (DA-3) from a clinical listeriosis case diagnosed in Arizona was provided by Joseph Lovett, Food and Drug Administration, Cincinnati, Ohio. A total of 19 cheese strains were evaluated. Ten isolates recovered from unopened cheese packages taken from the suspect cheese factory were provided by Judith Heisick, Food and Drug Administration, Minneapolis. These included 85-354-9185E, 83-354-91840A, 85-354-91818E, 85-354-91820D, 85-354-91839E, 85-354-91832A, 85-354-91838A, 85-354-91821C, 85-354-91815B, and 85-354-91833B. An isolate recovered from a patient's refrigerated cheese made by the suspect factory (F2365) and strains (F2379, F2380, F2381, F2382, F2385, F2386, F2387, F2392) recovered from the incriminated brand of cheese purchased in Los Angeles area markets were examined. The isolates recovered from cotija fresco (F2382)- and panela (F2387, F2392)-style cheeses manufactured by the incriminated plant were provided by Peggy Hayes. *L. monocytogenes* isolates recovered from the cheese plant environment (LALM-1, LALM-6, LALM-9), including insects (LALM-2, LALM-3), and from other dairy products made from the milk source incriminated in the outbreak (LALM-4, LALM-5, LALM-8, LALM-11) were examined (Table 2). An isolate from sodium caseinate (LALM-10), which was used in the manufacture of a sour cream-like product, was compared with an isolate recovered from that product (85-354-920). These strains were provided by Richard Ruby, Food and Drug Administration, Los Angeles, Calif.

TABLE 2. Isolates of *L. monocytogenes* associated with the 1985 California outbreak recovered from factory environment and additional cheese products

Isolate[a]	Origin
LALM-1	Cooler condensate
LALM-6	Pasteurizer
LALM-9	Floor drain
LALM-2	Ants, flies, debris
LALM-3	Ants
LALM-4	Cotija fresco
LALM-5	Cheese curd
(85-354-920)	Mexican sour cream
LALM-8	Mexican-style cheese
LALM-10	Sodium caseinate
LALM-11	Mexican-style cheese

[a] Provided by R. Ruby, Food and Drug Administration, Los Angeles.

Cultures were maintained on Trypticase soy agar slants supplemented with 0.6% yeast extract. All isolates were serotyped (19) and biochemically confirmed as *L. monocytogenes*, based on hemolysis and reactivity in xylose and rhamnose, by G. Wagner, Food and Drug Administration, Minneapolis.

DNA extraction and restriction enzyme analysis. Bacterial isolates were plated onto brain heart infusion agar containing 5% defibrinated bovine blood and incubated at 37°C for 24 h. Bacterial lawns from three plates each per isolate were harvested in phosphate-buffered saline (0.01 M, pH 7.2), pelleted (8,000 × g, 30 min, 4°C), suspended in 0.4 ml of 25% sucrose in TE buffer (1 mM EDTA, 10 mM Tris [pH 7.2]), and frozen (−20°C) until the time of DNA preparation. DNA was extracted as follows (27). The dense bacterial suspension (300 μl) was transferred to 13- by 51-mm polyallomer ultracentrifuge tubes (Beckman Instruments, Palo Alto, Calif.), and lysozyme (Sigma Chemical Co., St. Louis, Mo.) was

added (130 μl at 50 mg/ml). After incubation (37°C, 1 h), 20 μl of proteinase K (Sigma) at 50 mg/ml and 130 μl of 0.5 M EDTA (pH 8.0) were added and mixed gently. Cells were lysed by the addition of 130 μl of Sarkosyl (25%, sodium salt of *N*-laurylsarcosine; Sigma) and incubated overnight at 65°C. DNA was recovered by equilibrium centrifugation (416,000 × *g*, 4 h, 15°C) of the lysate in CsCl (1.25 g/ml of 50 mM Tris–5 mM EDTA–5 mM NaCl) in a VTi 65.2 rotor (Beckman). The viscous DNA band was harvested from the side of the centrifuge tube with a 16-gauge needle and dialyzed extensively against TE buffer (pH 8.0). The final DNA concentration was determined in an Ultra-Spec II spectrophotometer (model 4050; LKB Instruments, Gaithersburg, Md.), with an optical density at 260 nm of 1 being equal to 50 μg of DNA per ml (21).

