APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1994, p. 1547–1553
0099-2240/94/$04.00+0

Vol. 60, No. 5

Application of Multilocus Enzyme Electrophoresis and Restriction Fragment Length Polymorphism Analysis to the Typing of *Listeria monocytogenes* Strains Isolated from Raw Milk, Nondairy Foods, and Clinical and Veterinary Sources

J. HARVEY[1] AND A. GILMOUR[1,2]*

Food Microbiology, Department of Agriculture for Northern Ireland,[1] and The Queen's University of Belfast,[2] Agriculture and Food Science Centre, Belfast, Northern Ireland, United Kingdom

Received 23 November 1993/Accepted 17 February 1994

The powerful discriminatory typing capabilities of multilocus enzyme electrophoresis and restriction fragment length polymorphism analysis were applied to *Listeria monocytogenes* strains from raw milk, nondairy foods, and clinical and veterinary sources. The raw milk and nondairy food strains were sequential isolates obtained over a year-long period from a number of different producers and manufacturers. Results obtained by the two typing methods were in substantial agreement and showed that both raw milk and nondairy foods frequently contain recurrent *L. monocytogenes* strains, thus suggesting that the presence of these organisms in such commodities often arises because of contamination from within their respective processing environments. Most recurrent strains were serogroup 1/2, with only one instance of recurrent serogroup 4 strains. Some recurrent *L. monocytogenes* strains, including the serogroup 4 strains, were found by analysis of multilocus enzyme electrophoresis results to be closely related to clinical and veterinary strains, thus suggesting that strains adapted for survival in the food-processing environment retain their potential for pathogenicity.

Increasing recognition regarding *Listeria monocytogenes* as a food-borne pathogen has elicited a worldwide response by food industries, health agencies, and government bodies concerned with preventing infections caused by the presence of these organisms in food. Achievement of this goal is enhanced by the availability of satisfactory typing methods which allow the tracing of strains in the various links of the food chain from primary producer, raw materials, and processed and retailed products to the consumer. Established methods for characterizing *L. monocytogenes* such as serotyping (16) and phage typing (1) have suffered from insufficient discrimination or poor typeability of strains. More recently, greatly improved discrimination and typeability of *L. monocytogenes* have been attained following the development of molecular typing methods such as restriction enzyme analysis of chromosomal DNA (10, 15) and multilocus enzyme electrophoresis (MEE) (2, 13). MEE has the added advantage of allowing the construction of a genetic framework which can be used to estimate genetic distances between members of the same bacterial species (17).

In Northern Ireland, studies have been carried out to assess the incidence of *Listeria* strains in raw milk, dairy products, and a range of nondairy foods (6, 7). In the work reported here, selected *L. monocytogenes* isolates representative of those obtained over the 1-year period of each of these previous studies together with a number of veterinary and clinical *L. monocytogenes* strains were characterized by restriction enzyme analysis and MEE. The aim of work reported here was to determine the incidence and relatedness of *L. monocytogenes* subtypes from each of these sources.

* Corresponding author. Fax: 44 232 668376.

MATERIALS AND METHODS

Bacterial strains. A total of 141 *L. monocytogenes* strains were studied; of these, 78 were obtained from raw milk during a survey of milk and milk products carried out in 1988 and 1989 (6), 41 were from various foods during a survey of nondairy foods carried out in 1990 and 1991 (7), and 22 were clinical and veterinary strains from various culture collections. The clinical and veterinary strains were chosen to include a wide spectrum of serotypes. Further information on the strains is shown in Tables 1 and 2. All strains were examined by MEE procedures, and in addition, 26 strains from raw milk and 34 strains from nondairy foods were examined by restriction fragment length polymorphism (RFLP) analysis.

MEE. *L. monocytogenes* strains were grown in 150 ml of brain heart infusion broth at 30°C for 18 h on a shaking platform (100 rpm), and extracts for MEE were prepared from harvested cells by freezing and grinding with alumina powder (Sigma A2039) by using a mortar and pestle as described by Nørrung (11). Cell enzymes were extracted by the addition of 1.0 ml of buffer (10 mM Tris-HCl [pH 6.8], 1 mM EDTA, 0.5 mM NADP) followed by centrifugation (12,000 × *g* for 10 min) and storage of the resultant supernatant at −80°C. For each cell extract, the electrophoretic mobilities of 11 different, commonly occurring cellular enzymes were determined by a slight modification of the procedures described by Selander et al. (17). Extracts were subjected to horizontal electrophoresis at a constant 135 V in 11.5% starch gels (Tris-citrate buffer, pH 8.0) for 6 to 7 h until a marker dye (0.5% bromophenol blue in 80% glycerol and 50 mM Tris-HCl, pH 8.0) had moved 10 cm. Each gel was cut horizontally to yield five or six 2-mm-thick slices, and separate gel slices were stained for specific enzymatic activity as described by Selander et al. (17).

