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Listeria monocytogenes Isolates from Invasive Infections: Variation of Sero- and Genotypes during an 11-Year Period in Finland

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Listeria monocytogenes strains that were isolated from 314 human listeriosis cases in Finland during an 11-year period were analyzed by O:H serotyping and pulsed-field gel electrophoresis (PFGE). Serotyping divided the isolates into five serotypes, the most common being 1/2a (53%) and 4b (27%). During the study period, the number of cases caused by serotype 1/2a increased from 22% in 1990 to 67% in 2001, and those caused by serotype 4b decreased from 61 to 27%, respectively. PFGE with restriction enzyme Ascl divided the strains into 81 PFGE genotypes; among strains of serotypes 1/2a and 4b, 49 and 18 PFGE types were seen, respectively. PFGE type 1 (serotype 1/2a) was the most prevalent single type (37 strains). Together with six other, closely related PFGE types, PFGE type 1 formed a group of 71 strains, representing 23% of all 314 strains. Strains of PFGE type 1 have also been isolated from cold smoked fish, suggesting a source of human infections caused by this type. Moreover, PFGE type 24 (serotype 1/2c) was significantly associated with gender: 5% of 180 male subjects but none of 132 female subjects (P = 0.012). An electronic database library was created from the PFGE profiles to make possible the prompt detection of new emerging profiles and the tracing of potential infection clusters in the future.

Listeria monocytogenes is an important food-borne pathogen which is widely distributed in the environment and which may contaminate foodstuffs at any point of the food chain. It causes an infection, listeriosis, in certain well-defined high-risk groups, including pregnant women, neonates, and immunocompromised adults (10, 36). Usually the infection causes sepsis and may affect the central nervous system, causing meningitis, or may lead to abortion or stillbirth. Occasionally persons without any predisposing conditions may also have gastroenteritis (9, 19, 21, 29, 31, 34). The clinical symptoms take from a few days to several weeks to appear, making the source of the infection difficult to trace. Unlike most other common food-borne infections, listeriosis has a mortality rate of 20 to 30% (8, 10). In Finland, since the 1980s clinical microbiology laboratories have sent their L. monocytogenes findings to the Laboratory of Enteric Pathogens (LEP), National Public Health Institute (KTL); in 1994, such submission became obligatory. Also, physicians have been obligated to report any culture-confirmed cases of human listeriosis to the National Infectious Disease Registry since 1994.

From 1990 through 2001, the annual number of cases of invasive listeriosis in Finland varied between about 20 and 50 (M. Jahkola, unpublished data; http://www.ktl.fi/ttr). On the basis of only limited O serotyping of L. monocytogenes strains, listeriosis cases, excluding an outbreak caused by a rare serotype, 3a (26), have been thought to be sporadic.

Sero-typing is a classic phenotypic tool for epidemiological studies (11, 12, 32). Thirteen serotypes of L. monocytogenes have been identified. However, most isolates belong to only three serotypes, 1/2a, 1/2b, and 4b (10, 24). Although the epidemiological benefit of serotyping is limited, it has provided rapid information for the screening of isolates during suspected outbreaks. Various genotyping methods have been used successfully in more detailed epidemiological studies (2, 13, 18, 22, 26, 28, 31). Pulsed-field gel electrophoresis (PFGE) is one of these genotyping methods, and it has proven to be highly discriminating and reproducible (1, 6, 22).

To study retrospectively the potential occurrence of infection clusters caused by L. monocytogenes in Finland since 1990, both systematic serotyping for O and H antigens and genotyping by PFGE were initiated in 1997 and have been continued prospectively since then. An electronic database library of PFGE profiles and serotypes was created to make possible prompt comparison of the PFGE profiles of different strains and rapid identification of infection clusters.

