CONCISE COMMUNICATION

An Outbreak of *Yersinia enterocolitica* O:8 Infections Associated with Pasteurized Milk

Marta-Louise Ackers,[1,a] Susan Schoenfeld,[2] John Markman,[1,a] M. Geoffrey Smith,[3,a] Mabel A. Nicholson,[1] Wallis DeWitt,[1] Daniel N. Cameron,[1] Patricia M. Griffin,[1] and Laurence Slutsker[1]

[1]*Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia;* [2]*Vermont State Department of Health, Burlington;* [3]*New Hampshire State Department of Health and Human Services, Concord*

In October 1995, an outbreak of *Yersinia enterocolitica* O:8 infections occurred in the Upper Valley of Vermont and New Hampshire. Ten patients were identified, median age 9 years (range, 6 months–44 years). Three patients were hospitalized; 1 underwent an appendectomy. Consumption of bottled pasteurized milk from a local dairy was associated with illness (matched odds ratio undefined; lower 95% confidence interval, 1.9). No deficiencies in pasteurization procedures or equipment were detected. *Y. enterocolitica* O:8 was isolated from 1 raw-milk sample and from a fecal sample from 1 dairy pig. The route of contamination was not determined; this outbreak likely resulted from postpasteurization contamination of milk. Dairy pigs were the most likely source of contamination. Milk bottles were likely contaminated by rinsing with untreated well water prior to filling or by other environmental routes. Educating dairy owners about *Y. enterocolitica* and postpasteurization contamination is necessary to prevent further outbreaks.

Yersinia enterocolitica is an infrequently reported diarrheal pathogen in the United States, although it is commonly reported in other parts of the industrialized world. Illness is characterized by diarrhea, abdominal pain, and fever. Symptoms can mimic appendicitis and may result in unnecessary laparotomy. In Europe the majority of cases are due to *Y. enterocolitica* serogroups O:3 and O:9 and are caused by consumption of pork products [1]. In contrast, in the United States between 1976 and 1982, 4 of the 5 reported outbreaks of *Y. enterocolitica* infections were caused by serogroup O:8; tofu, chocolate milk, bean sprouts, and powdered milk were implicated as vehicles of transmission [2–6]. However, after 1988, several US outbreaks of *Y. enterocolitica* O:3 infections in infants attributed to household preparation of pig intestines (chitterlings) [7, 8] suggested a serogroup shift [9].

In October 1995, an outbreak of *Y. enterocolitica* O:8 infections occurred among residents of the Upper Valley of Vermont and New Hampshire. We describe the results of the epidemiologic investigation that linked illness to consumption of bottled pasteurized milk from a local dairy and discuss the implications of this outbreak for regulation of milk products.

Materials and Methods

Case ascertainment. We defined an outbreak-related case of *Y. enterocolitica* O:8 infection as a stool, blood, or mesenteric lymph node culture that yielded *Y. enterocolitica* O:8 in an Upper Valley, Vermont or New Hampshire resident with onset of illness during October 1995. The Upper Valley refers to the area of the Connecticut River Valley bordering New Hampshire and Vermont, including Grafton and Sullivan counties in New Hampshire and Windsor County in Vermont.

The infection-control practitioner at the Dartmouth-Hitchcock Medical Center in Lebanon, New Hampshire, reported an initial cluster of 3 cases of *Y. enterocolitica* infections to the Vermont State Health Department during the last week of October 1995. On October 27, the New Hampshire and Vermont state health departments began informing physicians and hospitals about the initial cluster and requested that providers contact state health departments about suspected cases.

Case-control study. The initial 6 patients identified with *Y. enterocolitica* O:8 infection, who were the first to become ill in their

Received 23 August 1999; revised 10 January 2000; electronically published 15 May 2000.

Presented in part: 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, September 1996 (abstract K145).

Informed consent was obtained from the patients or their parents or guardians, and the human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of the clinical research.

[a] Present affiliations: Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta (M.-L.A.); Department of Neurology, Massachusetts General Hospital, Boston (J.M.); Virginia Department of Health, Chatham (M.G.S.).

Reprints or correspondence: Dr. Laurence Slutsker, Foodborne and Diarrheal Diseases Branch, DBMD/NCID/CDC, 1600 Clifton Rd., MS A-38, Atlanta, GA 30333.

