Association of *Yersinia enterocolitica* with the Manufacture of Cheese and Occurrence in Pasteurized Milk

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Received for publication 12 April 1978

Raw milk in southern Ontario frequently contains *Yersinia enterocolitica*. The potential for transmission of this organism by cheese manufactured from unpasteurized milk was evaluated by examination of milk and cheese curd samples from cheese manufacturing plants and finished cheddar and Italian cheeses. The incidence of *Y. enterocolitica* was lower in cheese curd samples (9.2%) than in raw milk (18.2%). Most of the curd samples showed a positive phosphatase test, indicating production from raw milk. One curd sample yielded *Y. enterocolitica* after 4 weeks of storage at 4°C but was negative after 8 weeks. All samples of cheddar and Italian cheeses, most of which showed a positive phosphatase test, were negative for *Y. enterocolitica*. One out of 265 samples (0.4%) of pasteurized fluid dairy products contained *Y. enterocolitica*.

Yersiniosis is frequently suspected to be a food-borne disease; however, only one documented outbreak has been recorded in which the vehicle, chocolate milk, contained the same serotype of *Yersinia enterocolitica*, O:8, as that isolated from ill persons (2). There have been a number of outbreaks in which the epidemiology strongly implicated a common vehicle, but it was never identified. Zen-Yoji et al. (21) described an outbreak which involved 198 out of 1,086 pupils at a junior high school in Japan. *Y. enterocolitica* was recovered from 122 patients (62%), and the isolates were predominantly serotype O:3 Nilehn biotype 4. Asakawa et al. (1) reported two additional outbreaks in Japan, both involving school children and characterized by high fecal recovery rates of *Y. enterocolitica* serotype O:3 biotype 4. No secondary infections of family contacts were observed, and the authors suggested that these outbreaks were probably food or waterborne, but the vehicle was not identified. A suspected outbreak of yersiniosis in school children was reported in Montreal in 1976 (7). Although raw milk was first thought to be the vehicle, the serotype recovered was different than that from ill children. Olousky (13) implicated food, possibly contaminated by a food handler, in an outbreak in Czechoslovakia. Szita and Szidro (18) stated in their review of human *Y. enterocolitica* infections in Hungary that the epidemiological evidence suggested a food-borne infection in one outbreak, but the incriminated food was not available.

Most reported outbreaks of human yersiniosis have involved serotype O:3, which is also commonly found in swine (19, 20). Wauters (G. Wauters, 3rd International Symposium on *Yersinia*, Mont-Gabriel, Quebec, Canada, 25-28 Sept. 1977) found 56% of fresh pork tongues in Belgium positive for *Y. enterocolitica*. All isolates were serotype O:3 with the exception of a few which were serotype O:9. Pedersen (K.B. Pedersen, 3rd International Symposium on *Yersinia*, Mont-Gabriel, Quebec, Canada, 25-28 Sept. 1977) isolated serotype O:3 from 30% of the throat swab samples taken from slaughtered pigs. Surveys of market meat samples as potential vehicles of infection rarely find serotype O:3, and the majority of the isolates from food sources are atypical from types commonly associated with human infections (6, 10, 12). Consequently, the link between swine reservoir and transmission to humans remains unclear.

There have been very few reports on the isolation of *Y. enterocolitica* from milk and dairy products. In a recent review on *Y. enterocolitica* in foods, Lee (11) cited only two references to isolation from milk and one from ice cream. Schiemann and Toma (16) recently reported a high incidence of *Y. enterocolitica* in raw milk from southern Ontario. The majority of the isolates were atypical from common clinical strains, but a few were identical with infrequently reported human types, such as serotype O:6,30, the third most common human serotype in Canada (S. Toma, 3rd International Symposium on *Yersinia*, Mont-Gabriel, Quebec, Canada, 25-28 Sept. 1977). The presence of *Y. enterocolitica* in raw milk presents the possibility of transmission by cheese manufactured from unpasteurized milk.
milk. This report describes the results of a study undertaken to evaluate that transmission potential by examination of milk destined for manufacture of cheese, cheese curd, and finished cheeses for *Y. enterocolitica*. The results of a survey for the presence of *Y. enterocolitica* in pasteurized fluid dairy products are also presented.

**MATERIALS AND METHODS**

**Milk and cheese samples.** Raw milk and cheese curd samples were collected from manufacturing plants located in the province of Ontario. Samples of cheddar and Italian cheeses were collected either at the manufacturing plant or from retail outlets. All samples were collected at random and were not directly related in any way. Samples of pasteurized fluid dairy products were selected at random from regular submissions to our public health laboratory for routine bacteriological examinations. Most of these samples were collected at the dairy plant, and very few came from retail outlets.

