A Research Note

Occurrence of Yersinia enterocolitica in Raw and Pasteurized Milk

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ABSTRACT

Raw milk (100 samples) and pasteurized milk (100 samples) were assayed for presence of Yersinia enterocolitica, using four different enrichment and post-enrichment techniques. Isolation of Y. enterocolitica was made on MacConkey agar and Cefsulodin-Irgasan-Novobiocin (CIN) agar directly from incubated broths containing the milk, and after treatment of enrichment broths with a potassium hydroxide solution. Twelve samples of raw milk and one of pasteurized milk contained Y. enterocolitica. Nearly all isolates were found after KOH treatment. All isolates except that obtained from the pasteurized milk sample produced detectable levels of heat-stable enterotoxin. However, none of the 13 isolates agglutinated in WA-SAA, an antiserum specific for virulent Y. enterocolitica.

Yersinia enterocolitica is a ubiquitous bacterium indigenous to the gastrointestinal tract of warm-blooded animals and is associated with human diseases. The types of infection reported include gastroenteritis, terminal ileitis, mesenteric lymphadenitis, septicemia, meningitis and skin and eye infections (4,29). The bacterium has been isolated from a variety of foods (3,12,26), and is able to multiply to large populations at normal refrigeration temperatures (25).

Y. enterocolitica has been isolated from raw milk in Australia (13), Canada (20), Czechoslovakia (1,27) and the USA (24). Esseveld and Goudzwaard (9), in 1973, and Inoue and Kurose (15), in 1975, reported the isolation of Y. enterocolitica from caecal contents of healthy cows in Japan. Hughes (14) obtained 16 isolates of Y. enterocolitica from a dairy farm and from two raw milk collection depots in Australia. Other investigators had shown that the prevalence of Y. enterocolitica in raw milk was 18.2% in the southern Ontario region of Canada (21). Both environmental strains and serotypes associated with human illness were found. In another study conducted in Denmark, a recovery rate of 10% was obtained (6). However, none of the Danish isolates was identical to the serotype of Y. enterocolitica which has been implicated in human illness in Denmark.

There have been a few reports of isolation of Y. enterocolitica from pasteurized milk. In 1975, the Canadian National Reference Center for Yersinia received two strains of Y. enterocolitica that had been isolated from pasteurized milk (21). Hughes (13) repeatedly isolated Y. enterocolitica (biotype 1 serotype 5a) from pasteurized milk in the same holding vat at a particular dairy factory in Australia. She attributed survival of the organism to inadequate chemical disinfection of the holding vat, and reported that the problem was eliminated when chemical disinfection of the holding vat was replaced by a steam cleaning process. There have been only two well-documented outbreaks of foodborne infection caused by Y. enterocolitica, one resulting from consumption of contaminated chocolate milk (3).

Methodology for isolation of Y. enterocolitica from foods is still in the developmental stage. At this time there is no good selective enrichment medium for isolation of all clinically important serotypes of Y. enterocolitica. Some investigators have found that it is difficult to detect small numbers of Y. enterocolitica among large numbers of other microorganisms (25). Also, some of the selective media used do not recover all clinically important serotypes (4,5).

Based upon the relative tolerance of yersiniae to dilute alkali, isolation of the organism was improved when a potassium hydroxide treatment of the enriched culture was applied before streaking on the selective agar medium (2). We examined the influence of some variables in enrichment procedures and selective plating media on recovery of Y. enterocolitica from raw and pasteurized milk. Results of determining the enterotoxigenicity and potential virulence of isolates of Y. enterocolitica obtained from milk are also presented.

MATERIALS AND METHODS

Milk samples

Two hundred samples of raw and pasteurized milk (100 samples of each) were examined for presence of Y. enterocolitica. Raw milk samples originating from different dairy farms supplying the University of Wisconsin-Madison dairy plant and delivered to the plant laboratory for routine bacteriological examinations were examined for presence of Y. enterocolitica. Pasteurized milk samples (cartons) were obtained from different retailers in the city of Madison. One milliliter of each milk sample was transferred to 10 ml of enrichment media for examination.
TABLE 1. Y. enterocolitica isolated from raw and pasteurized milk by direct streaking of different enrichment broths, before and after treatment with KOH solution, onto MacConkey and CIN agars.

<table>
<thead>
<tr>
<th>No. positive sample</th>
<th>Enrichment procedure*</th>
<th>PBS</th>
<th>PBSSB</th>
<th>PBS + MRB</th>
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<tbody>
<tr>
<td></td>
<td>Mac</td>
<td>CIN</td>
<td>Mac</td>
<td>CIN</td>
</tr>
<tr>
<td>1</td>
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<td>+</td>
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<td>+</td>
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<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13b</td>
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<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

* +, – recovery of Y. enterocolitica with and without KOH treatment. No isolates were obtained by MRB technique.

b Y. enterocolitica isolate from a pasteurized milk sample.

