Low Prevalence of Listeria monocytogenes in Foods from Italy

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ABSTRACT

Listeria monocytogenes is an important foodborne pathogen that causes gastrointestinal disorders, and, especially in immunocompromised people, serious extraintestinal diseases, such as septicemia and meningitis, as well as abortion in pregnant women. Many foods, from both plant and animal origin, have been involved in listeriosis outbreaks. This article reports the results of a 12-year survey (1993 through 2004) on the presence of L. monocytogenes in several kinds of food marketed in Italy. Of 5,788 analyzed samples, 121 (2.1%) were contaminated with L. monocytogenes. The highest prevalence was found in smoked salmon (10.6%) and in poultry meat samples (8.5%) and the lowest in red meat (0.3%). L. monocytogenes was not found in 154 samples of fresh seafood products. Fifty-two isolates were also serotyped by the agglutination method. The most common serotypes detected in the 52 strains tested were 1/2a (36.5%), followed by 1/2c (32.8%), 1/2b (13.5%), 4b (11.5%), 3a (3.8%), and 3b (1.9%). The results of the present study showed low levels of L. monocytogenes in the analyzed samples. A total of 61.5% of the 52 L. monocytogenes strains analyzed belonged to serotypes 1/2a, 1/2b, and 4b, namely the serovars that are most commonly involved in extraintestinal human listeriosis outbreaks. In the ready-to-eat samples, these three serotypes were 40.0% (1/2a), 17.1% (1/2b), and 14.3% (4b). This finding highlights the need to implement strict hygienic measures during the production, distribution, and sale of foods to reduce the risk of foodborne listeriosis in humans to an acceptable level.

Listeria monocytogenes, the etiological agent of human listeriosis, is a gram-positive facultative intracellular pathogen. The significance of food as a primary source of infection for humans was recognized in the early 1980s (18, 28). Listeriosis primarily affects neonates and immunocompromised individuals, causing severe conditions such as septicemia, encephalitis, and meningitis; it also causes abortion and stillbirth in pregnancy (47). Infection with L. monocytogenes in healthy individuals causes fever, vomiting, and diarrhea (38). The infection process starts with colonization of the gastrointestinal tract; then it moves on to the liver, the principal site of bacterial multiplication, and finally results in septicemia involving multiple organs, mainly the brain and pregnant uterus. Progression of the infection depends on several factors, including the intestinal microbial population (29), the immune competence of the patient (27), and the virulence of the strain involved (10, 35).

In the last few years, several outbreaks of listeriosis due to consumption of contaminated food products have been reported (14). In 2000, the Centers for Disease Control and Prevention reported that L. monocytogenes had the second highest case-fatality rate (21%) and the highest hospitalization rate (90.5%) among foodborne pathogens (36). The bacterium often colonizes the intestinal tract of animals without causing illness and has been isolated from a wide range of healthy domestic and wild animals, including birds and fishes (5). L. monocytogenes is commonly found in the soil and water, and because it is widely present in the environment, it may contaminate foods during production and distribution (12). The ubiquitous distribution of this pathogen in nature, its ability to grow at refrigeration temperatures, and its tolerance to certain preservative agents make its eradication from food chain very difficult (3). Based on their physical and chemical characteristics as well as their storage, foodstuffs can be classified as high- and low-risk foods. In this sense, it is especially important to control the presence of this microorganism in these ready-to-eat (RTE) products that can support the growth of L. monocytogenes to high levels (15, 21). RTE foods are believed to be the major sources of human infection, so the U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition and the U.S. Department of Agriculture, Food Safety and Inspection Service recently completed an assessment of the relative risk of listeriosis associated with various types of these foods (50). The dose-response of L. monocytogenes is unknown, although it is believed to vary with the strain and host susceptibility (11, 34, 45).
TABLE 1. Detection of L. monocytogenes in analyzed food samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Food group</th>
<th>Food sample</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
<th>MPN/g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (NRTE)</td>
<td>Red meat</td>
<td>Beef, pork, horse, mutton, game, etc.</td>
<td>883</td>
<td>3 (1 pork, 2 bovine)</td>
<td>15–70</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Poultry meat</td>
<td>Chicken and turkey</td>
<td>284</td>
<td>24 (24 chicken)</td>
<td>3.6–93;</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Seafood products</td>
<td>Fishes, shellfishes, crustaceans</td>
<td>154</td>
<td>0</td>
<td>&lt;3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Raw sausages</td>
<td>Fresh sausages</td>
<td>384</td>
<td>9</td>
<td>3–90</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Rat treated meat</td>
<td>Hamburger, minced meat, etc.</td>
<td>123</td>
<td>3 (3 hamburger)</td>
<td>4–9</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Cured fish</td>
<td>Dried salted cod, shellfish frozen, etc.</td>
<td>133</td>
<td>8 (8 dried salted cod)</td>
<td>ND</td>
<td>6.0</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td></td>
<td>1,961</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (RTE)</td>
<td>Milk and cheese</td>
<td>Bovine milk and cheese</td>
<td>2,498</td>
<td>27 (3 milk, 19 soft cheese, 4 cheese, 1 casein)</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dried or cooked</td>
<td>Sausages, mortadella, frankfurters, etc.</td>
<td>641</td>
<td>25 (2 frankfurters, 23 seasoned and cured sausages)</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sausages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cooked or salted</td>
<td>Raw or cooked ham, bacon, etc.</td>
<td>151</td>
<td>4 (2 bacon, 2 cooked ham)</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>treated meats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoked salmon</td>
<td>Salmon</td>
<td>104</td>
<td>11</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other ready-to-eat</td>
<td>Stew, roast turkey, Russian salad, baked products,</td>
<td>433</td>
<td>7 (1 stew, 1 roast turkey, 1 Russian salad, 1 salad of sea food, 1 cooked ham-burger, 1 croissant, 1 tortellini)</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td></td>
<td>3,827</td>
<td>74</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>5,788</td>
<td>121</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