The Journal of Infectious Diseases 2000; 181:1834–7

0022-1899/2000/18105-0051$02.00

respective families, were included in the case-control study. Cases of *Y. enterocolitica* infection occurring within a household after an initial case were excluded. For each case, 3 age- and town-matched controls were interviewed. Except for 1 infant, age 6 months, all controls were randomly selected from public telephone directories from the case-patient's township, matched by telephone prefix. The controls for the 6-month-old were selected from an area hospital birth list. Eligible controls had no history of diarrheal illness, abdominal cramps, sore throat, or fever during October and were living in the Upper Valley during the outbreak.

Trained interviewers administered a questionnaire by telephone to patients and controls or their parents asking about illness, food consumption, and animal exposures associated with *Y. enterocolitica* infection. Interviewers asked patients about exposures in the 14-day period before illness onset and controls in the most recent 2-week period, beginning with the same day of the week as the day of illness onset for the patient.

Statistical analysis. Univariate matched odds ratios (MORs) were calculated by using maximum-likelihood estimates and exact 95% lower confidence intervals (CIs) by Fisher's exact test [10].

Laboratory investigation. Stool specimens from patients with suspected *Y. enterocolitica* infection were cultured by using MacConkey or cefsulodin-irgasan-novobiocin (CIN) agar. Porcine rectal, tonsillar, and pharyngeal swabs and other dairy environmental specimens were similarly cultured and cold-enriched in a peptone-sorbitol broth at the Centers for Disease Control and Prevention (CDC) and/or New Hampshire State Laboratory. Retail milk samples were examined for phosphatase (its presence indicates inadequate pasteurization). Retail milk, raw milk, and sweetwater (water passed through piping prior to pasteurization) samples were tested for total coliforms. State public health laboratories cultured milk samples for *Yersinia.*

Y. enterocolitica isolates were confirmed and biotyped at CDC. Tests were conducted to detect biochemical markers of pathogenicity [11], and isolates were serogrouped by somatic O antigens. Isolates were tested for susceptibility to chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, nalidixic acid, ampicillin, sulfisoxazole, streptomycin, kanamycin, gentamicin, ceftriaxone, and amoxicillin-clavulanic acid by disk diffusion.

The *Y. enterocolitica* O:8 isolates were subtyped by pulsed-field gel electrophoresis (PFGE) and restricted with *Not*I (New England Biolabs, Beverly, MA). The PFGE pattern of the outbreak-associated isolates was compared with background isolates from the Vermont and New York State health departments and Canada. The International *Yersinia* Reference Center (Pasteur Institute, Paris) determined phage types.

Environmental investigation. Dairy A pasteurized milk samples were collected from Upper Valley retail stores. Pasteurized-milk, raw-milk, and well-water samples were obtained during dairy routine testing and subsequent inspections.

Investigators from the Food and Drug Administration (FDA) regional office and the Vermont Department of Agriculture assisted in 2 dairy inspections. Environmental assessment included inspection and microbiologic sampling of dairy equipment, drainage collections, the milk bottle washer, delivery trucks, and other storage sites. Rectal, tonsillar, and pharyngeal swabs were obtained from 9 dairy pigs.

Results

Patient characteristics. Ten cases of *Y. enterocolitica* O:8 infection were identified in Upper Valley residents, 9 with symptom onset between October 9 and 26, 1995 (figure 1). The tenth patient had a chronic underlying gastrointestinal illness, so a date of illness onset could not be determined. The patient median age was 9 years (range, 6 months–44 years); 50% were male. *Y. enterocolitica* was isolated from the mesenteric lymph nodes of 1 person, from the blood and stool of 1 person, and from the stool of 8 persons. Three patients were hospitalized; 1 underwent an appendectomy. Symptoms reported among the 9 patients with known dates of illness onset included abdominal cramping (100%), fever (100%), median maximal temperature 40°C, diarrhea (78%), nausea (61%), headache (61%), bloody diarrhea (22%), and pharyngitis (22%). The median duration of illness was 6 days (range, 4–25 days).