The enzymes detected were as follows: 6-phosphogluconate dehydrogenase (EC 1.1.1.44), glucose 6-phosphate dehydroge-

TABLE 1. Origin, isolation date, serotype, MEE type, and RFLP type of *L. monocytogenes* strains isolated from raw milk and nondairy foods

Origin[a]	Isolation date(s)[b]	No. of strains	Serotype	ET and RFLP type[c]
A	Feb'89–Jul'89	9	1/2	37-Q (3, 1); 27-T (6, 3)
		2	4b	13 (1); 18 (1)
B	Dec'88–Sep'89	17	1/2	35-R (12, 4); 36-T (5, 1)
		1	4b	7 (1)
C	Dec'88–Nov'89	19	1/2	19-P (2, 1); 45-N (4, 1); 46-Q (2, 1); 47-M (4, 2); 26-N (3, 1); 30-S (4, 1)
		1	4b	20 (1)
D	May'89–Nov'89	13	1/2	40-N (10, 3); 15-O (3, 1)
E	Apr'89–Oct'89	5	1/2	46-U (5, 2)
F	Nov'89	7	1/2	21 (2); 28-S (5, 2)
G	Oct'89–Nov'89	4	1/2	28-S (4, 2)
H	Sep'90–Jan'91	4	1/2	11-H (2, 1); 42-A (1, 1); 23-I (1, 1)
I	Feb'91–Mar'91	3	1/2	23-B (3, 3)
J	Jul'90–Apr'91	5	1/2	10-K (1, 1); 38-A (1, 1); 24-H (1, 1); 48-J (1, 1); 41 (1)
		5	4	9 (5)
K	May'90–Mar'91	6	1/2	13-C (1, 1); 14-G (1, 1); 39-A (1, 1); 42-A (3, 3)
L	May'90–Feb'91	6	1/2	42-A (6, 6)
M	Jun'90–Sep'90	3	1/2	42-A (3, 3)
N	May'90–Nov'90	3	1/2	43-E (1, 1); 22-D (1, 1); 25-F (1, 1)
O	May'90–Jan'91	6	1/2	42-A (2, 2); 43-D (3, 3); 25-D (1, 1)

[a] Raw milk from milk processing plants A to D and dairy farms E to G, cooked poultry products from manufacturers H to J, cooked meat products from manufacturers K to M, cooked meals from manufacturer N, and processed vegetables from manufacturer O.
[b] Dates are indicated by the first three letters of the month and the last two digits of the year (e.g., Feb'89, February 1989).
[c] Numbers indicate the ET; letters indicate the RFLP type. Not all strains were subjected to RFLP analysis. The first number in parentheses indicates the number of strains examined by MEE; a second number in parentheses indicates the number of strains examined by RFLP analysis.

nase (EC 1.1.1.49), NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12), alanine dehydrogenase (EC 1.4.1.1), catalase (EC 1.11.1.6), phosphoglucomutase (EC 2.7.5.1), α-naphthyl propionate esterase 1 (EC 3.1.1.1), α-naphthyl propionate esterase 2 (EC 3.1.1.1), acid phosphatase (EC 3.1.3.2), fumarase (EC 4.2.1.2), and mannose phosphate isomerase (EC 5.3.1.8). The enzymes examined in the present study had previously been found to be polymorphic for *L. monocytogenes* food strains (7).

TABLE 2. Origin, serotype, and MEE type of clinical and veterinary *L. monocytogenes* strains obtained from various culture collections

Strain[a]	Source	Serotype	ET
NCTC 5214	Ovine	4a	2
CRA 198	Human	4b	4
CLIP 12505	Human	4b	5
CPHL 4	Human (blood)	4b	8
CPHL 5	Human (blood)	4b	8
CPHL 6	Human (CSF[b])	4b	8
VSD 3	Ovine (brain)	4b	8
CRA 433	Human	4b	16
NCTC 4885	Human	4b	17
VSD 4	Bovine (organs)	4b	20
NCTC 4883	Poultry	4c	1
CLIP 12506	Poultry	4c	1
NCTC 10888	Ovine	4d	6
CLIP 12508	Poultry	4e	12
CPHL 2	Human (blood)	1/2a	33
VSD 2	Bovine (blood)	1/2a	34
VSD 1	Ovine fetus (brain)	1/2a	29
VSD 5	Porcine (blood)	1/2a	29
CPHL 1	Human (CSF)	1/2b	3
CPHL 3	Human (CSF and blood)	1/2c	44
NCTC 5348	Human (CSF)	1/2	31
NCTC 5105	Human	3a	32

[a] Culture collections: VSD, Veterinary Science Division, Department of Agriculture for Northern Ireland; CPHL, Central Public Health Laboratory, London, England; NCTC, National Collection of Type Cultures; CLIP, Collection of the Pasteur Institute, Paris, France; CRA, Campden Food and Drink Research Association, Chipping Campden, England.
[b] CSF, cerebrospinal fluid.