MATERIALS AND METHODS

Strains. All human L. monocytogenes isolates (n = 314; one isolate per patient) available at LEP from 1990 to 2001 were studied. The median age of the patients was 56, ranging from 1 day to 93 years. Twenty-five of these isolates were connected with an outbreak of listeriosis (26). Most of the isolates (n = 289) originated in blood, cerebrospinal fluid, or other sterile sites (Table 1). Of the isolates, 180 (57%) were from male subjects and 132 (42%) were from female subjects; for 2 isolates (1%) information on gender was no longer available. Thirty-two isolates were reported to be from pregnancy-associated cases, and 14 of these isolates were from seven mother-child pairs. The strains were isolated in routine clinical microbiology laboratories and were subsequently submitted, with information regarding the patient, to LEP for verification and serotyping. Identification of the strains was carried out by standard methods (5, 37). All strains were stored at −70°C in sterilized skim milk.

Sero-typing. All strains were serotyped by using antisera against O and H antigens according to the instructions of the manufacturer (Denka Seiken Co., Japan).
The preparation of genomic DNA was brought into use, with slight modi-
fications. Chromosomal DNA was digested overnight with 5 U of proteinase K per ml. The washing of the plugs and the conditions for restric-
tion digestion were as described previously (27), with the following modi-
fications. The plugs were incubated overnight at 37 °C in a buffer containing 0.5 M NaCl and again overnight at 55 to 57 °C in a buffer containing 0.5 M NaCl, 100 mM EDTA, 0.5% Brij 58, 0.2% sodium deoxysilicate, and 0.5% sodium laurylsarcosine and supplemented with 1 mg of lysozyme per ml and again overnight at 55 to 57 °C in a buffer containing 0.5 M EDTA (pH 9.5) and 1% sodium laurylsarcosine and supplemented with 0.5 mg of protease K per ml. The washing of the plugs and the conditions for restric-
tion endonuclease digestion and PFGE were as described previously (25). Chro-
mosomal DNA was digested overnight with 5 U of *Aci I* (New England Bio-
Labs Inc., Beverly, Mass.).

In 2001, the shorter protocol described by Graves and Swaminathan (17) for
serotyping divided the 314 isolates into five O:H serotypes (Table 2 and Fig. 1a): serotypes 1/2a (53%), 1/2b (6%), 1/2c (3%), 3a (11%), and 4b (27%). From 1990 through 2001, the percentages of serotype 1/2a strains varied between 22 and
67%, those of serotype 1/2b varied between 0 and 24%, and those of serotype 1/2c varied between 0 and 12%. Strains of serotype 3a were rare, except in 1997, 1998, and 1999, when the percentages were 9, 44, and 22%, respectively. The percent-
ages of serotype 4b strains varied between 12 and 61%. In
1990, most of the strains were of serotype 4b. Since then, strains of serotype 1/2a have been the most common.

PFGE divided the isolates into 81 PFGE types (Table 3). Each type correlated with one serotype, except for PFGE types 2, 71, and 207, each of which included one strain belonging to a serotype different from the others. PFGE type 2 included strains of serotype 1/2a (8 strains) and 3a (1 strain), PFGE type 71 included strains of serotype 3a (32 strains) and 1/2a (1 strain), and PFGE type 207 included strains of serotype 1/2a (2 strains) and 3a (1 strain). PFGE divided serotypes 1/2a, 1/2b, 1/2c, 3a, and 4b into 49, 10, 2, 5, and 18 PFGE types, respec-
tively. Groups of closely related types were designated with the letter G or with the letters GT when a type containing at least five strains was not related to any other type; the group number indicates the PFGE type with which all of the other PFGE types were compared.

Statistical methods. Fisher's exact two-tailed test (Epi-Info 6.04 software; World Health Organization, Geneva, Switzerland, and Centers for Disease Control and Prevention, Atlanta, Ga.) was used for statistical analysis. A *P* value of <0.05 indicated statistical significance.

