Microbiology of a Major Foodborne Outbreak of Gastroenteritis Caused by *Yersinia enterocolitica* Serogroup O:8

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Gastrointestinal disorders of varying severity were observed in 239 (53%) of 455 campers and staff members at a coed summer camp in Sullivan County, New York, during July 1981. Five of seven hospitalized patients had appendectomies before the disease was recognized as yersiniosis. *Yersinia enterocolitica* serogroup O:8 (American strain) was isolated from 37 (54%) of 69 persons examined, including the head cook and 3 others of the 11-person kitchen staff. Of 48 food, water, and environmental samples collected from the camp area, *Y. enterocolitica* isolates belonging to the same serogroup and biogroup as the human isolates were recovered from dissolved powdered milk, a milk dispenser, and turkey chow mein. This laboratory finding supported the epidemiological data indicating a correlation between consumption of these foods and illness. *Y. enterocolitica* isolates of the same biogroup as the O:8 isolates but belonging to serogroup O:34 were also isolated from six campers and two samples of dissolved powdered milk. Pathogenicity studies on the *Yersinia* isolates were performed with three in vitro tests (calcium dependency, autoagglutination, and HeLa cell infection) and one in vivo test (intraperitoneal challenge of mice). Most of the serogroup O:8 human isolates and the chow mein isolate were positive in all four tests. Milk isolates of serogroup O:8 were positive in the in vitro tests but were relatively avirulent in mice, whereas serogroup O:34 isolates, regardless of source, were negative in all four tests.

Two major foodborne outbreaks of yersiniosis have been reported in the United States. Both were caused by *Yersinia enterocolitica* serogroup O:8, biogroups Niléhn 2, Wauters 1, and Knapp and Thal 2 (American strain) (13). Both occurred in New York State. The first, occurring in Holland Patent, Oneida County, N.Y., in September 1976, affected over 200 children attending five schools. Because the symptoms of yersiniosis can mimic those of appendicitis, 36 children were hospitalized, 16 of whom had appendectomies. Chocolate milk was epidemiologically and bacteriologically incriminated in the outbreak (2, 12, 14).

The second major outbreak occurred in a coed summer camp in Liberty, Sullivan County, N.Y., in July 1981. At least 35% of 455 campers and staff members had symptoms of abdominal pain and fever or abdominal pain and two other gastrointestinal disorders (nausea, vomiting, diarrhea); each was designated as a case in this report. A total of 53% complained of abdominal pain. Appendectomies were performed on five of the seven hospitalized camp members.

This report documents the microbiological aspect of the latter outbreak, with emphasis on the isolation and characterization of the causative organism.

MATERIALS AND METHODS

Specimens. As soon as the outbreak of gastrointestinal illness was reported, the New York State Department of Health collected samples for *Yersinia, Salmonella*, and *Shigella* testing. Stool samples were requested from camp members. Of 66 persons submitting stool samples, 43 were symptomatic and 23 were asymptomatic at the time of collection.

Of 48 food and environmental samples collected, 28 were food specimens from the camp kitchen, 16 were drinking and surface water samples from the kitchen and other areas of the camp, 1 was a soil sample from the camp's septic system, and 3 were stools from dogs (1 from the only animal in the camp and 2 others from the family pets of an ill person in the camp).

All specimens were collected in phosphate-buffered saline (pH 7.6), except for the first few, which were collected in buffered glycerol. All specimens were kept refrigerated during transport to the laboratory.

Three *Yersinia* isolates obtained from hospitalized camp members were received from the Community
General Hospital in Harris for confirmation and serogrouping and biogrouping.

Isolation and identification. Yersinia were isolated, identified, and characterized as previously described (13). In brief, all specimens were plated upon receipt and, if no Yersinia-like colonies were seen, after 1 and 3 weeks of cold enrichment in phosphate-buffered saline onto Endo, deoxycholate citrate, and deoxycholate citrate-sucrose agars. Suspected colonies were transferred to triple sugar iron and urea agar slants. If reactions typical of Yersinia were seen, 36 biochemical tests were performed to establish the species and biogroup of each isolate (1, 13). The isolates were serogrouped by slide agglutination with antisera (O:1 through O:34) prepared in our laboratory.