Electrophoretic mobilities were established for each enzyme by comparison with the mobilities of enzymes prepared from four control *L. monocytogenes* strains which were included in every electrophoresis run. A number corresponding to the relative anodal migration was assigned to each electromorph (mobility variant), and each unique combination of electromorphs was designated an electrophoretic type (ET). Analysis of the ET data was carried out using a computer program (Centers for Disease Control and Prevention, Atlanta, Ga.) which enables genetic distances between strains to be determined by means of constructing a matrix detailing the dissimilarity between all possible pairs of ETs (18). A dendrogram displaying these genetic relationships is produced by means of the unweighted pair group method using arithmetic averages (19). The program also determines the genetic diversity at each locus (h_i) and the mean genetic diversity over all loci (H) by the method of Nei and Roychoudhury (9). The formulae used for these calculations are as follows: $h_i = (1 - \Sigma x_i^2)[n/(n - 1)]$, where x_i is the frequency of the ith allele and n is the number of alleles, and $H = \Sigma h_i/L$, where L is the number of loci.

RFLP analysis. For preparation of chromosomal DNA, *L. monocytogenes* strains were grown in 50 ml of brain heart infusion broth on a shaking platform (100 rpm) for 18 h at 30°C. Cells were pelleted by centrifugation and lysed with guanidine thiocyanate as described by Saunders (14). Following digestion of DNA samples with the restriction enzyme *Nci*I (Stratagene Ltd., Cambridge, United Kingdom), restriction fragments were separated by electrophoresis in 0.8% agarose, followed by Southern blot hybridization with a DNA probe derived from an *L. monocytogenes* gene library in a λ phage vector (15). In preliminary work the probe was labelled with ^{32}P and subsequently by using an enhanced chemilumines-