Case-control study. Six patients and 18 matched controls were included in the case-control study (table 1). Overall, milk consumption was not associated with illness. However, consumption of dairy A bottled milk in the 2 weeks before illness onset was strongly associated with infection (MOR undefined; lower 95% CI, 1.9; $P < .01$). Five (83%) of 6 patients reported consuming dairy A bottled milk, compared with only 2 (12%) of 17 controls. Patients reported drinking bottled skim milk, 2% milk, and chocolate milk. No cases were traced to consumption of bottled cream, bottled half-and-half, or bulk milk packaged in plastic bags. Consumption of pork or well water or exposure to animals, including domestic livestock, was not linked with illness.

Patient specimens. Ten *Y. enterocolitica* isolates were confirmed as serogroup O:8; 5 isolates demonstrated resistance to ampicillin, and 5 demonstrated intermediate sensitivity to ampicillin. All isolates were biotype 1B and pathogenic.

Milk specimens. None of 43 pasteurized retail milk samples collected from October 27, 1995 through March 6, 1996 or the 8 pasteurized milk samples collected from dairy A on October 24, November 11, or November 16 yielded *Y. enterocolitica.* The absence of phosphatase in tested samples indicated ade-

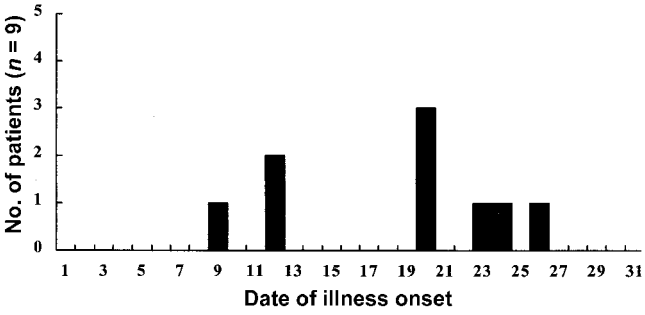

Figure 1. Patients with *Yersinia enterocolitica* O:8 infection, by date of illness onset, Upper Valley of Vermont and New Hampshire, October 1995.

Table 1. Selected exposures among patients with *Yersinia enterocolitica* O:8 infection and controls, univariate analysis, Upper Valley of Vermont and New Hampshire, October 1995.

Exposure	No. of patients/total (%), $n = 6$	No. of controls/total (%), $n = 18$	Matched odds ratio (95% confidence interval)	*P*
Food				
Any milk[a]	5/6 (83)	15/17 (88)	—	—
Dairy A bottled milk	5/6 (83)	2/17 (12)	Undefined (1.9–∞)	<.01
Any pork	4/4 (100)	8/17 (47)	Undefined (.6–∞)	.07
Well water	4/6 (67)	12/18 (67)	1.0 (.1–16.3)	.70
Environment				
Any animal exposure	6/6 (100)	18/18 (100)	—	—
Pigs	2/6 (33)	1/18 (6)	6.0 (.3–354.0)	.16
Sheep	2/5 (40)	0/18 (0)	Undefined (.6–∞)	.06
Cows	2/6 (33)	0/18 (0)	Undefined (.6–∞)	.06
Dogs	4/5 (80)	11/17 (65)	2.0 (.2–111.5)	.48
Cats	4/6 (67)	16/18 (89)	.2 (0–4.8)	.25
Mice	3/6 (50)	4/18 (22)	4.2 (.3–235.9)	.22

[a] No discordant sets.

quate pasteurization. However, 1 retail milk sample collected on November 1, evaluated by the most probable number of coliform organisms (MPN) method, had a coliform count ⩾120 MPN/mL; coliform levels should not be >10 per mL [12]. One raw-milk sample collected on November 14 yielded *Y. enterocolitica* O:8 resistant to ampicillin and amoxicillin-clavulanic acid. This isolate was biotype 1A and was nonpathogenic.