For restriction endonuclease digestion, 2 μg of purified DNA was incubated (37°C, 3 to 4 h) with *Hha*I (Bethesda Research Laboratories, Gaithersburg, Md.) in a 20-μl reaction mixture in buffer supplied by the manufacturer. DNA fragments were separated on 0.8% agarose gels (60 V, 16 h) in a horizontal gel bed (120 by 25 cm) with Tris-borate-EDTA (89 mM Tris borate, 89 mM boric acid, 2 mM EDTA) as the running buffer. At the completion of electrophoresis, gels were stained (1 h) with ethidium bromide (0.125 mg/ml), visualized with shortwave UV light, and photographed with a Kodak 23A red filter (Foto UV 300/mp-4 DNA Photographic Transilluminator System; Fotodyne, Inc., New Berlin, Wis.).

RESULTS

Restriction enzyme analysis. Thirty-one clinical isolates and two food isolates associated with the Canadian outbreak were examined. The restriction enzyme pattern of isolate 81-861, recovered from a patient's refrigerated coleslaw and incriminated as the food vehicle in the outbreak (Fig. 1), was selected as the reference standard against which the remaining strains were compared. After DNA digestion with *Hha*I, the restriction enzyme patterns of 28 clinical isolates were identical to that of the reference isolate (81-861). Because of their identical DNA electrophoretic pattern, these 28 isolates were considered to be of the epidemic strain. The restriction enzyme patterns of isolates 81-505 (Fig. 1A, lane 6), 81-507 (Fig. 1A, lane 7), and 81-859 (Fig. 1B, lane 11), however, were clearly different from that of the reference isolate. In addition, 16 clinical samples from Canada not associated with the outbreak were examined. None exhibited the restriction enzyme pattern of the epidemic strain (data not shown).

The nine clinical and five raw milk isolates associated with the Massachusetts outbreak were compared. The nine clinical isolates exhibited the identical restriction enzyme pattern after digestion of DNA with *Hha*I (Fig. 2A). However, the restriction enzyme profiles of the two representative human isolates F4641 and F4642 (Fig. 2B, lanes 1 and 2) differed from those of the five raw milk isolates (Fig. 2B, lanes 3 through 7). These milk isolates were recovered after the outbreak, during a survey of raw milk sources supplying the pasteurizer (12). The serotype 4b milk isolates V37CE, F1057, F1109, and F5069 (Fig. 2B, lanes 4 through 7) exhibited a restriction enzyme pattern that differed from that of the human strains F4641 and F4642 (Fig. 2B, lanes 1 and 2). The restriction enzyme pattern of the serotype 1/2A isolate V7 (Fig. 2B, lane 3) differed from that of the epidemic-associated strains. Therefore, unlike the patterns in the Canadian epidemic, the restriction enzyme patterns of the suspect food and clinical isolates associated with the Massachusetts outbreak differed.