**RESULTS**

Serotyping divided the 314 isolates into five O:H serotypes (Table 2 and Fig. 1a): serotypes 1/2a (53%), 1/2b (6%), 1/2c (3%), 3a (11%), and 4b (27%). From 1990 through 2001, the percentages of serotype 1/2a strains varied between 22 and 67%, those of serotype 1/2b varied between 0 and 24%, and those of serotype 1/2c varied between 0 and 12%. Strains of serotype 3a were rare, except in 1997, 1998, and 1999, when the percentages were 9, 44, and 22%, respectively. The percentages of serotype 4b strains varied between 12 and 61%. In 1990, most of the strains were of serotype 4b. Since then, strains of serotype 1/2a have been the most common.

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**TABLE 1. Origins of *L. monocytogenes* isolates**

<table>
<thead>
<tr>
<th>Category</th>
<th>No. (%) of isolates (total n, 314)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of infection</td>
<td></td>
</tr>
<tr>
<td>Blood or cerebrospinal fluid</td>
<td>278 (88)</td>
</tr>
<tr>
<td>Other sterile sites</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Other sites</td>
<td>14 (4)</td>
</tr>
<tr>
<td>Not known</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>180 (57)</td>
</tr>
<tr>
<td>Female</td>
<td>132 (42)</td>
</tr>
<tr>
<td>Not known</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Pregnancy associated</td>
<td>32 (10)</td>
</tr>
</tbody>
</table>

*a Brain, bursa, hip prosthesis, joint, pericardium, peritoneum, and pleural fluid.
*b Gastrointestinal tract, genit al mucosa, neck abscess, placenta, upper respiratory tract, and wound swabs.
*c Fourteen were from seven mother-child pairs.

**TABLE 2. Annual distributions of serotypes of *L. monocytogenes* strains isolated from infections in human subjects**

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2a</td>
<td>4 (22)</td>
<td>6 (35)</td>
<td>8 (50)</td>
<td>9 (56)</td>
<td>14 (56)</td>
<td>12 (60)</td>
<td>12 (57)</td>
<td>26 (55)</td>
<td>19 (44)</td>
<td>25 (56)</td>
<td>12 (63)</td>
<td>165 (53)</td>
</tr>
<tr>
<td>1/2b</td>
<td>2 (11)</td>
<td>4 (24)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>3 (16)</td>
<td>2 (7)</td>
<td>10 (3)</td>
</tr>
<tr>
<td>1/2c</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>2 (7)</td>
<td>10 (3)</td>
</tr>
<tr>
<td>3a</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>2 (7)</td>
<td>10 (3)</td>
</tr>
<tr>
<td>4b</td>
<td>11 (61)</td>
<td>5 (29)</td>
<td>6 (38)</td>
<td>7 (44)</td>
<td>4 (16)</td>
<td>5 (25)</td>
<td>6 (29)</td>
<td>16 (34)</td>
<td>5 (12)</td>
<td>10 (22)</td>
<td>3 (16)</td>
<td>7 (26)</td>
</tr>
</tbody>
</table>

*a At least 25 of 33 isolates (76%) were connected with an outbreak (26).

Electrophoresis was performed at 210 V with 1.0% Pronadisa D-5 agarose gels (Hispanlab, Madrid, Spain) by using a CHEF Mapper or CHEF-DR system (Bio-Rad Laboratories, Richmond, Calif.). Running conditions for *Aci I*-digested DNA were 1 to 28 s for 10 h, followed by 28 to 30 s for 10 h. Low-range PFGE markers (New England BioLabs Inc.) were used as molecular weight standards. The gels were visualized on a UV transilluminator and were photographed by using AlphaImager 1220 (Alpha Innotec Corporation, San Leandro, Calif.). The TIFF images were analyzed by using BioNumerics software (Applied Maths, Kortrijk, Belgium) and were normalized by using the low-range PFGE marker standards on each gel. Any difference between two PFGE profiles was consid-
ered sufficient to distinguish these profiles. The different PFGE profiles were marked with numbers based on the coding agreed upon with the National Veterinary and Food Research Institute (L. Rantala, S. Lukinmaa, A. Siitonen, and T. Honkanen-Buzalski, Abstr. ISPOL XIV Int. Symp. Problems Listeri osis, p. 155, 2001). Similarity values were calculated by the unweighted pair-group method with arithmetic averages and the Dice coefficient by using BioNumerics software.

