Does Sporadic *Listeria* Gastroenteritis Exist? A 2-Year Population-Based Survey in Nova Scotia, Canada

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**Background.** Febrile gastroenteritis due to *Listeria monocytogenes* (LM) has been primarily described in foodborne outbreaks. We decided to determine the incidence of sporadic, febrile gastroenteritis due to LM in a large, well-defined North American population over a 2-year period and to compare these cases to sporadic cases of *Campylobacter* and *Salmonella* infections occurring concurrently in the community.

**Methods.** From 1 September 2002 through 31 August 2004, all stool specimens submitted for evaluation of diarrheal illness to a public health laboratory and to a children’s hospital serving a population of ∼350,000 were examined for the presence of *Listeria* species. Patients identified as having LM in their stool samples were matched with 2 temporally-matched patients with cultures positive for *Campylobacter* and *Salmonella* species. Patients with LM and control patients were contacted by telephone, and they answered a questionnaire that examined clinical features and risk factors for diarrheal illness.

**Results.** A total of 7775 stool specimens were submitted during the period 1 September 2002–31 August 2004. Thirty-nine *Listeria* species were recovered. Seventeen of the species were LM, 13 were *Listeria innocua*, 3 were *Listeria welshimeri*, 1 was *Listeria grayi*, and 4 were other species. Pulsed-field gel electrophoresis results demonstrated no temporal or other clusters, and no seasonality was noted for isolates of LM. Preexisting gastrointestinal problems were much more common in patients with LM than in patients with *Campylobacter* or *Salmonella* infections.

**Conclusions.** Sporadic gastroenteritis due to LM appears to be an uncommon illness, and routine screening of stool samples for LM remains unwarranted. Preexisting gastrointestinal disease may be a risk factor for infection of the gastrointestinal tract with LM.

FoodNet data for 2002 in the United States identified >12,000 foodborne infections caused by bacteria [1]. *Campylobacter*, *Salmonella*, and *Shigella* infections were the most common, and invasive infections due to *Listeria monocytogenes* represented <1% of the total (101 infections). However, none of the FoodNet laboratories routinely seek to identify *Listeria* species from stool specimens submitted for evaluation, and for the majority of foodborne infections, the causative organism remains unidentified. Despite this, several outbreaks of foodborne listeriosis in healthy people have been identified in Europe [2–6] and the United States [7–9], where LM appears to have caused diarrhea and fever more characteristic of traditional foodborne infections caused by *Campylobacter* or *Salmonella* species. These outbreaks have been recently reviewed by Ooi and Lorber [10]. These syndromes were generally unaccompanied by bloodstream or CNS infections, which have been more commonly associated with previous outbreaks of listeriosis [11–16].

The identification of these outbreaks of milder illness suggests that foodborne, noninvasive LM infection (i.e., infection without bloodstream or CNS involvement) might also cause some cases of sporadic noninvasive foodborne diarrhea in the general population. We performed a 2-year, prospective, laboratory-based study of febrile gastroenteritis in a defined population to determine the incidence and clinical features of and risk factors for gastroenteritis due to LM.
PATIENTS AND METHODS

Population studied. The Capital District Health Authority serves a population of ~350,000 in 2 cities (Halifax and Dartmouth, Nova Scotia, Canada), and several outlying suburban and rural areas. Health care services in Nova Scotia are family practice–based, and family physicians have free access to transport media for stool culture to provide an opportunity to identify bacterial pathogens in stool samples if they wish to do so. No active surveillance for gastrointestinal infections is performed in Nova Scotia.

All stool samples submitted to the Queen Elizabeth II Health Sciences Laboratory (QEIIHSC) or the Izaak Walton Killam Hospital Clinical Microbiology Laboratory (IWK) for bacterial culture during the period 1 September 2002–31 August 2004 were included in this study. Physicians' offices or patients couriered or dropped off specimens at either laboratory.

All specimens were submitted in Cary-Blair transport media (Bebbington Bioclinical), which is available for distribution in individual 50-mL containers suitable for stool sample collection. These containers were labeled with instructions for the patients and were received within 72 h after sample collection. Stool specimens were identified as being hard, soft, or watery. Following delivery, specimens were processed on the same day or after overnight storage at 4°C.