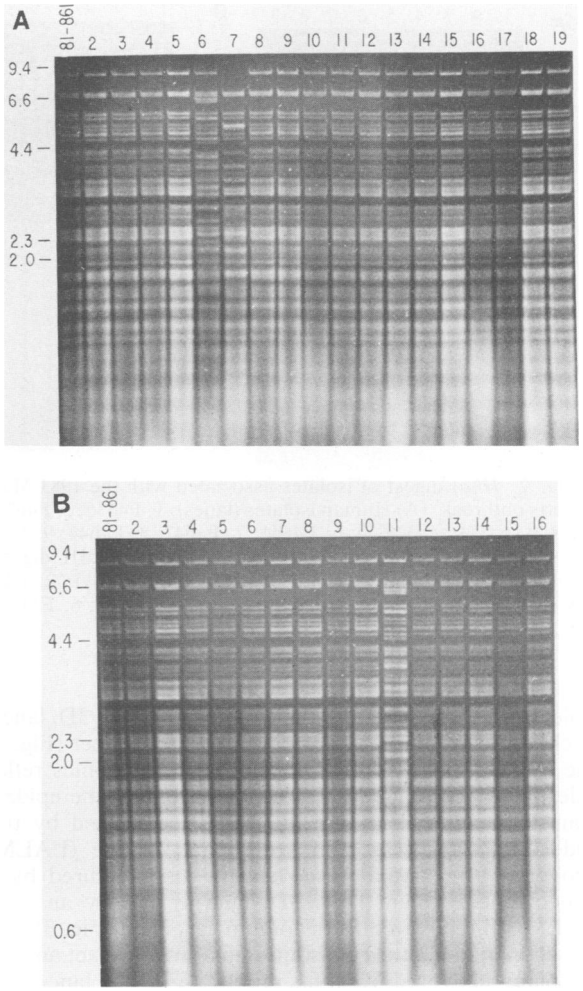

FIG. 1. *Hha*I digest of isolates associated with the 1981 Canadian outbreak. (A) Lanes: 1, 81-861; 2, 81-498; 3, 81-499; 4, 81-501; 5, 81-502; 6, 81-505; 7, 81-507; 8, 81-509; 9, 81-511; 10, 81-512; 11, 81-515; 12, 81-516; 13, 81-558; 14, 81-590; 15, 81-591; 16, 81-592; 17, 81-618; 18, 81-619; 19, 81-637. (B) Lanes: 1, 81-861; 2, 81-678; 3, 81-679; 4, 81-680; 5, 81-682; 6, 81-694; 7, 81-711; 8, 81-712; 9, 81-739; 10, 81-784; 11, 81-859; 12, 81-861; 13, 81-884; 14, 81-886; 15, 81-923; 16, 81-1087. Size markers (kilobases) were derived from a *Hin*dIII digest of bacteriophage λ.

L. monocytogenes isolates associated with the California outbreak were evaluated. Human isolates from Los Angeles County (n = 18) and an additional isolate recovered from Arizona (DA-3) were compared. The restriction enzyme patterns of these human isolates were identical (Fig. 3A). The restriction enzyme profiles of cheese isolates (n = 10) recovered from unopened cheese packages confiscated from the factory were identical (Fig. 3B) and indistinguishable from those of the human isolates (Fig. 3A). This restriction enzyme pattern was also exhibited by isolate F2365, recovered from a patient's refrigerated cheese (Fig. 3C, lane 1), by isolates recovered from packages purchased in supermarkets in Los Angeles (Fig. 3C, lanes 2 through 9), and by isolates recovered from the cheese plant (Fig. 3C, lanes 11 through 14). This restriction enzyme profile was also displayed by cheese plant environmental isolates recovered from the

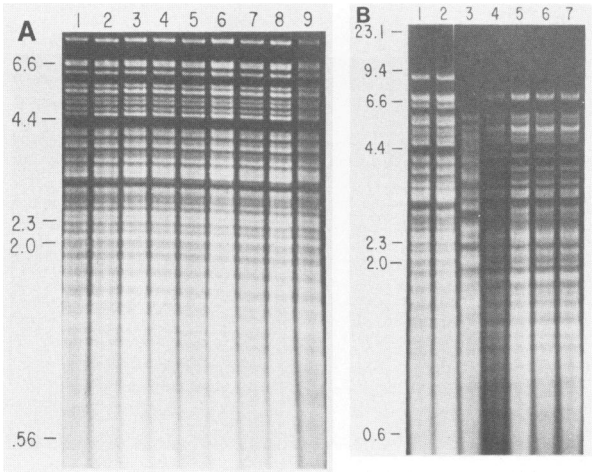

FIG. 2. *Hha*I digest of isolates associated with the 1983 Massachusetts outbreak. (A) Human isolates (lanes): 1, F4636; 2, F4637; 3, F4638; 4, F4639; 5, F4640; 6, F4641; 7, F4642; 8, F4644; 9, F4645. Size markers (kilobases) were derived from a *Hin*dIII digest of bacteriophage λ. (B) Human isolates (lanes): 1, F4641; 2, F4642. Raw milk isolates (lanes): 3, V7; 4, F5069; 5, F1057; 6, F1109; 7, V37CE.