Environmental specimens. Well-water samples collected on October 24 and November 14 revealed elevated levels of coliforms (5.1 and 3.6 MPN/100 mL, respectively); these values should be <1.1 [12]. One pig rectal-swab specimen collected on November 10 yielded a nonpathogenic strain of *Y. enterocolitica* O:8, biotype 1A, resistant to ampicillin and amoxicillin-clavulanic acid. Rectal-swab specimens from 3 other pigs yielded *Y. enterocolitica* O:6,30 and serogroup O:5, clinically nonpathogenic serotypes. Pig tonsillar and pharyngeal cultures were negative. None of the cultures from additional dairy environmental specimens yielded *Y. enterocolitica.*

Subtyping. Nine of the 10 patient *Y. enterocolitica* O:8 isolates had an indistinguishable PFGE pattern (outbreak strain). The 1 differing isolate was from the 1 patient who did not drink the implicated milk. The PFGE patterns of the pig rectal-swab isolate and the raw-milk isolate differed from each other and from the outbreak strain. None of the PFGE patterns from the background isolates matched the outbreak strain. All 7 *Y. enterocolitica* O:8 isolates (3 from patients, 3 from 1 pig, and 1 from raw milk) submitted to Pasteur Institute were phage type X_o, because they were not lysed by any phage.

Environmental investigation. Dairy A began pasteurizing milk in 1993. Each week the dairy sold ~1100 gallons of raw milk to a milk cooperative. Another 2300 gallons of pasteurized milk were bottled or bagged on the premises and distributed to Vermont and New Hampshire stores and institutions in 2 dairy-owned, refrigerated trucks. A mechanical bottle washer washed and rinsed the recyclable glass bottles before they were filled with pasteurized milk. The final rinse was performed with untreated well water. The plastic bags used for bagged milk were not recycled or rinsed. Returned bottles and crates were stored in a back room, where the pails used to feed returned milk to the pigs were also kept.

One milk-processing room at the dairy adjoined both a cooler and a bottle-washing room. A buried, sealed concrete reservoir in front of the dairy stored water obtained from 2 wells behind the dairy. A barn, located in a separate building, housed pigs and dairy cattle at 2 opposite ends. The person who cared for the pigs on the dairy did not process milk but did have some bottle-handling responsibilities.

The workers and their family members denied experiencing illness during October. On September 19, a routine inspection disclosed no irregularities. Environmental inspections conducted on November 10 and 15 revealed no pasteurization defects. However, investigators observed condensation collection in the cooler, which dripped onto sealed filled bottles awaiting shipment. Dairy A was not a member of the Interstate Milk Shippers Conference, a voluntary national program that follows the guidelines and regulations of the Grade A Pasteurized Milk Ordinance (PMO).

Discussion

We conclude that this outbreak of *Y. enterocolitica* O:8 infections in the Upper Valley of Vermont and New Hampshire was caused by consumption of dairy A bottled pasteurized milk. Although the exact mechanism of contamination of the milk is uncertain, postpasteurization contamination was the most likely route of contamination. Previous outbreaks of pasteurized-milk-associated *Y. enterocolitica* infections have been linked to a variety of sources, including the addition of contaminated ingredients after pasteurization, contamination of the exterior of the milk crate, poor bottle-washing techniques, or contamination of the final product by raw milk [3, 6, 13].

Therefore, it is important to identify all biologically plausible modes of contamination.

Dairy pigs were likely the ultimate source of the outbreak strain, given that pigs are a major reservoir of pathogenic *Y. enterocolitica* O:3 and may be carriers for other serogroups [1]. At this dairy, at least one pig was colonized with *Y. enterocolitica* O:8. Milk bottles were rinsed with untreated well water before they were filled with pasteurized milk. The PMO recommends rinsing bottles with either heat- or chemically treated water before filling with pasteurized milk [12]. Although the well water was not specifically tested for *Y. enterocolitica,* 2 samples had elevated levels of coliforms, suggesting *Y. enterocolitica* could have been present. If present in the rinse water used on milk bottles before filling, *Y. enterocolitica* could have easily contaminated the milk. Past epidemiologic investigations linked *Y. enterocolitica* O:8 infections to consumption of contaminated water or of products contaminated by water [2, 4, 14, 15].

A second possible route of postpasteurization contamination was through environmental contamination to milk bottles. Returned milk crates and bottles were stored in an area where they may have been handled or touched by a worker who also cared for the pigs. If milk cases were contaminated with *Y. enterocolitica,* the exterior of their milk bottles could have become contaminated. A previous milk-associated outbreak of *Y. enterocolitica* linked contamination of milk bottles to crates that had been on a pig farm [6].