Stools samples were plated directly from Cary-Blair transport media to MacConkey agar, Campylobacter agar (Skirrow's formula), and Sorbitol-MacConkey agar. An additional aliquot was placed in Selenite broth for 12–18 h incubation at 35°C and then subcultured to Xylose-Lysine-Deoxycholate agar. Cultures and broth were examined daily, and identifications were performed from single-colony subculture with use of a standard algorithm. No other routine testing was performed on these community-based specimens, such as routine occult blood tests, microscopic examination for fecal WBCs, viral culture, or electron microscopy. The laboratories also do not perform routine tests for Clostridium difficile toxin on stool specimens obtained from the community, although this test was requested for some patients in the survey.

Identification of LM from stool samples. After routine bacterial culture, the remainder of the stool specimen was transported to the Infectious Disease Research Laboratory. An aliquot of 0.1 mL of the specimen was directly plated to Oxford agar (Oxoid), including Listeria-selective agar base and Listeria-selective supplement (Oxford formulation) containing cycloheximide, colistin sulphate, acriflavin, cefotetan, and fosfomycin [17]. An additional aliquot was directly plated to Palcam agar (Palcam agar base plus Palcam-selective supplement containing polymyxin B sulfate, acriflavin hydrochloride, and ceftazidime; Oxoid). A 1-mL sample was then added to 9 mL of primary UVM broth (Oxoid) containing nalidixic acid and acriflavin hydrochloride (10 mg/mL concentration) and was incubated for 24 h. Subcultures on Oxford and Palcam agars were performed, and an additional 0.1 mL of the enrichment broth was added to 10 mL of UVM secondary selective enrichment broth containing nalidixic acid and acriflavin hydrochloride (25 mg/L concentration), with incubation for an additional 24 h.

The Palcam plates were incubated at 30°C for 48 h in microaerophilic conditions (5% oxygen, 7.5% carbon dioxide, 7.5% hydrogen, and 80% nitrogen); Oxford media was kept at 37°C for 48 h. Typical colonies of LM on Palcam (gray-green colonies with black centers) were transferred to tryptic-soy agar containing 5% sheep blood for incubation at 35°C and subsequent identification as LM. Black colonies (1–3 mm in diameter) with a black halo on the Oxford media were also transferred to TSA blood agar. After 18–24 h incubation, Listeria species were identified through standard analyses, including determination of the presence or absence of β-hemolysis, cyclic adenosine monophosphate reaction, acid production from deoxyglucose, esculin hydrolysis, and positive Voges-Proskauer and methyl-red reactions. LM were identified by deoxyxyllose negativity and α-methyl d-mannoside–positive reactions (API-Coryne; bioMérieux).

PFGE was performed on restriction products of LM DNA after cutting using Smal and ApaI endonucleases (New England Biolabs). Isolates that had identical PFGE patterns with use of Smal were further characterized using ApaI. Isolates of LM were also referred to the Canadian reference laboratory for Listeria confirmation, subsequent serotyping, and ribotyping. One isolate from the IWK and 1 from the QEIIHSC could not be recovered for further study.

After 1000 stool specimens were processed, the 4-h UVM-2 and potassium hydroxide steps were eliminated, because they did not provide additional sensitivity. For technical reasons, in November 2003, we also changed our quality control organisms to American Type Culture Collection (ATCC) strains of LM and Staphylococcus aureus. The S. aureus strain that we had been using produced black colonies similar to those of Listeria species, whereas the ATCC strain did not.

Quantitative testing. An aliquot of all stool samples was frozen at −80°C in anticipation of quantitative testing, if LM was identified in the initial specimen. These specimens were discarded if Listeria species failed to grow from the primary specimen. For specimens with positive culture results, a 1-g or 1-mL aliquot of stool was suspended in 9 mL of normal saline, and 10-fold dilutions were directly plated on Palcam or Oxford agar, whichever had grown the largest number of colonies in the qualitative assessment. Typical colonies were then counted using low-power microscopic examination, and the number of colony-forming units per gram or milliliter of stool was determined.

Case-control study. After the identification of LM in a stool
Figure 1. Number of stool samples growing Listeria species, by month, from samples collected as part of a population-based survey of diarrheal illness (n = 7775), 1 September 2002–31 August 2004.

Statistical analysis. The survey results were entered into a Microsoft Access database (Microsoft) and analyzed using SPSS software, version 11.5 (SPSS), for Windows. $\chi^2$ and Fisher’s exact test were used to determine differences between case patients with Listeria infection and their matched control patients with Salmonella and Campylobacter infections in regard to demographic and clinical features associated with each illness.