cooler condensate (Fig. 3D, lane 1), insects (Fig. 3D, lanes 2, 3), cheese curd (Fig. 3D, lane 5), and pasteurizer (Fig. 3D, lane 6). The identity of restriction enzyme profiles reflects widespread contamination of the factory with the epidemic strain. The restriction enzyme pattern exhibited by these epidemic strains was also seen in an isolate (LALM-4) recovered from cotija fresco cheese manufactured by the suspect cheese plant (Fig. 3D, lane 4) and by an isolate (LALM-10) recovered from sodium caseinate (Fig. 3D, lane 9) used in manufacturing a sour cream-like product and from an isolate recovered from this product (Fig. 3D, lane 7). The sour cream-like product was made by the plant supplying raw milk to the cheese plant in question. The restriction enzyme pattern of an isolate (LALM-9) from the cheese plant floor drain (Fig. 3D, lane 10) was clearly different, although it was of the epidemic serotype 4b. The restriction enzyme pattern of isolate LALM-11 of serotype 1a from a Mexican-style cheese product (Fig. 3D, lane 11) was also distinct.

Twenty-nine clinical isolates of serotype 4b originating from Los Angeles County and not associated with the 1985 epidemic were examined. None exhibited the DNA electrophoretic pattern of the epidemic strains (data not shown).

When the strains associated with each of the epidemics are compared by restriction enzyme analysis, their characteristic profiles are evident (Fig. 4).

DISCUSSION

The four major food-associated listeriosis outbreaks that occurred in North America involved the 4b serotype (10, 13, 17, 27). We have previously reported that the restriction enzyme *Hha*I offered the best discrimination of field isolates that were serologically indistinguishable (28). In this study we used *Hha*I to discriminate epidemic strains of *L. monocytogenes* of serotype 4b.

Thirty-three isolates associated with the Canadian epidemic, in which coleslaw was the vehicle of infection, were examined. The restriction enzyme pattern exhibited by the *L. monocytogenes* isolate cultured from a patient's refrigerated coleslaw (81-861) was also exhibited by 28 clinical strains recovered during that outbreak. The three clinical isolates (81-505, 81-507, 81-859) with markedly different restriction enzyme patterns may represent sporadic cases that occurred concurrently and thus are superimposed on the epidemic. Alternatively, human infection may have resulted from consumption of three different virulent *Listeria* clones in the coleslaw.

Phage typing (3, 10, 17, 25, 26) has been used to characterize *L. monocytogenes* isolates recovered from food-borne epidemics. In this study complete bacteriophage typing data were available for comparison with restriction enzyme profiles of isolates recovered from the Canadian outbreak. The restriction enzyme profile, phage type, and electrophoretic type of strains 81-505, 81-507, and 81-859 differed from those of strain 81-861. Strains 81-859 and 81-505 were both nontypable by phage typing but were of the same electrophoretic type and exhibited the same restriction enzyme pattern.

