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. Quantification 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, cook-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, 22-23). 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).

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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. monocyto
genocytogenes 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 Listeria spp. were isolated in higher numbers in the MPN tubes than on MOX agar plates. This is similar to the findings of Ronvik and Yndestad (17), which showed that samples with low numbers of Listeria spp. (< 10^2 CFU/g) were detected only after enrichment. In this study, MOX agar achieved quantitative recovery of ≥ 10^2 CFU/g of Listeria spp. in raw meat containing 10^2 to 10^3 CFU/g of background organisms. Similar results also were observed in fish slurry by Paranjpye et al. (15).

Raw meat might be expected to contain low numbers of Listeria, because the organisms occur widely in the environment, e.g., in water, sewage, and soil, and also in the gastrointestinal tract of food animals (1). Luppi et al. (12) 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). Breuer and Schopfer (2) estimated Listeria contamination of raw meat products at ca. 10^3 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, Breuer 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 < 10^2 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.

Rapid tests for the detection and quantification of L. monocytogenes in food products are among the top priorities for primary research (16). Direct plating on a selective medium is restricted in the recovery and enumeration of indigenous Listeria spp. in raw meat products, where the numbers of Listeria are often low and the competitive microflora are at a high level. The Fung-Yu tube MPN test, with the ability to isolate Listeria from heavily contaminated meat samples, could be used as a screening test throughout the production line to establish whether a possible survival of Listeria has taken place after food processing.

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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)</th>
<th>Viable cell count (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(No. of positives)</td>
<td>by MPN</td>
<td>by DPC</td>
</tr>
<tr>
<td>Ground beef (14)</td>
<td>4.0 × 10^6 - 4.3 × 10^7</td>
<td>ND (9), 2.4 × 10^1 - 3.0 × 10^1</td>
</tr>
<tr>
<td>(2)</td>
<td>1.9 × 10^7 - 5.6 × 10^7</td>
<td>2.4 × 10^3 - 4.5 × 10^3</td>
</tr>
<tr>
<td>Ground pork (6)</td>
<td>4.0 × 10^6 - 2.4 × 10^7</td>
<td>ND (3), 3.2 × 10^1 - 2.0 × 10^2</td>
</tr>
<tr>
<td>Ground turkey (1)</td>
<td>1.4 × 10^1</td>
<td>ND</td>
</tr>
<tr>
<td>Pork sausage (2)</td>
<td>2.4 × 10^2</td>
<td>1.4 × 10^1</td>
</tr>
<tr>
<td>(2)</td>
<td>&gt;2.4 × 10^2</td>
<td>3.1 × 10^3 - 2.1 × 10^4</td>
</tr>
<tr>
<td>Beef cube steak (1)</td>
<td>2.4 × 10^2</td>
<td>1.4 × 10^2</td>
</tr>
<tr>
<td>Pork cutlet (1)</td>
<td>9.3 × 10^1</td>
<td>1.2 × 10^1</td>
</tr>
<tr>
<td>Lamb patty (2)</td>
<td>4.0 × 10^6 - 5.6 × 10^7</td>
<td>ND (1), 4.0 × 10^1</td>
</tr>
</tbody>
</table>

*a* ND: not detected.

b: not done.