Pathogenicity in vitro. (i) Calcium dependency test. The calcium dependency test was performed by the method of Gemski et al. (4). The isolates were grown overnight on tryptic soy agar plates at 25°C. Three individual colonies were transferred to three tubes of tryptic soy broth, grown overnight at 25°C, and diluted in saline to approximately 10⁴ organisms per ml. Duplicate drops were placed on each of two plates of Blood Agar Base II (Oxoid USA Inc., Columbia, Md.) with and without magnesium oxalate. The plates were incubated overnight at 35 to 37°C. An isolate was considered calcium dependent if growth was inhibited only on Blood Agar Base II with magnesium oxalate and non-calcium dependent if the growth was similar on both plates.

(ii) Autoagglutination test. The method of Laird and Cavanaugh (7) was used for the autoagglutination test. The same three single-colony tryptic soy broth cultures used in the calcium dependency test were also used for this test. Two drops of the 18- to 24-h undiluted broth were placed in each of two tubes containing 2 ml of Eagle minimal essential medium with 10% fetal bovine serum. One tube was incubated at 25°C and the other at 35 to 37°C for 18 to 24 h. An isolate was considered positive if the growth agglutinated strongly at 35 to 37°C and slightly or not at all at 25°C; it was considered negative if growth was not agglutinated at either temperature.

(iii) HeLa cell infection test. The method of Devenish and Schiemann (3) was used with slight modification. A suspension of HeLa cells containing 5 × 10⁴ cells per ml was prepared in minimal essential medium—10% fetal bovine serum. A 0.4-ml portion was placed in each chamber of an eight-chamber tissue culture slide (Miles Laboratories, Inc., Lab Tek Div., Naperville, Ill.) and incubated overnight at 37°C in 5% CO₂. A sweep of growth of an isolate grown overnight on a tryptic soy agar plate at 25°C was used to inoculate two tubes of tryptic soy broth. One tube was incubated at 25°C and the other at 35 to 37°C for 18 to 24 h. Both cultures were centrifuged and diluted in minimal essential medium—10% fetal bovine serum to approximately 10⁸ organisms per ml.

A 0.4-ml portion of minimal essential medium—10% fetal bovine serum containing 150 Yersinia per HeLa cell was added to each chamber. After 1 h of incubation at 37°C in 5% CO₂, the slides were washed with warm phosphate-buffered saline, fixed with methanol, and stained with Giemsa. The isolate was considered positive if the bacteria adhered to the entire surface of the HeLa cell; it was considered negative if the bacteria were found only surrounding the HeLa cell on the surface of the slide.

Pathogenicity in vivo: mouse virulence test. A culture of Yersinia grown overnight in tryptic soy broth at 25°C was washed twice in saline, and the number of viable organisms was estimated from a previously established plot of turbidity versus viable count. For screening purposes, mice were inoculated intraperitoneally with 10⁴, 10⁵, or 10⁶ Yersinia. To determine the median lethal dose (LD₅₀), 10 representative isolates were inoculated into mice at five concentrations each, and the LD₅₀ was calculated by the procedure of Reed and Muench (10). All mice were observed for mortality for 21 days.

RESULTS

Epidemiology. The coed summer camp where the outbreak occurred is located in a rural area in Liberty, Sullivan County, N.Y., at an elevation of 2,000 feet (610 m). The camp has its own septic system but obtains its water from a nearby public supply. At the time of the outbreak, the population of the coed summer camp totaled 455 people: 327 campers (several of whom came from western and central Europe), 101 counselors, 16 administrative staff, and 11 kitchen staff. The campers ranged in age from 9 to 18 years, and the counselors ranged in age from the late teens to the early twenties. The campers were housed in cabins, each accommodating 8 to 15 campers and staff. Each cabin had its own bathroom and shower facilities.

Of the camp population, 239 individuals (53%) complained of abdominal pain. Of these patients, 159 (35% of the total camp population) complained of abdominal pain and fever or abdominal pain and two other gastrointestinal disorders and were designated as cases. Of the seven hospitalized patients, five had appendectomies before the disease was identified as yersiniosis.

The outbreak peaked between 4 and 10 July; during that week, 135 camp members reported symptoms. There was no significant difference in the attack rates between males and females, nor were the inhabitants of any specific cabins affected more than the rest. The foods consumed by camp members with epidemiologically significant attack rates were milk and turkey chow mein (D. L. Morse, M. Shayegani, and R. Gallo, manuscript in preparation).