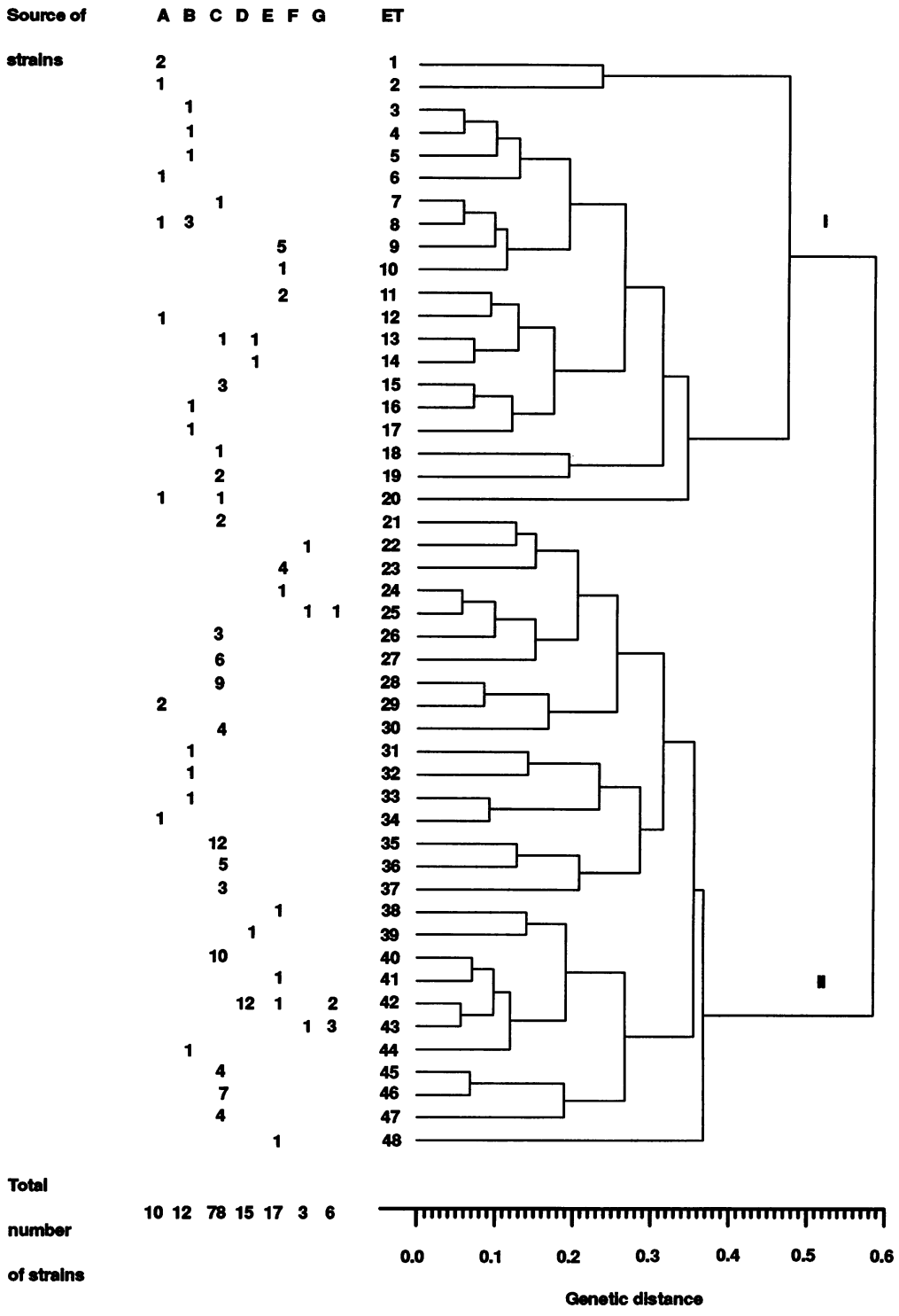

FIG. 1. Dendrogram showing the genetic relationships among 48 ETs of *L. monocytogenes* isolated from raw milk, food, and clinical and veterinary sources. A, veterinary strains; B, clinical strains; C, raw milk; D, cooked meat products; E, cooked poultry products; F, cooked meals; G, salad vegetables.

cence direct labelling system (Amersham, Aylesbury, United Kingdom). The resulting restriction patterns were visualized either by autoradiography or by using enhanced chemiluminescence detection reagents. Each distinct pattern of restriction fragments was designated an RFLP from type A to U. These designations are intended for use within our own laboratory, and their relationship to RFLP types designated by other workers was not determined.

TABLE 3. Distribution of 141 *L. monocytogenes* strains within ET groups I and II

ET group	Serotype(s) (no.) of strains from:		
	Clinical and veterinary sources	Raw milk	Nondairy foods
I	1/2b (1), 4a (1), 4b (9), 4c (2), 4d (1), 4e (1)	1/2 (5), 4b (4)	1/2 (5), 4 (5)
II	1/2 (1), 1/2a (4), 1/2c (1), 3a (1)	1/2 (69)	1/2 (31)

RESULTS

Division of strains into two primary groups. The 141 *L. monocytogenes* strains examined by MEE were found to contain 48 ETs which separated into two ET groups (I and II) at a genetic distance of 0.585 as shown in Fig. 1. Serovars 1/2b, 4a, 4b, 4c, 4d, and 4e were contained in ET group I, while serovars 1/2a, 1/2c, and 3a were in ET group II (Table 3). Serovars 4a and 4c were located in a subdivision of ET group I consisting of ETs 1 and 2 and separated from the remainder of the group at a genetic distance of 0.475. ET groups I and II contained 34 (24.1%) and 107 (75.9%) isolates, respectively, with the majority of milk and food strains in group II and the majority of clinical and veterinary strains occurring in group I.

Genetic diversity. All of the enzymes assayed were polymorphic, the number of alleles at each locus ranging between two and five. Genetic diversity (h) information for all the strains examined is shown in Table 4. Milk strains displayed greater genetic diversity (h = 0.530) than nondairy food strains (h = 0.431).

MEE and RFLP analysis identify recurrent strains. From the information presented in Fig. 2 and 3 it can be seen that a number of ETs were found to recur, for periods ranging from a few months up to a year, in samples of raw milk and nondairy foods obtained from the same manufacturer. This finding was confirmed by the results of RFLP typing. From the information shown in these figures it can be seen that when results of both methods are available, each method for the most part places the same strains into the same groups. Although there was good correlation between typing results obtained using MEE and RFLP analysis, there was not complete agreement. For strains from raw milk, RFLP types N and Q each yielded more than one ET. Similarly for strains from nondairy foods, RFLP types A, D, and H were each composed of more than one ET. Also in the case of isolates from nondairy foods, ETs 43 and 23 each contained more than one RFLP type.

Relatedness of ETs from different sources. From the MEE results shown in Fig. 1, it can be seen that ETs were not shared between strains from either of the three different sources (raw milk, nondairy foods, and clinical or veterinary sources) except in the cases of ET 13 (one strain from raw milk and one strain from nondairy food) and ET 20 (one strain from a veterinary source and one strain from raw milk). However, a number of clusters (ETs 7 to 10, ETs 11 to 17, ETs 21 to 23, ETs 24 to 27, ETs 28 to 30, and ETs 40 to 44), each closely related at a genetic distance of <0.18, were found to encompass strains from more than one source.

