Occurrence of *Listeria monocytogenes* in Raw Milk in Nebraska

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ABSTRACT

Raw milk samples were obtained from bulk storage tanks of individual dairy farms in eastern Nebraska during February and July of 1986. One hundred different farms were tested during each period. One-tenth ml of each sample was plated directly onto McBride’s Listeria Agar (MLA) and 30 ml was subjected to a four-week cold enrichment procedure. Suspect colonies from MLA were subjected to biochemical tests to confirm identity. Nine percent of all raw milk samples examined were determined to be positive for *Listeria* species after the cold enrichment procedure. Four percent contained *L. monocytogenes* and five percent contained *L. innocua*. Six percent and two percent of samples were found to contain *L. monocytogenes* in February and July respectively.

*Listeria monocytogenes* has been the causative agent in several recent foodborne disease outbreaks. During the summer of 1983, a listeriosis outbreak occurred in Massachusetts in which pasteurized milk was implicated as the vehicle of infection (5). In a California outbreak (8), a Mexican-style cheese was found to be contaminated with *L. monocytogenes*, resulting in a nationwide recall of the product. Mortality rates for both outbreaks were approximately 30%. During February and March of 1986, *L. monocytogenes* was detected in Brie cheese imported from France (1). No cases of listeriosis attributable to the cheese were reported in the United States, however, several cases did occur in both England and Canada. Additionally, there have been several major product recalls due to the presence of the organism in pasteurized dairy products. Post-processing contamination, resulting from raw milk or environmental contamination, was assumed to be the source of the bacterium.

The objective of this study was to determine the incidence of *L. monocytogenes* in raw milk obtained from bulk tanks on dairy farms in eastern Nebraska.

MATERIALS AND METHODS

Raw milk samples were randomly obtained from bulk milk tanks of individual dairy farms in eastern Nebraska during February and July of 1986 by a cooperating marketing organization. One hundred different samples were obtained during each test period. Samples were taken aseptically from tanks containing milk less than two d old which had been pooled from 10-150 cows depending upon herd size. Samples were transported on ice to the laboratory and refrigerated upon receipt.

One-tenth ml of each sample was plated directly onto McBride’s Listeria Agar (MLA) and incubated for 48 h at 35°C. A cold enrichment procedure was also used, in which 30 ml of sample was added to 150 ml Oxoid Nutrient Broth #2 (Oxoid, Columbia, MD) and incubated for 4 weeks at 4°C. Each week, one-tenth ml of the cold enrichment broth was plated onto MLA and incubated for 48 h at 35°C. The pH values of enrichment broths were monitored during incubation. After 4 weeks of cold enrichment, 1 ml of each sample was added to 9 ml of a selective broth (Oxoid Nutrient Broth #2 containing 100 mg/ml nalidixic acid and 3.75% potassium cyanide), incubated at 35°C for 18 h, plated onto MLA and incubated at 35°C for 48 h as described by Hayes et al. (6). MLA plates were examined for suspect colonies using the Henry method of oblique lighting (7). Suspect colonies were streaked onto MLA, examined microscopically for characteristic morphology, and differentiated and characterized as described in Bergey’s Manual of systematic bacteriology, Vol. 2 (11). Isolates recovered were checked for motility, gram stain, catalase production, oxidase production, MR-VP reaction, indole production, esculin hydrolysis, urease production, nitrate reduction, H₂S production (TSI), citrate utilization, hemolytic activity, CAMP test, and ability to ferment glucose, rhamnose and mannitol with acid production. Isolates giving responses typical of *L. monocytogenes* were serotyped using Bacto-Listeria O Antiserum Types 1 and 4 (Difco, Detroit, MI) by the slide agglutination method according to the manufacturer’s instructions.

Positive and negative controls were included in each trial. *L. monocytogenes* ATCC 7644 was used as the positive control.

RESULTS AND DISCUSSION

Nine percent (18/200) of all raw milk samples examined were positive for *Listeria* (Table 1). Four percent (8/200) contained *L. monocytogenes* and 5% (10/200) contained *L. innocua*. No other species of *Listeria* was isolated.

In the February trial, 6% (6/100) of all raw milk samples tested were positive for *L. monocytogenes* and 2% (2/100) were positive for *L. innocua*. In July, 2% (2/100) of samples were positive for *L. monocytogenes* and 8% (8/100) were positive for *L. innocua*. None of the samples yielded more than one *Listeria* isolate.
Serotypes of *L. monocytogenes* isolates are summarized in Table 2. Isolates incriminated in recent foodborne disease outbreaks have been serotype 4 (3,5,9,10).

No *Listeria* isolates were obtained by direct plating of raw milk onto MLA. Most of the positives were obtained after the first week of enrichment. The pH values of enrichment broths generally remained in the range of 5.9 - 6.1, although several broths had pH values of 4.9 - 5.0 at the end of the four-week cold enrichment. None of the broths where pH values fell below 5.6 yielded *Listeria* isolates.

*L. monocytogenes* is commonly and consistently present in Nebraska’s milk supply, occurring in 4% of all samples examined. Our results are similar to those reported by Lovett et al. (9) and Farber et al. (4) in the United States and Canada respectively. However, Dominguez Rodriguez et al. (2) reported an isolation rate of 45.3% for *L. monocytogenes* from raw milk in Spain. Additionally, a seasonal variation may exist for the occurrence of the bacterium with a larger number being isolated in winter. Lovett et al. (9) also noted an increase in occurrence of *L. monocytogenes* in the winter. A seasonal variation was also noted for the occurrence of *L. innocua*, with a larger number isolates occurring in summer. While Lovett et al. (9) reported a seasonal variation in occurrence of *L. innocua* with an increase during the winter months, Farber et al. (4) reported an increase during the summer months. It is unknown whether our values observed are real or due to our relatively small sample size.

Lovett et al. (9) postulated that seasonal variation may be related to feeding practices, herd management or unknown factors affecting animal-bacteria or bacteria-environment relationship, or both. Dairy cattle are often fed silage during the winter months and improperly fermented and old silage can reportedly be a source of *Listeria* (3).

Problems exist with current methodology for isolation of *Listeria* from food products. The method used in this study required observation and screening of large numbers of colonies and must be performed by experienced personnel. It is possible that some positives may have been missed. Furthermore, the pH of enrichment broths often dropped to levels that inhibit the growth of *Listeria*. It is recognized that other more efficient methods for isolation of *Listeria* are available.

The results of this study and those reported by other researchers indicate that *Listeria* commonly occurs in the raw milk supply. Consequently, all raw milk should be assumed to be contaminated with *Listeria* and handled accordingly.

**ACKNOWLEDGEMENTS**

The technical assistance of Dianne Peters and Jon Haakenstad is gratefully acknowledged.

**REFERENCES**


**TABLE 1. Isolation of *Listeria* from raw milk in Nebraska.**

<table>
<thead>
<tr>
<th>Listeria species</th>
<th>February 1986</th>
<th>July 1986</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. monocytogenes</em></td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>(4.0)*</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>(5.0)</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10</td>
<td>18</td>
<td>(9.0)</td>
</tr>
</tbody>
</table>

*From 100 samples/period.

*Expressed as a percent of the 200 total samples taken.*

**TABLE 2. Serotype of *Listeria monocytogenes* isolates.**

<table>
<thead>
<tr>
<th></th>
<th>Type 1</th>
<th>Type 4</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 1986</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>July 1986</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Did not react with type 1 or type 4 sera.*