* Group A comprised products to be cooked or heated before consumption (NRTE). Group B comprised preserved ready-to-eat products (RTE).
* One poultry sample had an MPN > 1,100/g.
* ND, samples analyzed only with qualitative method.

The Italian National Health System includes a network of highly specialized veterinary laboratories of the Experimental Zooprophilactic Institute that monitor the microbiological and chemical risks connected with foods of animal origin. This paper reports the findings of a 12-year survey on the occurrence and characterization of L. monocytogenes in different kinds of food.

MATERIALS AND METHODS

A total of 5,788 samples of different kinds of food, marketed in two Southern Italian regions, Apulia and Basilicata, were analyzed over a 12-year period (1993 through 2004) to detect L. monocytogenes. All the samples were randomly collected by Italian National Health System staff at retail outlets and in manufacturing plants, taken to the laboratory in sterile bags, stored in coolers at a suitable temperature (4°C–18°C), and processed within 24 h of collection. The samples were divided into two groups: group A, products to be cooked or heated before consumption (NRTE), and group B, preserved RTE products (Table 1). Group A included 1,961 raw samples of fresh or cured meats (pork, beef, horse, game, mutton, minced meat, chicken, and turkey) and raw seafood products (fishes, shellfishes, and crustaceans). Group B included 3,827 samples of RTE foods, such as salami, frankfurters, “mortadella,” ham, pasteurized milk, dairy products, ice creams, eggs products, pastries, treated fish, and smoked salmon. With the exception of smoked salmon, all the products were produced in Italy.

Detection. During the survey, the microbiological methods to assess L. monocytogenes contamination in foods were modified. From 1993 to 1997, dairy products were analyzed by the U.S. Food and Drug Administration/International Dairy Federation method (24). Briefly, a 25-g sample was homogenized in 225 ml of Listeria enrichment broth (Oxoid, Basingstoke, Hampshire, UK) and incubated at 30 ± 1°C. After 24 h, 0.1 ml of the incubated broth was transferred into 10 ml of Listeria enrichment broth (Oxoid) and incubated at 30 ± 1°C for 24 h. After the incubation period, the first and second enrichment broths were streaked onto Oxford agar and PALCAM agar plates (Oxoid) and incubated at 37°C for 24 to 48 h for isolation. From each plate presenting presumptive colonies of Listeria spp., up to five colonies were picked and identified to species with confirmation tests.