**Phosphatase.** Phosphatase analyses were completed by using the automated method described by Reynolds and Telford (14) on the supernatant from a 1:5 homogenate (wt/vol) of the cheese or cheese curd and applying the standards previously suggested by the author (15).

**Isolation of *Y. enterocolitica*.** Three enrichment techniques were used for the isolation of *Y. enterocolitica* from raw milk and cheese. (i) Modified Rappaport broth (MRB) (17) was inoculated at a ratio of 11 g of cheese or 11 ml of milk to 99 ml of broth, with incubation at room temperature (23 ± 1°C) for 5 days. Cheese and curd samples were first homogenized in a Stomacher homogenizer before incubation. (ii) Christenson’s cold enrichment medium (CE) (E.H. Christenson and G.P. Jansen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C43, p. 42) consisting of: K$_2$HPO$_4$, 0.2%; NaCl, 0.85%; mannnitol, 1.0% (pH 7.3) was inoculated at the same ratios as for MRB with incubation at 4°C for 14 days. (iii) The CE method followed by transfer of 1 ml of this enrichment medium to 10 ml of MRB, which was then incubated at room temperature for 2 days, was also used.

Pasteurized fluid dairy products were examined for the presence of *Y. enterocolitica* by transferring 1 ml of product to: (i) MRB (10 ml) with incubation at room temperature for 5 days; and (ii) phosphate-buffered saline (M/15, pH 7.6, 10 ml) with incubation at 4°C for 14 days and then transfer of 1 ml to MRB (10 ml), which was incubated at room temperature for two days.

All fluid enrichment media used in the raw milk and cheese study were streaked onto MacConkey and salmonella-shigella agars, and those used in the fluid dairy product survey were streaked onto MacConkey agar alone. The selective media were incubated at room temperature for 2 days. Colonies resembling *Y. enterocolitica* were fished to Kligler iron agar slants which were incubated overnight at 35°C. Isolates showing alkaline/acid reactions without hydrogen sulfide were confirmed further by the biochemical tests listed in Table 2. Those isolates biochemically identifiable as *Y. enterocolitica* were transferred for serotyping to the Canadian National Reference Centre for *Yersinia* located in our Central Public Health Laboratory.

**RESULTS**

*Y. enterocolitica* was isolated from raw milk samples collected from vats or holding tanks at cheese manufacturing plants with approximately the same frequency (18.2%) as that observed previously for individual producer samples (Table 1) (16). Two serotypes, O:16 and O:34, have not been isolated in prior surveys of raw milk, but serotype O:6,30 was previously isolated (16; D. A. Schiemann, 3rd International Symposium on *Yersinia*, Mont-Gabriel, Quebec, Canada, 25–28 Sept. 1977).

The isolation rate for cheese curd (9.2%) was

<table>
<thead>
<tr>
<th>Table 1. Isolation of <em>Y. enterocolitica</em> from raw milk and cheese</th>
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<tr>
<td><strong>Type of sample</strong></td>
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<tr>
<td></td>
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<tr>
<td>Raw milk</td>
</tr>
<tr>
<td>Cheese curd</td>
</tr>
<tr>
<td>Cheddar cheese</td>
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<tr>
<td>Italian cheese</td>
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</table>

* Enrichment methods: R, MRB at 23°C for 5 days; CE at 4°C for 14 days; CER, CE followed by R at 23°C for 2 days.

* Four curd samples yielding *Y. enterocolitica* tested for phosphatase and found positive.

* Provolone, caciocavallo, mozzarella, scamorza, provolette, and ricotta.
approximately half that for raw milk, although 80% of the samples tested for phosphatase indicated that the curd had been manufactured from raw milk. Four of the seven curd samples yielding Y. enterocolitica were tested for phosphatase and found positive. An identical serotype, O:16, to that found in raw milk was isolated from curd, and another serotype, O:4, from curd was previously isolated from raw milk with a high frequency (D. A. Schiemann, 3rd International Symposium on Yersinia, Mont-Gabriel, Quebec, Canada, 25-28 Sept. 1977). Six of the curd samples yielding Y. enterocolitica were stored at 4°C. Only one sample was still positive after 4 weeks of storage, and this sample was negative after 8 weeks.

All samples of cheddar cheese and Italian cheeses examined for Y. enterocolitica were negative. Phosphatase tests on a portion of these samples indicated that most of the cheddar cheese and approximately half of the Italian cheeses had been manufactured from raw milk (Table 1).