TABLE 4. Genetic diversity (h) at 11 enzyme loci among 48 ETs of *L. monocytogenes*

Enzyme locus[a]	h[b]			
	Of strains from:			Mean
	Clinical and veterinary sources	Milk	Food	
6PD	0.500	0.471	0.527	0.511
G6D	0.633	0.693	0.495	0.614
GP1	0.633	0.673	0.385	0.614
ALD	0.458	0.451	0.264	0.393
CAT	0.433	0.601	0.560	0.533
PGM	0.125	0.471	0.582	0.414
EST1	0.000	0.111	0.000	0.042
EST2	0.592	0.739	0.648	0.692
ACP	0.592	0.725	0.473	0.627
FUM	0.633	0.471	0.440	0.528
MPI	0.658	0.425	0.363	0.488
Mean	0.478	0.530	0.431	0.496

[a] 6PD, 6-phosphogluconate dehydrogenase; G6D, glucose 6-phosphate dehydrogenase; GP1, NAD-dependent glyceraldehyde 3-phosphate dehydrogenase; ALD, alanine dehydrogenase; CAT, catalase; PGM, phosphoglucomutase; EST, α-naphthyl propionate esterase; ACP, acid phosphatase; FUM, fumarase; MPI, mannose phosphate isomerase.

[b] See Materials and Methods for formula by which genetic diversity is calculated.

DISCUSSION

The division of the *L. monocytogenes* strains examined in this study into two ET groups and the location of all known 1/2b and 4b strains within group I accord with previous findings (2, 13). The occurrence of serovars 4a and 4c in a subdivision of ET group I has not previously been reported. It would be of value to examine a larger number of 4a and 4c *L. monocytogenes* strains to ascertain if these serovars constitute a genetically distinct group. Both MEE and RFLP analysis were sufficiently discriminatory to enable the recognition of recurrent *L. monocytogenes* strains in both raw milk and nondairy foods. That there was not complete agreement between the two typing methods is not surprising since each measures different cell characteristics. MEE detects differences in net electrostatic charge of proteins which arise from variations in their amino acid composition, and since some amino acid substitutions do not affect electrostatic charge (17), this would mean that some differences between strains may not be detected by this technique. In the case of RFLP analysis, strain differences could remain undetected if the varying nucleotide does not lie within an appropriate restriction site or if a specific nucleic acid probe which hybridizes with an inappropriate part of the genome is used (14).

Systematic contamination of cheese factories by *L. monocytogenes* has been reported in Germany (5) and the United States (21), while a study in Switzerland (3) concluded that *L. monocytogenes* strains isolated from meat originated mainly from the processing environment rather than directly from animals. The recurrent strains identified in the present study may represent *L. monocytogenes* clones adapted to particular processing environments. These strains were isolated at low levels from raw milk (<1.0 CFU ml^{-1}) and nondairy foods (<100 CFU g^{-1}) obtained from a number of different milk-processing plants, dairy farms, and food-processing factories throughout Northern Ireland (6, 7). It seems that the propensity of *L. monocytogenes* to persist in various food production and processing environments with subsequent contamination, albeit at low levels, of intermediate and final food products is quite widespread. Thus, our investigations confirm and extend the conclusions reached in some previous studies (3, 5, 21).

The findings with respect to recurrent strains in raw milk were different from those for nondairy foods. Recurrent strains