Groups of PFGE types. BioNumerics software was used to compare all PFGE types with PFGE types containing five or more strains. When the similarity value was over 80% and the number of fragment differences between the profiles was three or less, the PFGE type was regarded as being closely related to the one with which it was compared (40). Groups of closely related types were designated with the letter G or with the letters GT when a type containing at least five strains was not related to any other type; the group number indicates the PFGE type with which all of the other PFGE types were compared.
Only 11 of the 81 PFGE types contained five or more strains. Compared with the others, 8 of these 11 PFGE types (1, 2, 11, 21, 61, 71, 74, and 96) were closely related to several other PFGE types and consequently were grouped into G1, G2, G11, G21, G61, G71, G74, and G96, respectively (Table 3 and Fig. 2). The remaining three PFGE types (5, 24, and 65) were not closely related to any other PFGE type; therefore, each formed a group of its own, GT5, GT24, and GT65, respectively. Of the five most common PFGE groups, G1 included seven PFGE types (31 strains, 23%), G71 included two (34, 11%), G11 included three (32, 10%), G21 included eight (22, 9%), and GT5 included one (27, 9%).

Group G1 (71 strains) formed clusters every year from 1993 onward (Fig. 1b). From 1994 to 1996, most of the cases were caused by PFGE types 1 and 23 of group G1 (Table 3). In 1997 and 1999, large clusters were formed by PFGE type 1 of group G1 alone, and strains of this type were the most common. PFGE type 1 caused infections every year from 1994 onward (2 to 11 cases per year).

Group GT5 (27 strains, of type 5) formed clusters in 1997, 1998, and 2000 (Fig. 1b), and four strains in this group were pregnancy associated.

Strains belonging to group G11 (32 strains) formed clusters in 1990, 1993, 1994, 1995, 1997, and 2001 (Fig. 1b). In 1990, 1991, 1992, and 1993, a total of eight strains were pregnancy associated; four of these cases were caused by PFGE type 68.
and four were caused by PFGE type 11. All four cases caused by PFGE type 11 in 1994 occurred in the Helsinki metropolitan area.

Group G21 (29 strains) formed small clusters in 1991, 1992, 1997, 1998, 1999, and 2000 (Fig. 1b). Five strains were pregnancy associated; three of these cases were caused by PFGE types 72, 56, and 69 and two (a mother-child pair) were caused by PFGE type 21.

Group G71 (34 strains) formed clusters in 1997, 1998, and 1999 (Fig. 1b). The clusters were formed by serotype 3a, PFGE type 71. Serotype 1/2a, PFGE type 71, caused only one case, which was pregnancy associated, in 1992.

Group G61 (13 strains), PFGE type 61 (9 strains) formed a cluster of five cases in 1999 (Table 3). Four of these patients and one patient in 1998 were from the greater Tampere area. Also, PFGE type 65 (five strains), which belongs to group GT65, formed a cluster of three cases in 1997 in the Helsinki metropolitan area. However, two of these cases involved a mother-child pair.

The 32 pregnancy-associated cases were caused by 17 differ-
ent PFGE types. From 1990 to 1993, the pregnancy-associated cases were mostly (8 cases) caused by serotype 4b, PFGE type 68 or 11, each belonging to group G11 (Table 3). After 1993, cases caused by PFGE types in group G11 were not detected. PFGE type 24, which belonged to group GT24, was significantly associated with listeriosis in male subjects (9 of 180 male subjects [5%] versus 0 of 132 female subjects) \((P/H11005 0.012)\). Otherwise, PFGE types were distributed evenly between both genders and among different age groups.