RESULTS

Identification of Listeria, Salmonella, and Campylobacter species in stool samples. A total of 6785 stool specimens were submitted to the QEIHSC microbiology laboratory between 19 August 2002 and 31 August 2004. Thirty-four isolates of Listeria species were identified. Thirteen of these were LM (figure 1). Additional Listeria species identified included Listeria innocua (13 isolates), Listeria welshimeri (3), Listeria grayi (1), and other species (4). During the same period, 124 Salmonella and 128 Campylobacter isolates were recovered from the samples submitted. These numbers were similar to those for previous years. No patients had both Listeria and Salmonella or Campylobacter species in the same specimen.

At the IWK Children’s Hospital, 990 patients submitted stool specimens between 19 October 2002 and 31 August 2004. In 5 cases, LM was isolated, but no other Listeria species were isolated. One child had 2 isolates that were recovered 5 months apart but with different PFGE patterns. Campylobacter and Salmonella isolates were obtained from 13 and 12 stool samples, respectively, during the same period.

During the survey period, 3 cases of Listeria sepsis were identified from blood cultures performed on samples obtained from patients hospitalized at the QEIHSC. One woman who was receiving high-dose corticosteroid therapy and leflunomide died of Listeria sepsis but did not have diarrhea. However, culture of a stool sample obtained outside of the study grew LM. One case of fatal intrauterine infection was identified at the IWK/Grace Children’s Hospital in a woman residing in the study catchment area. She did not have a diarrheal illness but had a typical “flulike” prodrome. There were no cases of CNS infection in the Capital Health District during the study period.

Characterization of LM isolates. Of the 18 isolates of LM available for typing (2 of the 18 LM isolates were not recoverable), 10 different “fingerprints” were identified using the Smal digests (figure 2) when Tenover’s rules were applied [18]. One PFGE pattern was identified for 5 isolates, and another was identified for 3 isolates. The identical fingerprint in the group of 5 isolates was observed for isolates from adult patients; the identical triad was from a child and 2 unrelated adult pa-
Listeria Gastroenteritis

Figure 2. Smal digests of DNA from Listeria isolates recovered from stool samples obtained from 2 patients in a survey of febrile gastroenteritis. L. innocua, Listeria innocua; LM, Listeria monocytogenes.

Quantitative testing. Colony counts of LM in stool samples from patients positive for LM were highly variable. LM could not be reisolated from 2 specimens. In the other 16 cases, colony counts ranged from $2.0 \times 10^3$ to $1 \times 10^6$ cfu per gram of stool. There was no correlation in the case-control questionnaire between colony counts and severity of illness as determined by duration of diarrhea or presence or absence of fever or abdominal pain.

Case-control study. Of 13 adult case patients, 12 provided complete or partial information through the telephone interview or a questionnaire. One patient declined to participate. The caregivers of 2 of 4 pediatric patients also provided information. One parent refused, and 1 case patient could not be contacted, because he was placed in an unknown foster home in another province.

Matched control patients with Salmonella and Campylobacter infections were successfully identified for all case patients, but only control patients associated with the 15 case patients with LM in their stool specimens who provided data were contacted to complete the questionnaire. Twenty-four of 26 adult control patients subsequently provided data for analysis, as did 3 of 4 pediatric control patients.

Table 1 shows selected variables from the questionnaire and compares case patients and control patients. The 2 pediatric case patients and their matched control patients were not included in the analysis. Patients with Listeria species isolated from stool specimens had a significantly longer duration of diarrhea (mean duration, 29.6 vs 12.7 days; $P = .003$), fewer...


Table 1. Comparison of demographic and clinical characteristics of patients with *Listeria* species isolated from stool cultures (case patients) versus characteristics of patients with *Campylobacter* or *Salmonella* species isolated from stool cultures (control patients).