The nine human isolates associated with the 1981 Massachusetts epidemic exhibited the same restriction enzyme pattern. This pattern was clearly different from that exhibited by Canadian and California epidemic strains. We have observed this Massachusetts restriction enzyme pattern in other serotype 4b isolates, including three isolates recovered from ice cream in the Midwest, a bovine milk strain, and two isolates obtained from brains of two sheep that displayed clinical signs of listeriosis. This suggests that this strain of *L. monocytogenes*, with the potential to cause epidemics, is widespread. For the Massachusetts outbreak, pasteurized whole and 2% milk were incriminated as the vehicles of infection on the basis of epidemiological retrospective analysis. *L. monocytogenes* was not cultured from the pasteurized product, and the pasteurizer was operating at temperatures and holding times that exceeded the required limits (8, 10). Clinical symptoms compatible with the diagnosis of listeriosis had been reported earlier in cows supplying milk to the pasteurization facility, although no bovine isolates are available for study. Multiple serotypes of *L. monocytogenes* were recovered from raw milk supplying the pasteurizer months after the onset of the outbreak (12). However, the restriction enzyme profiles of the four serotype 4b raw milk isolates (V37CE, F1057, F1107, F5069), like their phage type profiles (10), differed markedly from those of the human epidemic strain pattern. That raw milk was sampled after the outbreak (10, 12) or was unrelated to this outbreak may explain the observed differences in restriction enzyme patterns of raw milk and clinical isolates.

The restriction enzyme profiles of 49 strains recovered from cheese, human, and environmental samples associated with the 1985 California epidemic involving Mexican-style soft cheese were compared. The restriction enzyme patterns of 18 human isolates from Los Angeles County were indistinguishable from that of an isolate (DA-3) obtained from an elderly diabetic who developed listeriosis after consuming the suspect brand of Mexican-style cheese purchased in Arizona. The restriction enzyme profiles of the *L. monocytogenes* strains recovered from unopened cheese packages confiscated at the factory and from assorted cheese varieties marketed under the suspect brand name purchased from supermarkets in Southern California were identical to those of the human isolates. The possible sources of *Listeria* contamination are diagrammed in Fig. 5. *L. monocytogenes* was not recovered from the dairy herds supplying the milk to the cheese factory (8). This has been ascribed to the delay in testing the dairy herds for nearly 2 weeks after the closure of