Y. enterocolitica obtained from humans. Sixty-eight fecal specimens from 66 campers and staff members (one person was sampled three times) and three isolates from locally hospitalized patients (sources, one fecal and two intraabdominal) were received by our laboratories. Specimens from 41 of these 69 people were culture positive for Y. enterocolitica. Of the isolates recovered, 39 were serogroup O:8 (including the three hospital isolates and the three isolates from the person sampled 3 times), and 6 were serogroup O:34. Specimens from two people were culture positive for both serogroups...
TABLE 1. Y. enterocolitica, serogroups O:8 and O:34, biogroups Niléhn 2, Wauters 1, and Knapp and Thal 2, isolated from human, food, and environmental specimens

<table>
<thead>
<tr>
<th>Source of specimen (no. tested)</th>
<th>No. of isolates recovered from serogroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O:8</td>
</tr>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Fecal specimens (68)</td>
<td>36²</td>
</tr>
<tr>
<td>Culture from patient (3)</td>
<td>3</td>
</tr>
<tr>
<td>Food and environmental</td>
<td></td>
</tr>
<tr>
<td>Milk suspensions (3)</td>
<td>1²</td>
</tr>
<tr>
<td>Milk dispenser (3)</td>
<td>1</td>
</tr>
<tr>
<td>Turkey chow mein (1)</td>
<td>1</td>
</tr>
<tr>
<td>Others (41)</td>
<td>0</td>
</tr>
</tbody>
</table>

² Specimens were received from 66 people; 1 person submitted specimens on three occasions.
² Isolates of both serogroups were obtained from two humans.
² The other 41 specimens were water (16), cold cuts (5), vegetables (5), chicken parts (4), dog fecal specimens (3), milk powder (2), and chocolate powder, orange juice, sweetener, mayonnaise, mustard, and septic system soil (1 each). Y. enterocolitica, serogroup O:4,33, was isolated from one of the dog fecal specimens, and Y. intermedia, serogroup O:5, was isolated from a celery sample.

(Table 1). No Salmonella or Shigella organisms were isolated.

Of the individuals positive for Y. enterocolitica, 25 (61%) were cases, 8 (19.5%) had only abdominal pain, and 8 were asymptomatic when the specimen was collected. The camp session ended before follow-up studies could be done on asymptomatic people.

On three occasions over a 5-week period, fecal specimens submitted by the head cook were tested. Y. enterocolitica serogroup O:8 was isolated all three times on primary plating. Four other kitchen workers were also culture positive for Y. enterocolitica, three for serogroup O:8 (one isolated on primary plating, one after 1 week, and one after 3 weeks of cold enrichment) and one for serogroup O:34 (isolated after 3 weeks of cold enrichment). Y. enterocolitica obtained from food and environmental samples. From a total of 48 food and environmental specimens, Y. enterocolitica serogroup O:8 was isolated from 3 food specimens: a milk suspension prepared from powdered milk (no isolates were recovered from dry powdered milk), a milk dispenser, and turkey chow mein (Table 1).

No Y. enterocolitica serogroup O:8 was isolated from 16 water samples taken from various parts of the camp nor from other foods. Y. enterocolitica serogroup O:34 was isolated from two milk suspensions, one of which contained an O:8 isolate. Y. enterocolitica serogroup O:4,33 was isolated from the only dog in the camp, and Yersinia intermedia serogroup O:5 was isolated from celery in the camp refrigerator. No Salmonella or Shigella were isolated.

Characteristics of Yersinia isolates. All 50 isolates of Y. enterocolitica obtained from human and food specimens had the following characteristics when tested at 35 to 37°C (or at 25°C where indicated): positive test reactions for urease, indole, methyl red (25°C), Voges-Proskauer (25°C), motility (25°C), nitrite reduction, lecithinase (25°C), ornithine decarboxylase, β-galactosidase (o-nitrophenyl-β-D-galactopyranoside, 25°C), L-arabinose, D-glucose, iso-inositol, maltose, D-mannitol, D-sorbitol, sucrose, D-trehalose, D-xylose, and oxidation of lactose (25°C); negative test reactions for oxidase, hydrogen sulfide (triple sugar iron agar), Simmons' citrate, motility, esculin, phenylalanine deaminase, arginine dihydrolase, lysine decarboxylase, adonitol, α-methyl-d-glucoside, dulcitol, gas from glucose, lactose, D-melibiose, D-raffinose, L-rhamnose, and salicin.

These reactions placed the isolates in biogroups Niléhn 2, Wauters 1, and Knapp and Thal 2 (American strain, when serogroup O:8). Forty-two of the isolates belonged to serogroup O:8 and eight to serogroup O:34.