This outbreak has several important implications for dairies. First, it reminds dairy operators of the public health hazards posed by the presence of *Y. enterocolitica* and other pathogenic bacteria. Secondly, it illustrates postpasteurization contamination opportunities if the interior or exterior of the bottles, cartons, or cases become contaminated and emphasizes the use of control measures to prevent contamination from environmental sources. Finally, this outbreak should encourage dairy operators to participate in voluntary programs (e.g., the Interstate Milk Shippers Conference) to ensure the safety and quality of pasteurized milk shipped across states.

Acknowledgments

We thank John Modlin (Dartmouth-Hitchcock Medical Center), Ruth Cameron and Elizabeth Hansen (Vermont State Health Department), and Amy Burns, Valerie Benoit, and Mary Lee Greaves (New Hampshire Department of Health and Human Services) for their assistance in case identification and the epidemiologic investigation; William Bolton (New Hampshire Department of Health and Human Services), Mary Celotti (Vermont State Health Department), Julie Kiehlbauch (New York State Health Department), and Al Borczyk (Canadian National Reference Centre for *Yersinia*) for their support and participation in the laboratory investigation; Elisabeth Carniel (Institut Pasteur) for phage typing and serotyping of selected study strains; and Clifford McGinnis (New Hampshire Department of Health and Human Services), Stephen Pierson (State Programs, Food and Drug Administration [FDA]), and Joseph Smucker (Milk Safety Branch, FDA) for their participation in the environmental investigation.

References

1. Tauxe RV, Vandepitte J, Wauters G, et al. *Yersinia enterocolitica* infections and pork: the missing link. Lancet **1987**; 1:1129–32.
2. Tacket CO, Ballard J, Harris N, et al. An outbreak of *Yersinia enterocolitica* infections caused by contaminated tofu (soybean curd). Am J Epidemiol **1985**; 121:705–11.
3. Black RE, Jackson RJ, Tsai T, et al. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. N Engl J Med **1978**; 298:76–9.
4. Aber RC, McCarthy MA, Berman R, et al. An outbreak of *Yersinia enterocolitica* among members of a brownie troop in Pennsylvania [abstract]. In: Program and abstracts of the 22d Interscience Conference on Antimicrobial Agents and Chemotherapy (Miami Beach). Washington, DC: American Society for Microbiology, **1982**:219.
5. Shayegani M, Morse D, DeForge I, Root T, Parson LM, Maupin PS. Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* serogroup O:8. J Clin Microbiol **1983**;17::35–40.
6. Tacket CO, Narein JP, Sattin R, et al. A multistate outbreak of infections caused by *Yersinia enterocolitica* transmitted by pasteurized milk. JAMA **1984**; 251:483–6.
7. Lee LA, Gerber AR, Lonsway DR, et al. *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. N Engl J Med **1990**; 322:984–7.
8. Kondracki S, Balzano G, Schwartz J, Kiehlbauch J, Ackman D, Morse D. Recurring outbreaks of Yersiniosis associated with pork chitterlings [abstract]. In: Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy (New Orleans). Washington, DC: American Society for Microbiology, **1996**:259.
9. Ostroff, S. *Yersinia* as an emerging infection: epidemiologic aspects of Yersiniosis. Contrib Microbiol Immunol **1995**; 13:5–10.
10. Rothman KJ. Modern epidemiology. Boston: Little, Brown and Co, **1986**.
11. Farmer JJ III, Carter GP, Miller VL, Falkow S, Wachsmuth IK. Pyrazinamidase, CR-MOX agar, salicin fermentation-esculin hydrolysis, and D-xylose fermentation for identifying pathogenic serotypes of *Yersinia enterocolitica.* J Clin Microbiol **1992**; 30:2589–94.
12. Food and Drug Administration. Grade A pasteurized milk ordinance, 1995 revision. US Department of Health and Human Services, Public Health Service, Food and Drug Administration, **1995**.
13. Greenwood MH, Hooper WL. Excretion of *Yersinia* spp. associated with consumption of pasteurized milk. Epidemiol Infect **1990**; 104:345–50.
14. Keet EE. *Yersinia enterocolitica* septicemia: source of infection and incubation period identified. NY State J Med **1974**; 74:2226–30.
15. Thompson JS, Gravel MJ. Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype O:3 from well water. Can J Microbiol **1986**; 32: 700–1.