A Research Note

Isolation and Identification of *Listeria monocytogenes* from Retail Meats in Beijing

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ABSTRACT

To determine the contamination of retail meats by *Listeria monocytogenes* in Beijing, 70 meat samples (25 pork, 10 beef, 14 lamb, and 21 chicken) were analyzed between January and March in 1990. Eight (11%) samples were positive for *L. monocytogenes*, and 39 (56%) samples were found to contain other *Listeria* spp. Seven pork and one chicken sample contained *L. monocytogenes*, whereas all beef and lamb were free of *L. monocytogenes*. Meanwhile, 15 (60%) pork, 11 (52%) chicken, 7 (70%) beef, and 6 (43%) lamb samples were positive for other *Listeria* spp. The eight confirmed isolates of *L. monocytogenes* were serologically typed. Six (5 from pork and 1 from chicken) were serotype 1/2a, and the other two isolates (2 pork) were serotype 1/2c and 1/2b. No isolates were of serotypes 3 and 4. All positive samples for *L. monocytogenes* were frozen meats, and the incidence of *Listeria* spp. was greater for frozen samples than for fresh. Sixty of 70 samples (22 pork, 8 beef, 11 lamb, and 19 chicken) were used to compare the effectiveness of Oxford medium and Palcam medium for the isolation of *L. monocytogenes* from meat samples. *L. monocytogenes* was isolated from seven samples by Palcam medium, while five samples were positive by Oxford medium, indicating that Palcam medium was more effective for recovering *L. monocytogenes* than Oxford medium.

In recent years, outbreaks of serious foodborne listeriosis involving many deaths have been associated with consumption of raw vegetables, coleslaw, pasteurized milk, and Mexican-style cheese. This has led to a renewed scientific interest on *L. monocytogenes* (2,4,5,11). Thus, *L. monocytogenes* is now considered as a dangerous pathogenic bacterium, and research on the organism has increased in industrialized countries (16).

*Listeria monocytogenes* is a gram-positive asporogenous coccobacillus which can grow and multiply at refrigeration temperatures, so it can be a potential micro-

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in 1990. Thirty-five samples were frozen meats, and 35 fresh meat samples were obtained after slaughtering about 7-8 h. Among the 70 samples, 60 samples (22 pork, 8 beef, 11 lamb, and 19 chicken) were used to compare the effectiveness of Oxford medium and Palcam medium for the isolation of *L. monocytogenes*; the other 10 samples (3 pork, 2 beef, 3 lamb, and 2 chicken) were only analyzed by Oxford medium because of the lack of Palcam medium. Each sample (250 g) was aseptically removed from the belly of retail pork, beef, and sheep carcasses and the breast of unpackaged chicken placed into sterile plastic bags, and transported to the laboratory at about 0°C within 2 h. Samples were frozen at -4°C until the day before being examined, then stored at 4°C to thaw. No more than 7 d passed from collecting the samples until microbiological analyses were initiated.

**Preparation of Oxford medium, Palcam medium, and the other related medium**

Listeria selective agar base (Oxford medium, Oxoid, Basingstoke, U.K.), Palcam-Listeria-selective agar (Palcam medium, Merck, Federal Republic of Gomoy), LPM medium (Difco, Detroit, MI), and tryticide soy agar (BBL, Cockeysville, MD) were prepared according to the manufacturers instructions.

**Listeria monocytogenes analysis**

The procedure used for the isolation and identification of *L. monocytogenes* was similar to that described by Kokubo (9) (Fig. 1). Each sample (10 g) was mixed in 50 ml of Listeria enrichment broth (LEB, Difco) and incubated at 30°C for 7 d. After 2 and 7 d of enrichment samples were streaked onto Oxford medium (Oxoid), and Palcam medium (Merck), respectively. The plates were incubated at 30°C for 24-48 h, then five typical esculin positive colonies per plate were streaked onto LPM medium (Difco). The plates were incubated at 30°C for 24 h, then observed by Henry’s oblique lighting technique (7). Greyish-blue typical bacterial bodies were picked and streaked onto tryticide soy agar (BBL) and incubated at 30°C for 24 h. Picked typical single suspicious *Listeria* spp. colony was again observed by Henry’s oblique lighting technique. Every colony was confirmed by biochemical reactions and morphology, including Gram stain, catalase production, motility, and VP reaction. For the further confirmation of *L. monocytogenes* other biochemical reactions including the ability to ferment rhamnose, xylose, and mannitol with acid production, β-haemolytic activity, and CAMP test were used (7). Whether enriched 2 or 7 d, samples positive for *L. monocytogenes* on Oxford medium or Palcam medium were designated as positive. Confirmed isolates of *L. monocytogenes* were serologically typed by Dr. Kokubo, who works at the Department of Food Hygiene and Nutrition, Tokyo Metropolitan Research Laboratory Public Health, Japan.