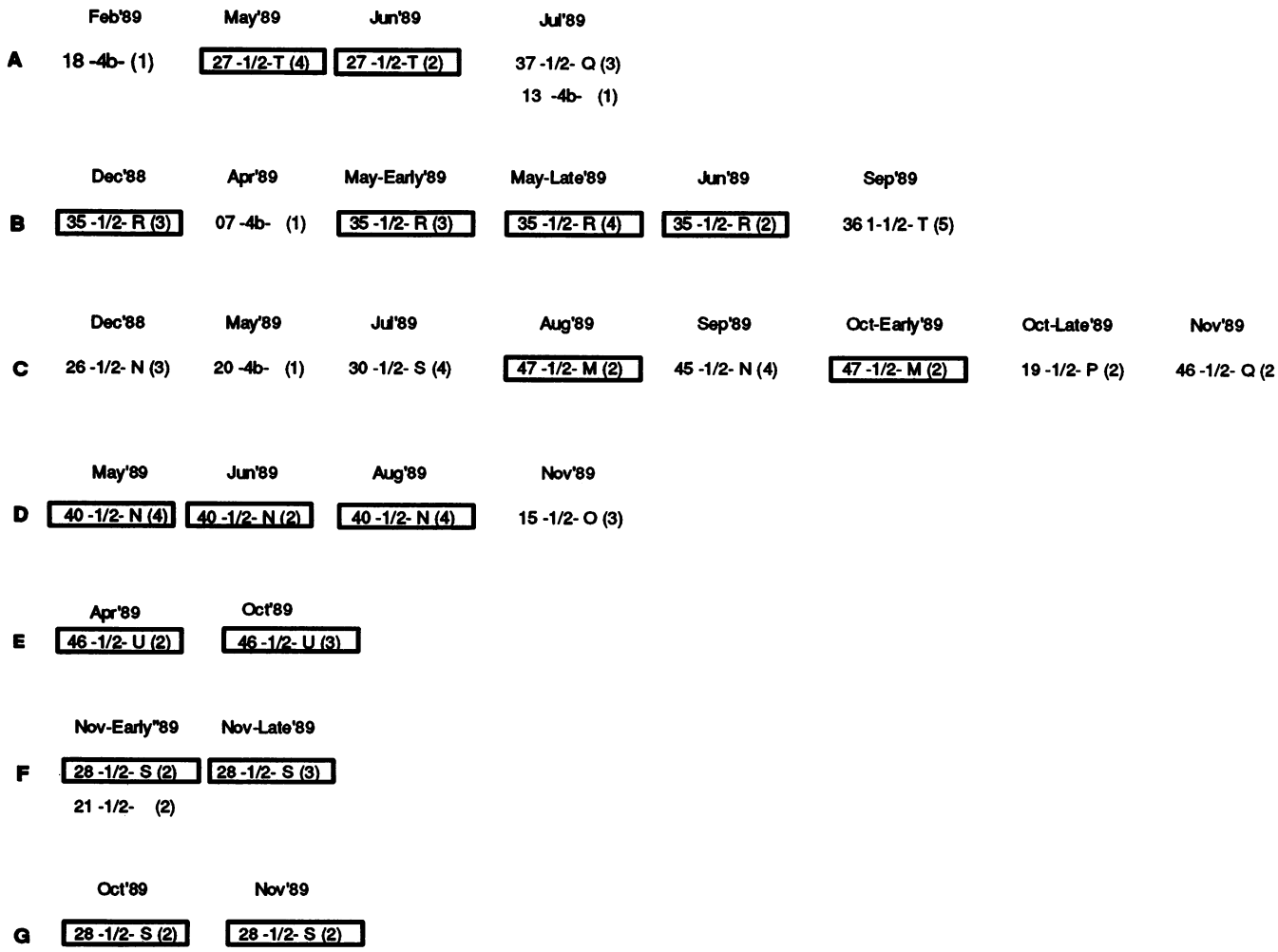

FIG. 2. Identification of recurrent and nonrecurrent *L. monocytogenes* strains in sequential samples of raw milk from processors A to D and dairy farms E to G by means of MEE, serotyping, and RFLP typing. Recurrent strains are boxed. Typing results are shown in the sequence ET (7 to 47)-serotype (1/2 or 4b)-RFLP type (M to U). The numbers of strains examined by MEE and serology are shown in parentheses. Not all strains were typed by RFLP analysis. See Table 1 for the numbers of strains examined by RFLP typing.

in raw milk were, in the main, specific to each manufacturer, although raw milk from two dairy farms (F and G) did have the same recurrent strain. In the case of nondairy foods, however, the *L. monocytogenes* type 42-1/2-A strain was found to be widespread and in several cases recurrent in a wide range of nondairy foods from different manufacturers (Fig. 3). The frequent occurrence of this type in nondairy foods accounts for the lower genetic diversity revealed by MEE for strains from this source compared with that for *L. monocytogenes* strains from raw milk (Table 4). Nørrung and Skovgaard (12) in their study of the epidemiology of *L. monocytogenes* in Denmark also found strains from cooked meat products to be less genetically diverse than those from dairy products.

The question of whether all *L. monocytogenes* strains isolated in food must be regarded as a threat to human health has been posed (8) and to date remains open. Evidence gained from studies using MEE and based on epidemiological information first indicated the existence of especially pathogenic clones which are marked by serovar 4b (2, 13). However, Hof and Rocourt (8) have commented that serovar 4b strains were reported for only 40 to 44% of sporadic human listeriosis cases in Sweden and the United States in 1989, indicating that serogroup 1/2 isolates are also often involved in human disease. In addition Brosch et al. (4) have demonstrated, using a mouse pathogenicity test, that a proportion of both serovar 1/2 (including 1/2c strains which would be classified in ET group II) and 4b *L. monocytogenes* strains are highly virulent.

In our own studies, recurrent *L. monocytogenes* strains are classified into both serogroups 1/2 and 4. Serogroup 4 *L. monocytogenes* strains (ET 9) located in ET group I and closely related to ETs representative of known pathogenic strains (Fig. 1) were identified as recurrent in poultry products from processor J (Fig. 3). Studies in Denmark (12) and Australia (20) showed that certain ET group I, serogroup 4 *L. monocytogenes* clinical strains were also commonly found in food. However, the majority of our recurrent strains were serogroup 1/2 and located in ET group II and thus may be thought to have a reduced virulence compared with that of ET group I strains. Nevertheless, their potential threat to health cannot be disregarded since from information shown in Fig. 1 and Table 2 it can be seen that the cluster comprising ETs 40 to 44 includes the recurrent strains 42-1/2-A from nondairy foods and 40-1/

