Most Probable Numbers of Listeria Species in Raw Meats Detected by Selective Motility Enrichment†

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ABSTRACT

We evaluated the methods of direct plate count (DPC) on Modified Oxford agar and most probable number (MPN) using Fung-Yu tubes for the detection of natural contamination by Listeria spp. by examining 100 retail samples of raw meat (beef, lamb, pork, and turkey). Listeria monocytogenes was isolated from 10 samples. Other Listeria spp. (L. innocua, L. murrayi, and L. welshimeri) were isolated from 27 samples. Despite the fact that very low numbers of Listeria were found on these products, the MPN method was significantly more sensitive than the DPC method. The isolation efficiencies of the MPN and DPC methods were 31 and 17%, respectively. Quantitation of indigenous Listeria showed that many meat samples contaminated with ≤ 4 colony-forming units (CFU) per g tested negative by the DPC method but positive by the MPN method.

Key words: Listeria, most probable number, direct plate count, meat

Listeria monocytogenes is a foodborne pathogen that has been implicated in three major listeriosis outbreaks in the 1980s in which pasteurized milk (8), coleslaw (19), and soft cheese (11) were the incriminated foods. A number of meat products, including chicken nuggets, turkey frankfurters, cooked and chill chicken, homemade sausage, Cajun meat and rice sausage, and pork sausage, have been associated with sporadic cases of listeriosis (6). Incidences of L. monocytogenes in meat have been studied in Canada, China, Japan, Norway, Spain, Taiwan, and Yugoslavia (4, 5, 7, 17, 18, 221-23). However, little has been published about its incidence and levels in raw meats in the Midwest of the United States. The organism is notoriously difficult to isolate from meats because of the large numbers of competitive organisms, and enrichment procedures often are used. Data on the prevalence of Listeria spp. in foods could be estimated by the most probable number (MPN) method (9).

The objectives of this report were (a) to determine the incidence and initial levels of Listeria spp. in raw meats sold at the retail level in Manhattan, Kansas and (b) to correlate results with the Fung-Yu tube MPN method (24) for enumeration using a selective plating medium without preenrichment of the meat samples.

MATERIALS AND METHODS

Meat samples

The meat samples, including 43 of beef, 45 of pork, 10 of turkey, and 2 of lamb, were collected from local supermarkets in Manhattan, Kansas. The samples were analyzed as soon as they arrived at the laboratory.

Sample preparation and quantitation

Two subsamples (25 g each) were taken from the same package, and each was homogenized 1:10 in 225 ml of Butterfield’s phosphate buffer in a Stomacher (Tekmar Co., Cincinnati, OH). Each homogenate was diluted 1:10 and 1:100 in the buffer. A 1-ml portion of each homogenate and dilution was inoculated onto modified Oxford agar (MOX) (13) and into double-strength Fraser broth (FB) (24) in arm A of the Fung-Yu tube (three tubes per dilution). The agar plates and tubes were incubated at 35 °C for 24 and 48 h. The MPN values (numbers of colony-forming units (CFU) per g) were obtained by referring to published MPN tables (14). The preparation and inoculation of the Fung-Yu tube to quantitate Listeria were as previously described (24). To estimate the number of other bacteria growing on the meat samples, plates of plate count agar (PCA) (Difco Laboratories, Detroit, MI) were also inoculated and incubated at 35 °C; and colonies were counted after 48 h.

Isolation and identification of genus and species

All MOX plates were examined at 24 h and reincubated for an additional 24 h if no typical colonies were present. Blackened colonies were streaked onto tryptic soy agar (Difco) supplemented with 0.6% yeast extract (TSA-YE) and incubated for 18 h at 35 °C for further confirmation. After 24 h of incubation at 35 °C, any black-
RESULTS AND DISCUSSION

Listeria spp. were isolated from 31 out of 100 meat samples, with L. monocytogenes occurring in 10 of the samples (Table 1). That species was isolated most frequently from ground pork. Other Listeria spp. isolated were L. innocua (15%), L. murrayi (5%), and L. welshimeri (7%). DPC detected 17 positives out of 100 samples while MPN detected 31 (Table 1).