Enrichment methods

Four types of enrichment procedures were used: (1) 1/15 M phosphate-buffered saline solution (PBS), pH 7.6 (11), incubated at 4°C for 3 weeks; (b) PBS + 1% sorbitol + 0.15% bile salts (PBSSB) (16), incubated at 4°C for 3 weeks; (c) modified Rappaport broth (MRB) (11), incubated at room temperature (23 ± 1°C) for 5 d; and (d) MRB inoculated with 1 ml of PBS (incubated at 4°C for 3 weeks) (PBS + MRB), incubated at 27°C for 2 d (11, 23).

Isolation and identification of Y. enterocolitica

After incubation, a loopful of each enrichment broth was streaked directly onto a MacConkey agar plate (10, 11) and CIN agar plate (22). Also, 0.5 ml of these cultures was added to 4.5 ml of 0.5% KOH in 0.5% NaCl, the mixture was swirled with a Vortex Mixer, and both selective agar media were streaked with the sample within 1 min. Agar plates were incubated for 48 h at 27°C. After incubation, colonies having characteristics of Y. enterocolitica (either translucent on MacConkey agar plates or having a deep red center with a rather sharp border and translucent outer zone on CIN agar plates), were subcultured on TSI agar slants and incubated for 2 d at 26°C. If a typical reaction was obtained (i.e., acid/acid without gas or H₂S), the organism was further tested for urease production at 37°C, and also for motility (11) at 22 and 37°C. Isolates giving a urease-positive reaction and that were motile at 22°C but not at 37°C were examined further with API-20E strips (Analytab Products, Plainview, NY).

Enterotoxin assay

All isolates identified with the API-20E system as Y. enterocolitica were examined for ability to produce heat-stable enterotoxin, as determined with the infant mouse assay (7, 19).

Sero logical testing

All isolates obtained in this study were submitted to the Food Research Institute at the University of Wisconsin-Madison for serological testing with WA-SAA antisem that agglutinates specifically with mouse-virulent Y. enterocolitica (8).

RESULTS AND DISCUSSION

Y. enterocolitica was recovered from 12 samples of raw and from one of pasteurized milk (Table 1). Schiemann and Toma (21) found a greater prevalence of Y. enterocolitica in raw milk. Also, Vidon and Delmas (28) found that 81.4% of raw milk samples contained Y. enterocolitica. Our results are somewhat similar to those obtained by Norbert (17) and Christensen (6) for raw milk. Schiemann (20) reported that 265 samples tested, one sample of a pasteurized fluid dairy product contained Y. enterocolitica.

Data on the recovery rate of Y. enterocolitica from raw and pasteurized milk with each of the enrichment broths on both selective agars are in Table 1. The organism was only recovered from samples given cold enrichment. Postenrichment treatment with dilute alkali was effective for recovery of nearly all Y. enterocolitica isolated; only one isolate was recovered without alkaline treatment (Table 1).

In our experiments, we found that cold-enrichment in PBSSB gave the greatest recovery of Y. enterocolitica from milk samples, followed by PBS and PBS + MRB enrichments. Eleven isolates were recovered by enrichment with PBSSB compared to nine with PBS and six with PBS + MRB procedures.

Direct enrichment in MRB was ineffective for recovering Y. enterocolitica, as no isolates were obtained by this technique. This may have resulted from absence of certain serotypes which usually grow in Modified Rappaport broth (18).

We also compared MacConkey and CIN agar media for isolation of Y. enterocolitica (Table 1). The organism was recovered from three different enrichment broths by CIN agar alone, from an additional enrichment broth by MacConkey agar alone, and from 9 enrichment broths by both selective media.

The CIN medium gave a greater frequency of isolation of Y. enterocolitica than MacConkey agar. These results agree with those of Schiemann (22). This demonstrates the value of using more than one enrichment procedure and isolation medium for recovery of Y. enterocolitica.

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In addition to screening for presence of *Y. enterocolitica* in milk, we determined the enterotoxigenicity of our isolates by using the infant mouse model. All isolates originally from raw milk were enterotoxigenic, giving a ratio of intestinal weight/remaining body weight of $\geq 0.113$ (0.147 ± 0.014), while that obtained from a pasteurized sample was not enterotoxigenic, giving a ratio of 0.069.

We also tested the isolates for their potential virulence by determining their ability to agglutinate with WA-SAA antiserum that agglutinates specifically with mouse-virulent *Y. enterocolitica* (8). All of our isolates were avirulent by this assay.

Our results suggest that enterotoxigenic *Y. enterocolitica* is not a common contaminant in pasteurized milk at the retail level. Presence of this organism in milk of high bacteriological quality is uncommon, but it may appear in milk when there is a breakdown in sanitation procedures, as may have happened in the gastroenteritis outbreak traced to chocolate milk (3).

Also, consumption of raw milk and the manufacture of cheese or other dairy products from raw milk are practices that can allow for transmission of human yersiniosis.

In conclusion, it is important that a standard laboratory procedure for isolation of virulent *Y. enterocolitica* from milk and milk products be developed. The PBSSB enrichment-KOH post-enrichment treatment used in this study appears to be a reliable procedure for avirulent *Y. enterocolitica*.

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**REFERENCES**