Although of the same biogroup, representatives of the two serogroups had slightly different antibiograms. Ten representative O:8 isolates (seven from humans and one each from chow mein, a milk suspension, and a milk dispenser) and three representative O:34 isolates (two from humans and one from a milk suspension) were tested by the antibiotic disk susceptibility method as described by the National Committee for Clinical Laboratory Standards (9). The O:8 isolates were susceptible to cephalothin and ampicillin, and the O:34 isolates were resistant to cephalothin and of intermediate susceptibility to ampicillin. All isolates of both serogroups were susceptible to amikacin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, nitrofurantoin, polymyxin B, streptomycin, sulfisoxazole, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole. All were resistant to novobiocin and of intermediate susceptibility to carbencillin.

Two other Yersinia isolates were obtained from farm-related samples. One from a dog was identified as Y. enterocolitica serogroup O:4,33, biogroups Niléhn 1, Wauters 1, and Knapp and Thal 3, and was salicin and esculin positive. The other, isolated from a celery sample, was identified as Y. intermedia serogroup O:5.

Effect of cold enrichment on isolation of Yersinia. Only 47% of the total Yersinia isolates were obtained from the primary plating (Table
2). An additional 32% became positive after 1 week of cold enrichment; the rest required 3 weeks of cold enrichment.

Of the 36 serogroup O:8 isolates recovered from human fecal specimens, 20 were not seen on primary plating. The O:8 isolates from a milk suspension and from turkey chow mein required 3 weeks of cold enrichment. However, the organism from the milk dispenser was recovered on primary plating.

Pathogenicity studies. Four pathogenicity tests were performed on all isolates of *Y. enterocolitica* serogroups O:8 and O:34; some results are shown in Table 3, in which representative isolates of both serogroups are listed in order of their virulence as measured by the LD₅₀ test in mice.

Of the human isolates, the lowest LD₅₀ was for a serogroup O:8 isolate from the head cook. The food isolate with the lowest LD₅₀ was the serogroup O:8 isolate from turkey chow mein. Milk isolates of each serogroup had high LD₅₀.

All serogroup O:8 isolates tested were positive in the HeLa cell test but variable in the other in vitro tests. Of the human serogroup O:8 isolates, 89% were virulent in mice, killing mice at ≤10⁵ organisms per mouse. In contrast, all serogroup O:34 isolates, regardless of source, were negative in the in vitro tests and were much less virulent in mice. These isolates did not kill mice at 10⁷ organisms per mouse; their LD₅₀s were ~10⁹ organisms per mouse.

All *Y. enterocolitica* serogroup O:8 isolates from the outbreak could be divided into four groups, depending on the results of the pathogenicity tests (Table 4). Most of the human isolates (64%) and the isolate from chow mein were positive in all four tests (group I). Ten of the human isolates (26%) were negative for calcium dependency and autoagglutination (group II), and four other isolates (10%) were positive only in the HeLa cell infection test (group III). Milk isolates were positive in all in vitro tests, but 10⁷ organisms did not kill the mice (group IV).

Of serogroup O:8 isolates from eight camp members asymptomatic at the time of the specimen collection, five were in group I, one was in group II, and two were in group III. All other isolates were from symptomatic people.

Antibody studies. When tested by a reference laboratory (Centers for Disease Control, Fort Collins, Colo.) with stock typing antigens, sera

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**TABLE 2.** Isolation of *Y. enterocolitica* from human and food specimens during primary plating or after cold enrichment

<table>
<thead>
<tr>
<th>Specimen and serogroup</th>
<th>No. of isolates recovered after</th>
<th>Primary plating</th>
<th>Cold enrichment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human feces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O:8</td>
<td>16</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>O:34</td>
<td>4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Milk suspensions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O:8</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>O:34</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Milk dispenser, O:8</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey chow mein, O:8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

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**TABLE 3.** Pathogenicity test results of representative isolates of *Y. enterocolitica* serogroups O:8 and O:34

<table>
<thead>
<tr>
<th>Serogroup and source</th>
<th>LD₅₀ for mice</th>
<th>Calcium dependency at 37°C</th>
<th>Autoagglutination at 37°C</th>
<th>HeLa cell infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serogroup O:8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>1.4 × 10²</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Turkey chow mein</td>
<td></td>
<td>1.9 × 10²</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>3.3 × 10³</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>3.7 × 10³</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>7.9 × 10³</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td>3.8 × 10⁸</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Milk dispenser</td>
<td></td>
<td>5.5 × 10⁸</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serogroup O:34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td>1.2 × 10⁹</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>1.4 × 10⁹</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td>1.5 × 10⁹</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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from 23 symptomatic persons were negative for Yersinia antibody. However, varied results were obtained when these samples were tested with serogroup O:8 antigens prepared from a strain isolated during the outbreak. Sera from five people gave serodiagnostically significant reactions (titer, \( \geq 256 \)); sera from nine people gave reactions of borderline significance (titer, \( \leq 128 \)), and sera from nine people did not significantly react with the O:8 antigens.