Of the 31 positive samples detected by MPN, 23 samples were found to contain < 100 CFU/g, six ≥ 200 and < 2,400, and the remainder were contaminated with ≥ 2,400 (Table 2). The two raw pork sausage samples contained Listeria murrayi at a level of ≥ 2,400 MPN/g, which was the highest incidence found, suggesting contamination by human contact and/or additional processing steps. Our results showed that L. monocytogenes were isolated in higher numbers in the MPN tubes than on the TSA-

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Ground beef (39)
Beef cube steak (4)
Ground pork (20)
Pork cutlet, links (8)
Pork sausage (17)
Ground turkey (10)
Lamb patty (2)
Total (100)

<table>
<thead>
<tr>
<th>Raw meats (No. of samples)</th>
<th>MPN</th>
<th>DPC</th>
<th>Positive for</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ground beef (39)</td>
<td>16</td>
<td>23</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Beef cube steak (4)</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ground pork (20)</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Pork cutlet, links (8)</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Pork sausage (17)</td>
<td>4</td>
<td>13</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Ground turkey (10)</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Lamb patty (2)</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total (100)</td>
<td>31</td>
<td>69</td>
<td>17</td>
<td>83</td>
</tr>
</tbody>
</table>

<sup>a</sup> LI, L. innocua; LM, L. monocytogenes; LMu, L. murrayi; LW, L. welshimeri.  
<sup>b</sup> Both LI and LW isolated from the same samples twice.  
<sup>c</sup> Both LI and LMu isolated from the same sample.  
<sup>d</sup> Both LM and LMu isolated from the same sample.  
<sup>e</sup> Both LI and LM isolated from the same sample.

examined 113 raw meat samples in Italy and reported that 12% were contaminated with Listeria spp. Nine of the 13 isolates were L. monocytogenes, with the remainder being L. innocua. Listeria monocytogenes was isolated from 28% retail ground beef samples in Denmark (20). Breer and Schoepfer (2) estimated Listeria contamination of raw meat products at ca. 10<sup>5</sup> CFU/g. Listeria monocytogenes was isolated in 17.3% of minced meats in Barcelona, with the most frequent serovars being 1/2 and 4. Other species isolated were L. innocua (66.6%) and L. welshimeri (0.6%) (5). In Japan, Listeria spp. were isolated from 43 (56.6%) of 76 samples of meat products. Listeria monocytogenes occurred in 26 (34%) of the samples (18). Using an MPN method, Breer and Prandl (3) estimated that 81% of the minced meat samples they examined contained < 110 Listeria per g. Karches and Teufel (10) assayed 117 ground meat samples and determined that Listeria was usually present at < 10<sup>5</sup> MPN/g, with ground pork having lower Listeria spp. populations than ground beef. Results from different studies could not be compared directly, because incidence data were taken from several geographic regions of the world and determined by various media and procedures.

ACKNOWLEDGMENTS

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REFERENCES

**TABLE 2. Numbers of indigenous Listeria spp. on 31 raw meats**

<table>
<thead>
<tr>
<th>Meat sample</th>
<th>Listeria spp. (CFU/g) by MPN</th>
<th>Viable cell count (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(No. of positives)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground beef (14)</td>
<td>$4.0 \times 10^0$ - $4.3 \times 10^1$</td>
<td>$ND$ (9), $2.4 \times 10^1$ - $3.0 \times 10^1$</td>
</tr>
<tr>
<td>(2)</td>
<td>$1.9 \times 10^0$ - $5.6 \times 10^2$</td>
<td>$2.4 \times 10^2$ - $4.5 \times 10^2$</td>
</tr>
<tr>
<td>Ground pork (6)</td>
<td>$4.0 \times 10^0$ - $2.4 \times 10^2$</td>
<td>$ND$ (3), $3.2 \times 10^1$ - $2.0 \times 10^2$</td>
</tr>
<tr>
<td>Ground turkey (1)</td>
<td>$1.4 \times 10^1$</td>
<td></td>
</tr>
<tr>
<td>Pork sausage (2)</td>
<td>$2.4 \times 10^2$</td>
<td>$1.4 \times 10^2$</td>
</tr>
<tr>
<td>(2)</td>
<td>$&gt;2.4 \times 10^2$</td>
<td>$3.1 \times 10^3$ - $2.1 \times 10^4$</td>
</tr>
<tr>
<td>Beef cube steak (1)</td>
<td>$2.4 \times 10^2$</td>
<td>$1.4 \times 10^2$</td>
</tr>
<tr>
<td>Pork cutlet (1)</td>
<td>$9.3 \times 10^1$</td>
<td>$1.2 \times 10^1$</td>
</tr>
<tr>
<td>Lamb patty (2)</td>
<td>$4.0 \times 10^0$ - $5.6 \times 10^1$</td>
<td>$ND$ (1), $4.0 \times 10^1$</td>
</tr>
</tbody>
</table>

*ND: not detected.

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