During the same period, the other foods were analyzed by the U.S. Department of Agriculture, Food Safety and Inspection Service method (31). A 25-g sample was homogenized in 225 ml of University of Vermont Medium I (UVM I; Oxoid) and incubated at 30 ± 1°C. After 24 h, 0.1 ml of the incubated broth was transferred into 10 ml of University of Vermont Medium II (UVM II) and incubated at 30 ± 1°C for 24 h. After the incubation period, the first (UVM I) and the second (UVM II) enrichment broths were streaked onto Oxford agar and PALCAM agar plates (Oxoid) and incubated at 37°C for 24 to 48 h for isolation. From each plate presenting presumptive colonies of Listeria spp., up to five colonies were picked and identified to species with confirmation tests.
After 1997, a standard procedure (26) was adopted to analyze RTE foods. A 25-g sample was homogenized in 225 ml of half Fraser broth (Oxoid) and incubated at 30 ± 1°C for 24 h. Then, 0.1 ml of the incubated broth was transferred into 10 ml of Fraser broth (Oxoid) and incubated at 30 ± 1°C for 48 h. After the incubation period, the first (half Fraser) and the second (Fraser) enrichment broths were streaked onto Oxford agar and PALCAM agar plates (Oxoid) and incubated at 37°C for 24 to 48 h for isolation. From each plate presenting presumptive colonies of *Listeria* spp., up to five colonies were picked and identified to species with confirmation tests. According to Italian rules, no level of *L. monocytogenes* contamination was tolerated in RTE foods.

As of 1994, NRTE foods were analyzed by a most-probable-number (MPN) method provided for in the Food Italian regulations (41). A 10-g sample was homogenized in 90 ml of buffer peptone water (Oxoid); decimal dilutions (up to 10⁻³) of homogenate were prepared in the same medium; 1 ml of the three dilutions (10⁻¹, 10⁻², 10⁻³) was transferred, in triplicate, into tubes containing 9 ml of Fraser broth (Oxoid), which were then incubated at 32 ± 1°C for 24 to 48 h. To confirm the MPN of *L. monocytogenes*, the broth cultures that changed color to black were streaked for isolation onto Oxford agar plates (Oxoid) at 37°C for 24 to 48 h. Five typical colonies from each plate were subjected to tests to confirm their identity as *L. monocytogenes*. The results were calculated by using the MPN table in the range of <3.0 to >1,100 MPN/g. Three unit collections (u.c.) were analyzed for each sample, and the presence of the pathogen was tolerated if the MPN of *L. monocytogenes* was lower than 110/g in two u.c. and 11 in the third u.c.

**Identification.** At least one isolate from each sample was identified by assessing morphological characteristics and performing biochemical tests, including Gram stain, motility at 25°C on motility agar (Oxoid), catalase test, oxidase test (Oxoid), fermentation of xylose and rhamnose (Oxoid), hemolysis test on sheep blood agar plates (Oxoid), CAMP test against *Staphylococcus aureus* and *Rhodococcus equi*, and, finally, the API Listeria (bioMérieux, Marcy l’Etoile, France).

Genomic DNA of the strains biochemically identified as *L. monocytogenes* was extracted with GenomicPrep cell and tissue isolation kit (Amersham, Piscataway, N.J.) following the manufacturer’s instructions. PCR was performed, as previously described (30), using PCR primers (lmo0733F and lmo0733R) derived from a specific gene (lmo0733) for the detection of *L. monocytogenes*. The amplified products were separated by electrophoresis in 1% agarose gel containing ethidium bromide and visualized under UV.

**Serotyping of the strains.** Serotyping was carried out on 52 collected strains with commercial specific antisera (Denka Seiken, Tokyo, Japan) against the serovars 1/2a, 1/2b, 1/2c, 3a, 3b, and 4b following the manufacturer’s instructions.

**Statistical analysis.** Chi-square test was performed with StatView 5.0 software (SAS Institute Inc., Cary, N.C.) in order to compare nominal variables between groups A and B as well as multiple subgroups of foods within the single groups.