**DISCUSSION**

In Finland, infections caused by *L. monocytogenes* have been thought to be sporadic. This insight is based on diagnostic tests in which only the O antigen of the strains was occasionally determined, and more discriminating methods, such as genotyping, were not used before the present study was undertaken.

In order to gain new information on infection clusters and on the distributions of subtypes of *L. monocytogenes*, 314 clinical isolates from an 11-year period in Finland were analyzed by PFGE and serotyping.

It should be mentioned that not all strains in Finland during the 4-year period from 1990 to 1993 were available for this study, since before 1994 physicians and clinical laboratories notified KTL and submitted strains to LEP only on a voluntary basis. However, the strains studied represented almost 60% of all 119 listeriosis cases diagnosed in the early 1990s (Jahkola, unpublished). Also, the proportion of pregnancy-associated cases might have been underestimated, since information on pregnancy was not always required in the laboratory notes.

From 1990 through 2001, the most common *L. monocytogenes* serotypes were 1/2a and 4b, accounting, respectively, for 53 and 27% of the 314 isolates from cases of human listeriosis. Since 1990, the number of cases caused by serotype 4b has been fairly constant, at about 4 to 7 cases per year, except in 1997, when the number of cases was 16, and in 1999, when it was 10. However, the number of listeriosis cases caused by serotype 1/2a has increased; therefore, the percentage share of this serotype has also increased. These results support findings in the United Kingdom (30), Denmark (14), Switzerland (33), and Sweden (23) suggesting that serotype 1/2a is replacing serotype 4b in human infections.

In the present study, when an electronic database library was constructed by using BioNumerics software, any difference between two PFGE types was considered sufficient to distinguish two different PFGE types. Therefore, PFGE types containing at least five strains were compared with all other PFGE types to approximate whether they were closely related to any other type according to the criteria of Tenover et al. (40). These
criteria are stringent and generally appropriate for studies of strains collected over a short period. However, in the present study, grouping of closely related types collected over a long period yielded more information on clusters. For example, PFGE type 1 was the most prevalent single type, with 37 strains. It belonged to a major cluster, group G1, representing 23% of all 314 strains studied. Furthermore, 27 strains of PFGE type 5 were not related to any other type and formed several clusters over a period of 3 years.

The most prevalent single type, PFGE type 1, alone was previously associated with a vacuum-packed cold smoked rainbow trout product, and it caused a small infection cluster of several clusters over a period of 3 years. PFGE type 5 were not related to any other type and formed during the 1990s clusters of cases of the same PFGE type or different strains was constructed by using BioNumerics software. In this new electronic library, PFGE profiles can be compared with each other more rapidly; therefore, clusters can be detected more rapidly and at an early stage. This database library also makes possible continuous surveillance of invasive L. monocytogenes infection clusters in Finland. After the library was constructed, a bilateral computer-based network for comparison of PFGE profiles of L. monocytogenes isolates from human subjects, food, and food production environments was created (Rantala et al., Abstr. ISOPOL XIV Int. Symp. Problems Listeriosis) by the National Veterinary and Food Research Institute and the KTL. This kind of national cooperation during a suspected outbreak will probably help in recognizing sources of infections. It will also enable authorities to track down food production plants that need to improve production hygiene for their food products or to give direct information on food hygiene to specific risk groups of consumers. During the 1990s, clusters of cases of the same PFGE type or clusters of groups of closely related types were seen every year, indicating the need for and the importance of timely typing of human L. monocytogenes strains. Furthermore, on a larger scale, an electronic network of PFGE profiles for human L. monocytogenes strains has been in use as PulseNet since 1996 in the United States (38), where it has demonstrated its value in the early recognition of outbreaks and the rapid identification of their sources.

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