Only 12 of these 23 patients submitted stool specimens for culture. Y. enterocolitica serogroup O:8 was isolated from eight specimens, serogroup O:34 from two specimens, both serogroups from one specimen, and no Yersinia from one specimen. There was no obvious relationship between development of significant antibody and the pathogenicity of the isolate obtained from the same patient. Isolates obtained from patients with serodiagnostically significant titers ranged from positive in all four pathogenicity tests to negative in all four tests.

**Plasmid studies.** Virulence of Y. enterocolitica has been correlated with the presence of a 42-megadalton plasmid (4, 16). To test for the correlation, eight representative serogroup O:8 isolates were tested for plasmids (6) at the Centers for Disease Control, Atlanta, Ga. Plasmids of 42 and 88 megadaltons were found in seven isolates: four from symptomatic patients, one from an asymptomatic patient, one from a milk suspension, and one from turkey chow mein. Four of these seven isolates were positive in all four pathogenicity tests (three from symptomatic patients and one from turkey chow mein), two were positive in the HeLa cell infection and the mouse virulence tests (one from a symptomatic and one from an asymptomatic patient), and one was positive only in the three in vitro tests (from a milk suspension). The one isolate which was negative for plasmids was isolated from a symptomatic patient and was positive only in the HeLa cell infection test.

Sera from two of the patients with plasmid-containing isolates were tested. Both had serodiagnostically significant titers with antigens from the outbreak.

**DISCUSSION**

During the last two decades, many individual cases of yersiniosis have been reported, particularly in Europe, North America, and Japan, but reports of large-scale outbreaks have been few. Since 1972, seven such outbreaks have been reported from Japan, with seven schools and several hundred students involved (18). The causative agent was determined to be Y. enterocolitica serogroup O:3, but no source of infection was found.

In the United States, a small-scale outbreak caused by serogroup O:8, the strain of Y. enterocolitica most frequently isolated from symptomatic humans in this country, was reported from North Carolina in 1973 (5). It involved 16 of 21 persons in four related and neighboring families. Two appendectomies and two deaths resulted. Dogs were suspected as vehicles of transmission of the infection, but any relationship between the illnesses observed in the dogs and the humans was circumstantial.

The only two large outbreaks caused by serogroup O:8 (American strain) occurred in New York State. One in Oneida County in 1976 involved 222 schoolchildren; contaminated chocolate milk was incriminated as the source of infection (2, 14). The second outbreak is the subject of the present report. Contaminated milk suspension, a milk dispenser, and turkey chow mein were epidemiologically and bacteriologically associated with the outbreak, which involved 239 members of a summer camp population of 455. Transmission of the disease most likely involved contamination of foods by the food handlers during the preparation of food, the cleaning of a 6-gallon plastic milk dispenser, or occasional manual repair of a leaking milk dispenser.

Cold enrichment was helpful to obtain serogroup O:8 isolates, 53% of which were recovered only after 1 or 3 weeks of cold enrichment. Van Noyen et al. (15) found cold enrichment not to be essential for isolation of pathogenic serogroups O:3 and O:9, which are most commonly found in Europe, Japan, and Canada. However,
these and other investigators have reported on the usefulness of cold enrichment for isolation of other strains of *Yersinia* (15, 17).

The relationship among various pathogenicity tests for *Yersinia* isolates has been previously investigated (8, 11). In our study, 26 (62%) of the 42 serogroup O:8 isolates were positive in all four pathogenicity tests; the other 16 isolates were positive in one to three of the tests.

In summary, our laboratory data demonstrated that *Y. enterocolitica* serogroup O:8, biogroups Nilöhn 2, Wauters 1, and Knapp and Thal 2 (American strain), was isolated from 54% of the 69 persons examined in the outbreak. The same serogroup and biogroup was isolated from humans, a milk suspension made from powdered milk, a milk dispenser, and turkey chow mein. Most of the human isolates and the turkey chow mein isolate had similar virulence properties, as determined by four pathogenicity tests, and the isolates from human and food sources had identical plasmid profiles. These laboratory data, combined with epidemiological data, suggest an association between the outbreak and consumption of milk or turkey chow mein or both. The role of *Y. enterocolitica* serogroup O:34, isolated from six patients and two milk suspensions, is unclear, since the isolates were negative in the four pathogenicity tests.

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LITERATURE CITED