**RESULTS**

The occurrence of *L. monocytogenes* in the analyzed samples is shown in Table 1. *L. monocytogenes* was detected in 121 (2.1%) of the 5,788 food samples tested. In group A, the highest incidence of *L. monocytogenes* was in poultry meat samples (24 of 284 [8.5%]; MPN per gram ranged from 3.6 to 93, with one sample with an MPN per gram >1,100/g) and cured fish samples (8 of 133 [6.0%]; 2.3% of the raw sausages (9 of 384; MPN per gram ranged from 3 to 90) and 2.4% of the treated meat were positive (3 of 123; MPN per gram ranged from 4 to 9). Three of 883 (0.3%; MPN per gram ranged from 15 to 70) red meat samples contained *L. monocytogenes*. No raw seafood products were contaminated.

In group B, the highest incidence of *L. monocytogenes* was detected in smoked salmon (11 of 104 [10.6%]). A lower proportion of contamination was observed in dried or cooked sausages (25 of 641 [3.9%]), cooked or salted treated meat (4 of 151 [2.6%]), other RTE foods (7 of 433 [1.6%]), and milk and cheese samples (27 of 2,498 [1.1%]).

The results of the PCR assay were consistent with those obtained with the phenotypical and biochemical identification methods used. The distribution of the detected serovars was 1/2a (36.5%), 1/2c (32.8%), 1/2b (13.5%), 4b (11.5%), 3a (3.8%), and 3b (1.9%) (Table 2). Two different *L. monocytogenes* serotypes were found in a croissant (3b and 1/2b) and in a sausage sample (1/2c and 1/2b).

**DISCUSSION**

In our study, 121 (2.1%) of 5,788 samples analyzed over 12 years were contaminated by *L. monocytogenes* (Table 1). Two different kinds of food were analyzed. A total of 1,961 were products to be cooked or heated before consumption and 3,827 were samples of preserved RTE products. The occurrence of *L. monocytogenes* was 2.4% in the foods to be cooked or heated and 1.9% in the RTE products. No statistically significant difference was observed between the two groups (χ² = 1.36, *P* > 0.05). The data showed a statistically significant difference in the distribution of positive samples in the different food categories of both group A (χ² = 142.83, *P* < 0.001) and B (χ² = 64.26, *P* < 0.001).

In group A (products to be cooked or heated), poultry meat (8.5%) and cured fish (6.0%) exhibited the highest incidence of *L. monocytogenes* (χ² = 29.69, *P* < 0.005 and χ² = 6.38, 0.005 < *P* < 0.05), whereas the occurrence of positive samples in raw sausages (2.3%) and raw treated meat (2.4%) was similar to the rates recorded in all the samples analyzed. Red meat samples showed the lowest incidence of *L. monocytogenes* (0.3%) (χ² = 14.92, *P* < 0.005), and none of the raw seafood products were contaminated with the pathogen (Table 1). As of 1994, these products were analyzed by an MPN method, and only one poultry sample had an MPN per gram >1,100/g, which exceeded the limit set by Italian law (Table 1).

In group B (preserved RTE products), as expected, smoked salmon (10.6%) and dried or cooked sausage (3.9%) had the highest levels of *L. monocytogenes* (χ² = 35.75, *P* < 0.005 and χ² = 9.80, *P* < 0.005). The rates of positive cooked or salted treated meat (2.6%) and other RTE foods (1.6%) were similar to those recorded in all the samples analyzed (2.1%) (*P* > 0.05). The lowest incidence of the pathogen was detected in milk and cheese (1.1%) (χ² = 6.99, *P* < 0.05) (Table 1).

Compared with another relevant Italian survey conducted between 1990 and 1999 on 4,185 food samples
TABLE 2. Results of serotyping of L. monocytogenes isolates