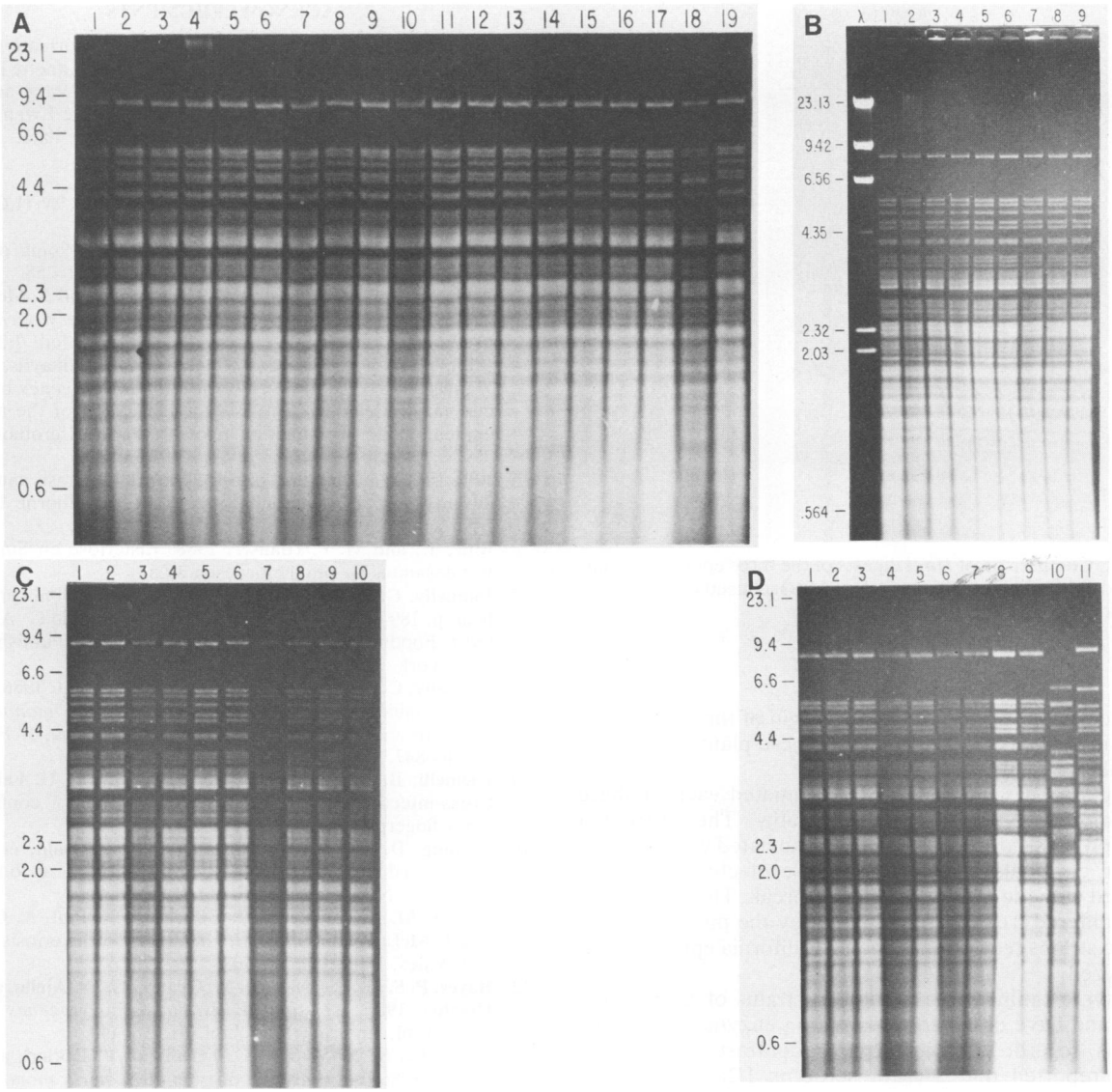

FIG. 3. *Hha*I digest of isolates associated with the 1985 California outbreak. (A) Human isolates (lanes): 1, F7149; 2, F7150; 3, F7257; 4, F7206; 5, F7207; 6, F7213; 7, F7214; 8, F7215; 9, F7223; 10, F7224; 11, F7225; 12, F7226; 13, F7231; 14, F7243; 15, F7244; 16, F7245; 17, F7248; 18, F7250; 19, DA-3. (B) Cheese isolates (lanes): 1, F2365; 2, F2379; 3, F2380; 4, F2381; 5, F2382; 6, F2385; 7, F2386; 8, F2387; 9, F2392. (C) Cheese isolates from unopened packages (lanes): 1, 85-354-9185E; 2, 85-354-91840A; 3, 85-354-91818E; 4, 85-354-91820D; 5, 85-354-91839E; 6, 85-354-91832A; 7, 85-354-91838A; 8, 85-354-91821C; 9, 85-354918B; 10, 85-354-91833B. (D) Environmental isolates (lanes): 1, LALM-1; 2, LALM-2; 3, LALM-3; 4, LALM-4; 5, LALM-5; 6, LALM-6; 7, 85-354-920; 8, LALM-8; 9, LALM-10; 10, LALM-9; 11, LALM-11. Size markers (kilobases) were derived from a *Hin*dIII digest of bacteriophage λ.

the cheese plant, to the insensitivity of culture methods (17, 22), and to generalized plant contamination unrelated to bovine listeriosis (15). Although the dairy cows were reportedly fed silage 3 months before the outbreak, bovine listeriosis was not reported in the herds (17). Mascola et al. (22) reported that the milk plant supplying raw milk to the cheese factory produced a sour cream-like product that was contaminated with *L. monocytogenes* of the same phage type seen in the Mexican-style soft cheese. This isolate (85-354-970) and that recovered from the sodium caseinate (LALM-10) used in sour cream production at the milk supplier exhibited the RE pattern of the epidemic type. Whether the *L. monocytogenes* in the milk plant originated from human carriers or from a long-standing environmental source is unknown. The identical restriction enzyme patterns of strains from the cooler condensate, pasteurizer, and cheese curd indicate widespread contamination of the cheese plant with epidemic strain. *L. monocytogenes* may have originated from contaminating sources already present in the cheese factory, including floor drains, factory workers, and cooler condensates. That *L. monocytogenes* could also be recovered from ants and flies in the cheese plant reflects the level of factory sanitation. The cheese factory records indicated that milk deliveries exceeded the capacity of the pasteurizer (17). In reviewing food-borne listeriosis, the World Health Organization working committee concluded that *L. monocytogenes* should be considered an environmental contaminant (1). In the absence of a documented