<table>
<thead>
<tr>
<th>Demographic or clinical characteristic</th>
<th>Case patients (n = 12)</th>
<th>Control patients (n = 24)</th>
<th>( \chi^2 ) or Fisher’s exact statistic</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years</td>
<td>48.9</td>
<td>46.1</td>
<td>NA</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex</td>
<td>4</td>
<td>11</td>
<td>.491</td>
<td>NS</td>
</tr>
<tr>
<td>Mean duration of diarrhea, days</td>
<td>29.6</td>
<td>12.7</td>
<td>NA</td>
<td>.003</td>
</tr>
<tr>
<td>Fever</td>
<td>2</td>
<td>13</td>
<td>5.26</td>
<td>.035</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>18</td>
<td>6.84</td>
<td>.015</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
<td>11</td>
<td>3.43</td>
<td>.083</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9</td>
<td>18</td>
<td>.142</td>
<td>NS</td>
</tr>
<tr>
<td>Use of GI drugs at onset</td>
<td>1</td>
<td>4</td>
<td>.581</td>
<td>NS</td>
</tr>
<tr>
<td>Underlying GI problems</td>
<td>10</td>
<td>5</td>
<td>11.0</td>
<td>.002</td>
</tr>
<tr>
<td>Use of immunosuppressive drugs</td>
<td>1</td>
<td>2</td>
<td>.005</td>
<td>NS</td>
</tr>
<tr>
<td>Isolation of other potential pathogens</td>
<td>2</td>
<td>1</td>
<td>1.42</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment with antibiotics</td>
<td>1</td>
<td>16</td>
<td>11.95</td>
<td>.003</td>
</tr>
<tr>
<td>Travel</td>
<td>0</td>
<td>6</td>
<td>8.98</td>
<td>.011</td>
</tr>
<tr>
<td>Exposure to cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any cheese</td>
<td>6</td>
<td>17</td>
<td>3.36</td>
<td>NS</td>
</tr>
<tr>
<td>Soft cheese</td>
<td>2</td>
<td>2</td>
<td>2.46</td>
<td>NS</td>
</tr>
<tr>
<td>Exposure to deli meats</td>
<td>5</td>
<td>8</td>
<td>2.13</td>
<td>NS</td>
</tr>
<tr>
<td>Exposure to raw vegetables</td>
<td>10</td>
<td>18</td>
<td>2.21</td>
<td>NS</td>
</tr>
<tr>
<td>Exposure to chicken</td>
<td>12</td>
<td>23</td>
<td>2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Exposure to hamburger</td>
<td>7</td>
<td>11</td>
<td>2.39</td>
<td>NS</td>
</tr>
<tr>
<td>Exposure to hot dogs</td>
<td>3</td>
<td>9</td>
<td>2.45</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients, unless otherwise indicated. \( P \) values are shown for statistically significant characteristics only. \( P \) values < .05 were considered to be statistically significant. GI, gastrointestinal; NA, not applicable; NS, not significant.

instances of fever (2 vs. 13 patients; \( P = .035 \)), fewer instances of nausea (4 vs. 18 patients; \( P = .015 \)), and more instances of underlying gastrointestinal illness (10 vs. 5 patients; \( P = .002 \)). These included 4 cases of previously diagnosed irritable bowel syndrome, 5 cases of inflammatory bowel disease, 1 case of rectal carcinoma, and 1 case of *C. difficile* colitis. Patients with cases of *Listeria* infection were less likely to be treated with antibiotics before receiving a diagnosis (\( P = .003 \)). A travel history was more common in patients with *Salmonella* or *Campylobacter* infections than in patients with *Listeria* infection (\( P = .011 \)).

**Selected Case Histories**

**Case 1.** A 71-year-old woman developed a diarrheal illness that persisted for 9 weeks. She had an extensive medical history, including a diagnosis of irritable bowel syndrome 2 years earlier. Other preexisting conditions included hiatal hernia, lactose intolerance, 4 cerebrovascular accidents, a myocardial infarction, and pseudogout. The diarrheal illness was worse during the first 3 weeks of illness, during which time the patient experienced episodes of incontinence. She also experienced intermittent cramps, nausea, headache, myalgias, joint pain, sore throat, and fatigue, as well as a 9-kg weight loss. She visited her physician several times, did not receive any treatment, but was sent for a colonoscopy 8 weeks after onset. On colonoscopy, a slight area of inflammation was seen in the rectum. Stool culture grew only LM serotype 1/2b. No blood cultures were performed at any time during the patient’s illness.

**Case 2.** A previously healthy 79-year-old man with no underlying medical problems developed diarrhea without fever, cramps, or other associated symptoms. The diarrhea persisted for 14 days. The patient saw his family physician, who ordered stool samples for culture and examination for ova and parasites. Stool samples grew only LM serotype 1/2a, and no parasites were identified. A subsequent sigmoidoscopic examination revealed rectal carcinoma, and a CT scan demonstrated liver metastases.