<table>
<thead>
<tr>
<th>Group</th>
<th>Food group</th>
<th>No. of samples</th>
<th>1/2a</th>
<th>1/2b</th>
<th>1/2c</th>
<th>3a</th>
<th>3b</th>
<th>4b</th>
<th>Total strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (NRTE)</td>
<td>Red meat</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Poultry meat</td>
<td>7</td>
<td>—</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Seafood products</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Raw sausages</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>4</td>
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<tr>
<td></td>
<td>Raw treated meat</td>
<td>4</td>
<td>1</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cured fish</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal no. (%)</td>
<td></td>
<td>5</td>
<td>(29.4)</td>
<td>1</td>
<td>(5.9)</td>
<td>8</td>
<td>(47.1)</td>
<td>2</td>
<td>(11.8)</td>
</tr>
<tr>
<td>B (RTE)</td>
<td>Milk and cheese</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Dried or cooked sausages</td>
<td>15</td>
<td>7</td>
<td>2</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>16$^b$</td>
</tr>
<tr>
<td></td>
<td>Cooked or salted-treated meats</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Smoked salmon</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Other ready-to-eat products</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6$^b$</td>
</tr>
<tr>
<td>Subtotal no. (%)</td>
<td></td>
<td>14</td>
<td>(40.0)</td>
<td>6</td>
<td>(17.1)</td>
<td>9</td>
<td>(25.7)</td>
<td>0</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Total no. (%)</td>
<td></td>
<td>50</td>
<td>19</td>
<td>(36.5)</td>
<td>7</td>
<td>(13.5)</td>
<td>17</td>
<td>(32.8)</td>
<td>2</td>
</tr>
</tbody>
</table>

$^a$ Group A comprised products to be cooked or heated before consumption (NRTE). Group B comprised preserved ready-to-eat products (RTE).

$^b$ Two different strains were isolated from the same sample.

(meat and meat products, sauces, vegetables, dairy products, fish, and fish products) that reported a total contamination rate of 12.8% (20), lower levels of L. monocytogenes were recorded in the samples we analyzed. Interestingly, our findings are consistent with the comment made in that survey that Southern Italian foods are less frequently contaminated by L. monocytogenes (20).

Other studies carried out in different countries reported broadly different occurrence rates for L. monocytogenes in foods, ranging from 5.1 to 40.0% in raw meats, including poultry (4, 7, 32, 33, 40, 48, 49) and from 0.3 to 15.4% in raw and preserved seafood products examined in Poland (37), the United States (50), Japan (40), and Canada (17). Different results were also reported for contamination of minced meat and raw sausages ranging from 1.6 to 42% (13, 25, 40, 46). These differences can be explained by the different sampling strategies and detection protocols used in these studies.

Our study showed a lower rate of contamination of dry sausages (3.9%) than other studies from the United States (6.4%) (50), Chile (10.6%) (9), or France (10%) (48). For milk and dairy products, previous surveys reported contamination ranging from 1 to up to 10% (6, 17, 19, 40, 42, 50, 52), whereas the rate recorded in our study was as low as 1.1% (Table 1). In smoked salmon the incidence rate of L. monocytogenes reported by other investigators ranged from 5 to 60% (1, 2, 8, 25, 29, 44, 50), while it was 10.6% in our study (Table 1).

The presence of L. monocytogenes has been demonstrated in a wide variety of RTE food samples with varying rates. In the United States from 2000 to 2001, the observed range was 0.17 to 4.7% (22); in France, the total proportion of contaminated RTE foods from 1995 to 1996 was 6.7% (23). Similarly, our results showed that these products had a contamination rate of 1.9% (Table 1).

Serootypes 1/2a, 1/2b, and 4b are the most frequently involved in human listeriosis (16, 43, 51). In our study, these serotypes were predominant in RTE products and their distribution was as follows: 1/2a (40.0%), 1/2b (17.1%), and 4b (14.3%). In total, these serotypes represented 61.5% of the 52 L. monocytogenes strains typed. The serotype distribution was similar to that of another study concerning Italian foods from 1990 to 1999 (20) and to those elsewhere in the world (39).

The microbiological risk associated with the presence of L. monocytogenes in products requiring heat treatment before consumption is much lower than with preserved RTE products. L. monocytogenes is inactivated by common cooking processes and milk pasteurization in spite of its relative heat resistance. Therefore, Italian law tolerates the presence of the pathogen in food to be cooked before consumption within the limits (MPN per gram lower than 11 in one u.c. and 110/g in two u.c.) established by the Ministerial Ordinance of 13 December 1993.

Further studies on the occurrence of L. monocytogenes are needed to establish international microbiological criteria for food control purposes. It is very difficult to avoid cross-contamination in one or more steps of the food chain from production to distribution because the organism is so widespread in food processing plant environments. However, the implementation of validated hazard analysis critical control point plans may act to decrease the contamination of foods. Appropriate education of consumers, especially of groups at risk (such as immunocompromised individuals and pregnant women), regarding safe food handling and cooking practices may lead to a reduction of listeriosis in humans.

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