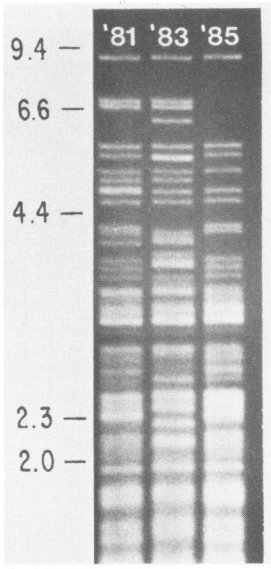

FIG. 4. Comparison of *Hha*I digests of the three epidemic strains associated with the Canadian (1981), Massachusetts (1983), and California (1985) outbreaks.

animal source in this outbreak, the origin of the *L. monocytogenes* in the milk supply and the cheese plant is left open to speculation (15, 22).

Restriction enzyme analysis differentiated each of these three major food-borne epidemic strains. The restriction enzyme profiles exhibited by strains associated with the 1981 Canadian outbreak differed from those of clinical isolates associated with the Massachusetts outbreak. These patterns, in turn, differed from those exhibited by the majority (48 of 49) of the strains recovered from the California epidemic that we analyzed.

We have examined more than 200 strains of *L. monocytogenes* and have obtained a restriction enzyme pattern for all strains, regardless of serotype. In contrast, McLaughlin (23) has reported that 37% of serotype 1/2a and 82% of serotype 4b strains can be characterized by bacteriophages. The ease with which restriction enzyme analysis can be applied to all strains of *L. monocytogenes* warrants its application in deciphering the molecular epidemiology of listeriosis outbreaks or suspected cases of cross-infection.

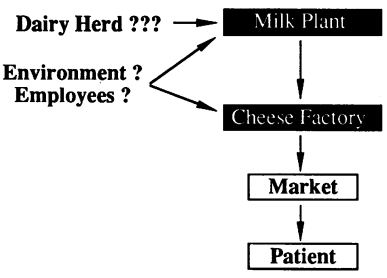

FIG. 5. Flow chart depicting possible routes of *L. monocytogenes* contamination. Although dairy cows are listed, no isolates were recovered from the milk herd supplying the milk plant. The possible recontamination of the milk and cheese plant from employees or environmental sources is indicated.