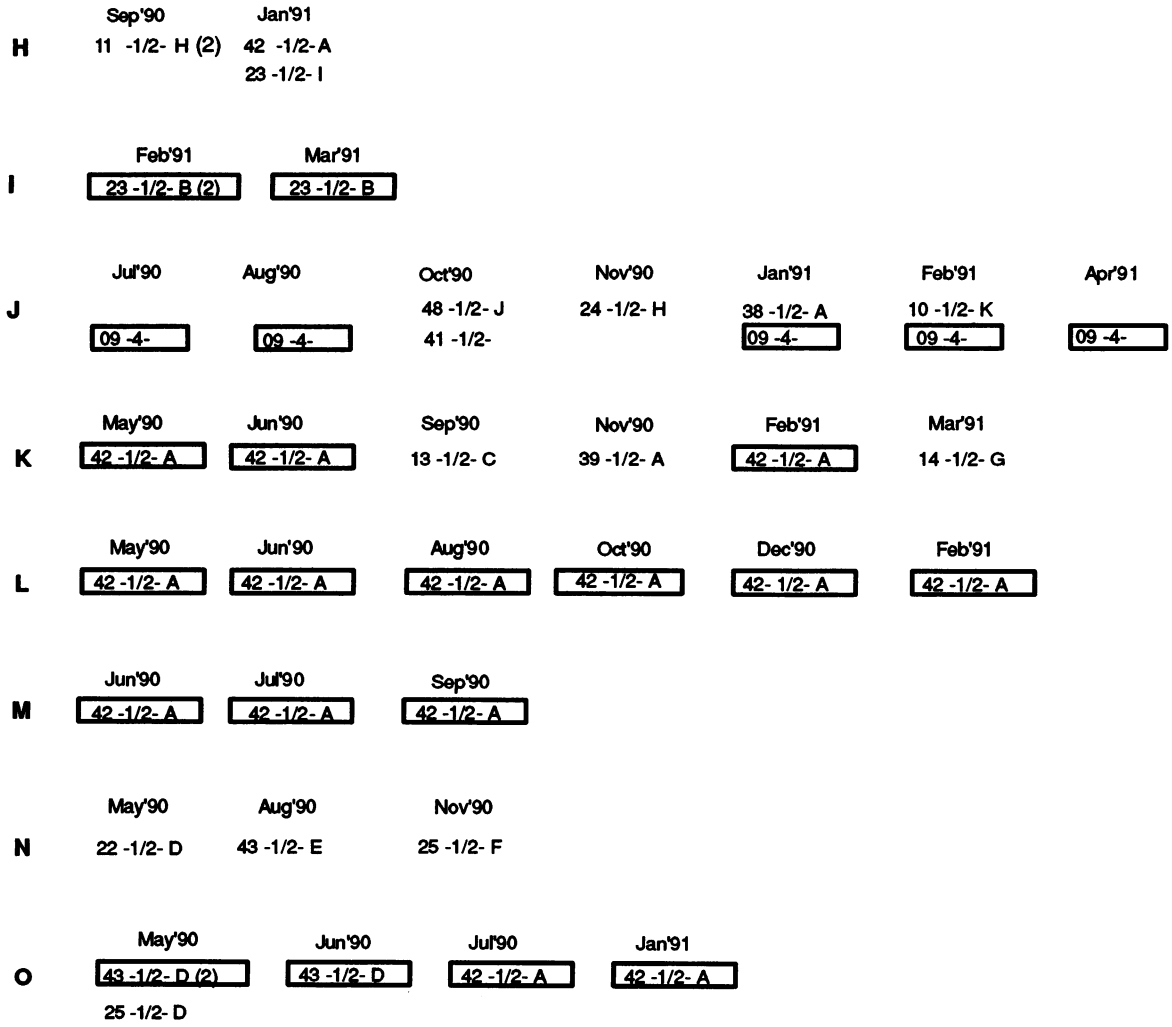

FIG. 3. Identification of recurrent and nonrecurrent *L. monocytogenes* strains in sequential samples of products from manufacturers H to J (cooked poultry), K to M (cooked meat), N (cooked meals), and O (processed vegetables) by MEE, serotyping, and RFLP typing. Recurrent strains are boxed. Typing results are shown in the sequence ET (9 to 48)-serotype (1/2 or 4)-RFLP type (A to K). The numbers of strains examined by MEE and serology are shown in parentheses. Not all strains were typed by RFLP analysis. See Table 1 for the numbers of strains examined by RFLP typing.

2-N from raw milk as well as strain 44-1/2c from a human listeriosis case. Similarly, the cluster containing ETs 28 to 30 contains recurrent strain 28-1/2-S from raw milk at two dairy farms and the strain 29-1/2a from cases of ovine and porcine listeriosis. The study in Denmark (12) also demonstrated that a number of *L. monocytogenes* strains from clinical cases and cooked meat products in ET group II shared the same ET. The findings reported in the present study indicate that the possible colonization of food production and processing environments by *L. monocytogenes*, resulting in contamination of foods being processed, occurs more frequently than has hitherto been reported. Adaptation of *L. monocytogenes* for survival in such environments may not necessarily result in a loss of pathogenic potential.