No single enrichment method for isolation of Y. enterocolitica appeared obviously superior to any other used in this study (Table 1); however, the number of positive samples was too small for a valid comparison of the methodologies.

The strains of Y. enterocolitica isolated from raw milk and cheese curd were mostly rhamnose positive (Table 2), resembling so-called “environmental strains” which have been infrequently associated with human infections. Most of the isolates were positive for indole and lecithinase, placing them in Wauters’ biotype 1, and were uniformly positive for salicin and aesculin hydrolysis, resembling Nileh’s biotype 1.

One isolation of Y. enterocolitica was obtained from 265 pasteurized fluid dairy products, representing an isolation rate of 0.4% (Table 3).

### Table 2. Characteristics of 28 isolates of Y. enterocolitica from raw milk and cheese curd

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>%</th>
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<tbody>
<tr>
<td>Motility, 36°C</td>
<td>2</td>
<td>7.1</td>
</tr>
<tr>
<td>Lactose, 36°C</td>
<td>21</td>
<td>75.0</td>
</tr>
<tr>
<td>Rhamnose, 36°C</td>
<td>24</td>
<td>85.7</td>
</tr>
<tr>
<td>Indole, 30°C</td>
<td>27</td>
<td>96.4</td>
</tr>
<tr>
<td>Voges-Proskauer, 23°C</td>
<td>23</td>
<td>82.1</td>
</tr>
<tr>
<td>Citrate, 23°C</td>
<td>24</td>
<td>85.7</td>
</tr>
<tr>
<td>Lecithinase, 23°C</td>
<td>23</td>
<td>82.1</td>
</tr>
</tbody>
</table>

*All isolates were positive at 23°C for motility, ornithine decarboxylase, sucrose, xylose, and aesculin hydrolysis and negative for malonate. All isolates were positive at 36°C for dextrose, beta-galactosidase, salicin, mannitol, maltose, urease, and nitrate reductase, and negative for lysine, arginine, and dulcitol.

The product was 2% homogenized milk which, according to the phosphatase test, had been properly pasteurized. The isolate was serotype O:34, and was biochemically unusual in being positive for indole, salicin, aesculin, and citrate, but negative for rhamnose, Voges-Proskauer, lactose, and lecithinase.

### DISCUSSION

The largest proportion of cheddar cheese manufactured in Ontario is made from raw milk. Legal requirements specify that this cheese must be aged for 60 days before sale, a procedure intended for the destruction of potential disease agents which may be present. The study reported here supports the effectiveness of aging for destruction of Y. enterocolitica, and suggests that cheese is not likely to present an important vehicle for transmission of this agent. When six curd samples which contained Y. enterocolitica were aged at 4°C, only one was positive after 4 weeks, and this sample was negative after 8 weeks storage. All of the finished cheddar and Italian cheese samples examined, most of which had been manufactured from raw milk according to positive phosphatase tests, failed to yield Y. enterocolitica.

The cheese manufacturing process itself appears to be deleterious to Y. enterocolitica in that the isolation rate from curd was about half that for raw milk. The destruction of Y. enterocolitica during both the manufacture and aging of cheese is not unexpected because the changes effected by the lactic bacteria used in the production process are detrimental to many other bacteria (3, 9). This antagonism results not only from a lowering of the pH by the production of lactic acid but also from antibiotic substances produced by certain lactic bacteria (4, 8). The final fermented product is not favorable to either multiplication or survival of gram-negative bacteria such as Y. enterocolitica.

In the only documented food-borne outbreak
of yersiniosis (2), pasteurized chocolate milk was identified as the vehicle. In the survey reported here, only one out of 265 samples (0.4%) of pasteurized fluid dairy products contained Y. enterocolitica. Because it is unlikely that Y. enterocolitica survives the pasteurization process (5), the organism more probably is a post-pasteurization contaminant which occurs with a low frequency. The presence of Y. enterocolitica in the dairy plant is quite understandable in view of its common occurrence in raw milk, the excellent growth medium that milk provides, and the ability of the organism to grow well in milk at refrigeration temperatures. Although milk may not be a frequent vehicle for transmission of Y. enterocolitica, the circumstances in the New York outbreak implicating chocolate milk and the one isolation reported here indicate that the organism can occasionally occur in milk which has been adequately pasteurized. 

ACKNOWLEDGMENTS

I gratefully acknowledge the technical assistance of Maija Latvala, the serotyping and manuscript review provided by S. Toma, and the assistance of J. Sterns in arranging for sample collections.

LITERATURE CITED