**RESULTS AND DISCUSSION**

When 60 of 70 samples were enriched at 30°C for 2 d, 5 were positive for *L. monocytogenes* on Palcam medium, while only 2 were positive on Oxford medium. When enriched at the same temperature for 7 d, 7 were positive on Palcam medium and 5 were positive on Oxford medium. This indicates the greater recovery of *L. monocytogenes* with 7 d of enrichment on Palcam medium than that on Oxford medium. However, the incidence of *Listeria* spp. from samples enriched at 30°C for 7 d was lower than that from samples at the same temperature for 2 d (Table 1). When 2 and 7 d enrichment samples were streaked onto Oxford medium and Palcam medium, esculin negative bacteria did not grow on Palcam medium, whereas a limited number grew on Oxford medium. This indicates that Palcam medium has greater inhibition on esculin negative bacteria than Oxford medium. But for certain samples, conflicting results were occasionally obtained between Palcam medium and Oxford medium. Therefore, if possible, Palcam and Oxford medium should be simultaneously used to isolate *L. monocytogenes* from retail meats.

<table>
<thead>
<tr>
<th>Enrichment procedure</th>
<th>Meat sample: 10 g + LEB medium: 50 ml</th>
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| Enrichment temperature | Oxford medium: 30°C, 2 d  
 Palcam medium: 30°C, 2 d |
| Confirmation procedure | Gram stain, Catalase, Motility, VP reaction |
| Serological identification | CAMP test, Rhamnose, Xylose, Mannitol |

**Table 1. Comparison of incidence of *Listeria* organism from 60 retail meats by Oxford medium and Palcam medium.**

<table>
<thead>
<tr>
<th>Enrichment procedures</th>
<th>No. (%) of positive samples</th>
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<tr>
<td>Oxford</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>30°C, 2 d</td>
<td>32 (53.3)</td>
</tr>
<tr>
<td>30°C, 7 d</td>
<td>30 (50.0)</td>
</tr>
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Of 70 samples, 8 were positive for *L. monocytogenes* and 39 were positive for *Listeria* spp. Of 25 pork samples, 7 (28%) were positive for *L. monocytogenes*. One (5%) of 21 chicken samples contained *L. monocytogenes*. All beef and lamb samples were negative. For *Listeria* spp., 15 (60%) of 25 pork, 11 (52%) of 21 chicken, 7 (70%) of 10 beef, and 6 (43%) of 14 lamb samples were positive (Table 2). Some survey data have shown *L. monocytogenes* to be present in approximately 30-50% of raw meats with incidence up to 80% reported (2). Meanwhile, because of the frequent occurrence of *L. monocytogenes* in bird feces, 14.7-60% of chicken were positive for *L. monocytogenes* (13). The low recovery of *L. monocytogenes* especially from chicken, beef, and lamb in our study may be due in part to the cold weather conditions (maximum temperature 0-2°C), low total bacterial counts of the examined samples, and an insufficient number of samples or differences in methods of isolation and identification.

Cultures of *L. monocytogenes* isolated from retail meats were also determined (Table 3). Six samples including 5 pork and 1 chicken contained serotype 1/2a; each of the
other two positive pork samples contained serotype 1/2b or 1/2c. No serotypes 3 and 4 were found. Johnson et al. (6) summarized a large number of reports about serotype of L. monocytogenes recovered from meat and poultry and found the majority serotype was serotype 1/2, with a smaller proportion of serotype 3 and 4 isolates. Our result appears to be agreeable with the conclusion of Johnson et al.

Of the 70 samples, all positive samples for L. monocytogenes were frozen meats, whereas the fresh meats were negative. Furthermore, the incidence of Listeria spp. was greater in frozen meats than in fresh meats (Table 4). The reason may be that the animals were slaughtered in the slaughter house every morning, and the fresh meats were quickly transported to the shop for sale, therefore, decreasing the possibility of contamination of L. monocytogenes. For freezing, additional handling was required, much more exposure for Listeria contamination of the meat samples was encountered. A second study conducted during the summer months in 1990 gave similar isolation results, indicating that cold weather can be discounted as a contributing factor for low isolation rates of L. monocytogenes.

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REFERENCES