ACKNOWLEDGMENTS

We thank W. McBurney and D. Norwood for excellent technical assistance; N. A. Saunders of the Central Public Health Laboratory, Colindale, London, United Kingdom, for the gift of the DNA probe used in this study; and B. D. Plikaytis of Centers for Disease Control and Prevention, Atlanta, Ga., for the provision of the program used to analyze ET data.

REFERENCES

1. **Audurier, A., R. Chatelain, F. Chalons, and M. Piechaud.** 1979. Lysotypie de 823 souches de *Listeria monocytogenes* isolees en France de 1958 à 1978. Ann. Microbiol. (Paris) **130B:**179–189.
2. **Bibb, W. F., B. G. Gellin, R. Weaver, B. Schwartz, B. D. Plikaytis, M. W. Reeves, R. W. Pinner, and C. V. Broome.** 1990. Analysis of clinical and food-borne isolates of *Listeria monocytogenes* in the United States by multilocus enzyme electrophoresis and application of the method to epidemiological investigations. Appl. Environ. Microbiol. **56:**2133–2141.
3. **Boerlin, P., and J.-C. Piffaretti.** 1991. Typing of human, animal, food, and environmental isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis. Appl. Environ. Microbiol. **57:**1624–1629.
4. **Brosch, R., B. Catimel, G. Milon, C. Buchrieser, E. Vindel, and J. Rocourt.** 1993. Virulence heterogeneity of *Listeria monocytogenes* strains from various sources (food, human, animal) in immunocompetent mice and its association with typing characteristics.

J. Food Prot. **56**:297–301, 312.
5. **Busse, M.** 1990. Preventive measures against *Listeria*. Eur. Dairy Magazine **1**:66–72.
6. **Harvey, J., and A. Gilmour.** 1992. Occurrence of *Listeria* species in raw milk and dairy products produced in Northern Ireland. J. Appl. Bacteriol. **72**:119–125.
7. **Harvey, J., and A. Gilmour.** 1993. Occurrence and characteristics of *Listeria* in foods produced in Northern Ireland. Int. J. Food Microbiol. **19**:193–205.
8. **Hof, H., and J. Rocourt.** 1992. Is any strain of *Listeria monocytogenes* detected in food a health risk? Int. J. Food Microbiol. **16**:173–182.
9. **Nei, M., and A. K. Roychoudhury.** 1974. Sampling variances of heterozygosity and genetic distance. Genetics **76**:379–390.
10. **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.
11. **Nørrung, B.** 1992. Characterisation of Danish isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis. Int. J. Food Microbiol. **15**:51–59.
12. **Nørrung, B., and N. Skovgaard.** 1993. Application of multilocus enzyme electrophoresis in studies of the epidemiology of *Listeria monocytogenes* in Denmark. Appl. Environ. Microbiol. **59**:2817–2822.
13. **Piffaretti, J.-C., H. Kressebuch, M. Aeschbacher, J. Bille, E. Bannerman, J. M. Musser, R. K. Selander, and J. Rocourt.** 1989. Genetic characterization of clones of the bacterium *Listeria monocytogenes* causing epidemic disease. Proc. Natl. Acad. Sci. USA **86**:3818–3822.
14. **Saunders, N. A.** 1991. Analysis of restriction fragment length polymorphisms in the study of bacteria, p. 227–244. *In* J. M. Grange, A. Fox, and N. L. Morgan (ed.), Genetic manipulation techniques and applications. Blackwell Scientific Publications Ltd., Oxford.
15. **Saunders, N. A., A. M. Ridley, and A. G. Taylor.** 1989. Typing of *Listeria monocytogenes* for epidemiological studies using DNA probes. Acta Microbiol. Hung. **36**:205–209.
16. **Seeliger, H. P. R., and K. Höhne.** 1979. Serotyping of *Listeria monocytogenes* and related species. Methods Microbiol. **13**:31–49.
17. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51**:873–884.
18. **Selander, R. K., R. M. McKinney, T. S. Whittam, W. F. Bibb, D. J. Brenner, F. S. Nolte, and P. E. Pattison.** 1985. Genetic structure of populations of *Legionella pneumophila*. J. Bacteriol. **163**:1021–1037.
19. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco.
20. **Trott, D. J., I. D. Robertson, and D. J. Hampson.** 1993. Genetic characterisation of isolates of *Listeria monocytogenes* from man, animals and food. J. Med. Microbiol. **38**:122–128.
21. **Wesley, I. V., and F. Ashton.** 1991. Restriction enzyme analysis of *Listeria monocytogenes* strains associated with food-borne epidemics. Appl. Environ. Microbiol. **57**:969–975.