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REFERENCES

1. **Anonymous.** 1988. Foodborne listeriosis. Bull. W.H.O. **66**:421–428.
2. **Audurier, A., and C. Martin.** 1989. Phage typing of *Listeria monocytogenes*. Int. J. Food Microbiol. **8**:251–258.
3. **Audurier, A., A. G. Taylor, B. Carbonelle, and J. McLaughlin.** 1984. A phage typing system for *Listeria monocytogenes* and its use in epidemiological studies. Clin. Invest. Med. **7**:229–232.
4. **Bibb, W. F., B. Schwartz, B. G. Gellin, B. D. Plikaytis, and R. E. Weaver.** 1989. Analysis of *Listeria monocytogenes* by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. Int. J. Food Microbiol. **98**:233–239.
5. **Bille, J.** 1989. Anatomy of a foodborne listeriosis outbreak, p. 29–36. *In* E. H. Kampelmacher (ed.), Foodborne listeriosis. Behr's, Verlag GmbH, Wiesbaden, Germany.
6. **Bille, J., and M. P. Glauser.** 1988. Listeriose en Suisse. Bull. Bundesamtes Gesundheitswesen **3**:28.
7. **Donnelly, C. W.** 1990. Resistance of *Listeria monocytogenes* to heat, p. 189–193. *In* A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), Foodborne listeriosis. Elsevier Science Publishing, Inc., New York.
8. **Donnelly, C. W., E. H. Briggs, and G. J. Baigent.** 1986. Analysis of raw milk for the epidemic serotype of *L. monocytogenes* linked to an outbreak of listeriosis in California. J. Food Prot. **49**:846–847.
9. **Facinelli, B. P., E. Varaldo, C. Casolari, and U. Fabio.** 1988. Cross-infection with *Listeria monocytogenes* confirmed by DNA fingerprinting. Lancet **ii**:1247–1248.
10. **Fleming, D. W., S. L. Cochi, K. L. Macdonald, et al.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312**:404–407.
11. **Hall, S. M., N. Crofts, R. J. Gilbert, P. N. Pini, A. G. Taylor, and J. McLaughlin.** 1988. Epidemiology of listeriosis, England and Wales. Lancet **ii**:502–503.
12. **Hayes, P. S., J. C. Feeley, L. M. Graves, G. W. Ajello, and D. W. Fleming.** 1986. Isolation of *Listeria monocytogenes* from raw milk. Appl. Environ. Microbiol. **51**:438–440.
13. **Ho, J. L., K. N. Shands, G. Friedland, P. Eckind, and D. W. Fraser.** 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. **146**:520–524.
14. **James, S. S. M., et al.** 1985. Listeriosis outbreak associated with Mexican-style cheese-California. Morbid. Mortal. Weekly Rep. **34**:357–359.
15. **Jasper, E. E.** 1989. Epidemic listeriosis. N. Engl. J. Med. **320**:538.
16. **Kaczmarski, E. B., and D. M. Jones.** 1989. Listeriosis and ready-cooked chicken. Lancet **i**:549.
17. **Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, et al.** 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. **319**:823–828.
18. **Loessner, M. J., and M. Busse.** 1990. Bacteriophage typing of *Listeria* species. Appl. Environ. Microbiol. **56**:1912–1918.
19. **Lovett, J.** 1987. FDA bacteriological analytical manual, suppl. 6 ed. Association of Analytical Chemists, Arlington, Va.
20. **Malinverni, R., J. Bille, C. L. Perret, F. Regli, F. Tanner, and M. P. Glauser.** 1985. Listeriose epidemique. Klin. Lab. **115**:2–10.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Mascola, L., S. L. Fannin, and M. Linnan.** 1989. Epidemic listeriosis. N. Engl. J. Med. **320**:538.
23. **McLaughlin, J.** 1987. *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in humans. J.

Appl. Bacteriol. **63**:1–11.

23a. **Nocera, D., E. Bannerman, J. Rocourt, K. Jaton-Ogay, and J. Bille.** 1990. Characterization by DNA restriction endonuclease analysis of *Listeria monocytogenes* strains related to the Swiss epidemic of listeriosis. J. Clin. Microbiol. **28**:2259–2263.

24. **Olander, A., S. Harlander, and B. Swaminathan.** 1990. Abstr. Annu. Meet. Am. Soc. Microbiol. 1990, P47, p. 286.

25. **Ortel, S.** 1989. Phage typing of *Listeria*. Int. J. Food Microbiol. **8**:241–244.

26. **Rocourt, J., A. Audurier, A. L. Courtieu, et al.** 1985. A multi-center study on the phage typing of *Listeria monocytogenes*. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A **259**:489–497.

27. **Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, et al.** 1983. Epidemic listeriosis—evidence for transmission by food. N. Engl. J. Med. **308**:203–206.

27a. **Wesley, I. V., R. Ruby, J. Heisick, and F. Ashton.** 1988. Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, P54, p. 287.

28. **Wesley, I. V., R. D. Wesley, J. Heisick, F. Harrel, and D. Wagner.** 1990. Restriction enzyme analysis in the epidemiology of *Listeria monocytogenes*, p. 225–238. *In* J. L. Richard (ed.), Symposium on cellular and molecular modes of action of selected microbial toxins in foods and feeds. Plenum Publishing Corp., New York.