**Case 3.** A healthy 36-year-old woman had *C. difficile*-associated diarrhea after treatment for a peritonsillar abscess with clindamycin. She was treated with a 10-day course of
metronidazole but experienced relapse after 3 days. Stool samples were submitted for culture, which revealed *C. difficile* toxin again, as well as LM. The patient responded to another course of metronidazole and was healthy thereafter.

**Case 4.** A healthy 24-year-old woman developed acute diarrhea with mild abdominal pain but no nausea, vomiting, or fever. The diarrhea lasted 30 days. Stool cultures were positive for LM (not typed), and no treatment was given. She lost 9 kgs during the illness and was waiting for colonoscopic examination to assess the possibility of inflammatory bowel disease or irritable bowel syndrome. Her mother had Crohn disease.

**Case 5.** A 37-year-old man was admitted to the hospital for ulcerative colitis refractory to coticosteroid treatment. He had had persistent diarrhea for 2 weeks. He underwent a total colectomy, and corticosteroid therapy was tapered. Culture of a stool sample obtained at admission grew LM serotype 4b, but culture was negative for other enteric pathogens.

**DISCUSSION**

This study is, to our knowledge, the only population-based survey of a representative North American population to determine the incidence of gastrointestinal illness attributable to LM. However, our hypothesis that microbiology laboratories may be missing cases of febrile gastroenteritis due to LM in the general population because they do not routinely look for LM using selective culture of stool samples is clearly not the case. We examined almost 8000 stool specimens that were submitted to determine an etiologic diagnosis for diarrhea, and we detected only 17 cases of LM infection or carriage. Previous studies of stool carriage of LM have suggested rates of 0.8–3.4 cases per 100 population [19–32], and this would be consistent with our results, even though we selected for patients with diarrhea. Techniques for isolating *Listeria* species from non-sterile environments, such as food and stool, have evolved both in sensitivity and selectivity, and we chose a method that was highly selective with acceptable sensitivity in food [17], although direct comparisons using stool samples have been infrequently performed before this study [28].

Of those patients with LM isolated from stool samples, only a few had a syndrome consistent with a febrile gastroenteritis. The case-control study suggested that patients with *Salmonella* and *Campylobacter* infections had a more severe presentation, typical of febrile gastroenteritis. Patients with *Campylobacter* or *Salmonella* isolated from stool samples had more instances of fever and were also more likely to be treated with antibiotics, suggesting that they were more severely ill than other patients. We found other potential pathogens more commonly in patients with *Listeria* infection, suggesting that the LM was simply a "fellow traveler" and was not responsible for the diarrheal illness. The fact that diarrhea persisted significantly longer in patients with LM infection than in those with *Campylobacter* or *Salmonella* infection also supports this hypothesis, because in patients with LM, the average duration of gastroenteritis is 2 days [10].

Underlying gastrointestinal problems were more frequently noted in patients who were infected with LM than in patients with *Campylobacter* and *Salmonella* infections. A particularly strong association was found with the presence of inflammatory bowel disease, suggesting a possible causal association of LM with inflammatory bowel disease. Liu et al. [33] found O antigens of LM in 75% of fixed tissues from French patients with Crohn disease by means of immunohistochemical staining, but more-sensitive techniques using PCR have failed to detect LM in specimens from patients with Crohn disease at a rate higher than that seen in control specimens [34, 35]. A noncausal hypothesis would be that the presence of inflammatory bowel disease or other gastrointestinal problems might alter motility or change the properties of the intestinal mucosa, making it easier for LM to remain in the gastrointestinal tract once it has been ingested and has successfully colonized the gut.

Throughout the 24 months of the project, it was noted that there was no clustering of LM isolates on a temporal basis, and no seasonal pattern of isolation was detected. Other studies have suggested that carriage of *Listeria* organisms [32] and invasive listeriosis [36, 37] are more common in the summer months. PFGE results suggest that some strains of LM may have persisted over prolonged periods in the Capital Health District population, but there was no clustering characteristic of an outbreak of febrile gastroenteritis or invasive listeriosis.

The limited food survey that we performed did not reveal any particular difference in food exposure between patients infected with LM and those infected with *Salmonella* or *Campylobacter* species. In the absence of an outbreak of febrile gastroenteritis in our community during the study, it was unlikely that an association could have been identified. Finally, the infrequent isolation of LM in our study would suggest that selective culture for LM should not be added to the algorithm used to process stool samples for the diagnosis of diarrheal illness. These studies may still be indicated during investigation of outbreaks of febrile gastroenteritis in which the cause remains elusive.

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