Yersinia enterocolitica infections associated with improperly pasteurized milk products: southwest Pennsylvania, March–August, 2011

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SUMMARY

In July 2011, a cluster of *Yersinia enterocolitica* infections was detected in southwestern
Pennsylvania, USA. We investigated the outbreak's source and scope in order to prevent further
transmission. Twenty-two persons were diagnosed with yersiniosis; 16 of whom reported
consuming pasteurized dairy products from dairy A. Pasteurized milk and food samples were
collected from this dairy. *Y. enterocolitica* was isolated from two products. Isolates from both
food samples and available clinical isolates from nine dairy A consumers were indistinguishable by
pulsed-field gel electrophoresis. Environmental and microbiological investigations were performed
at dairy A and pasteurization deficiencies were noted. Because consumption of pasteurized milk is
common and outbreaks have the potential to become large, public health interventions such as
consumer advisories or closure of the dairy must be implemented quickly to prevent additional
cases if epidemiological or laboratory evidence implicates pasteurized milk as the outbreak source.

Key words: Foodborne infections, outbreaks, *Yersinia enterocolitica*.

INTRODUCTION

Enteropathogenic *Yersinia enterocolitica*, a food-
borne pathogen, causes an estimated 116 716 human
infections annually in the USA [1]. Yersiniosis can
manifest as diarrhoea, abdominal pain, fever, acute
mesenteric lymphadenitis mimicking appendicitis,
and systemic infection. Severe and even fatal disease
can occur. Diagnosis is determined by using stool or
blood culture, although a limited number of labora-
tories routinely screen human clinical specimens for
Yersinia species [2].

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The epidemiology of *Y. enterocolitica* is complex and poorly understood because outbreaks are infrequent and investigation of sporadic infections rarely identifies an apparent exposure source. Raw or undercooked pork consumption is considered a primary mode of sporadic transmission, although other food items have been implicated in outbreaks, including pasteurized milk, tofu, and chitterlings [3–10]. Consumption of unpasteurized milk and untreated ground water are also considered risk factors for infection [11, 12].

Since the late 1980s, serogroup O:3 has been the predominant strain associated with human infections in the USA, although other serogroups including O:8 and O:9 are well established human pathogens [2–6]. Healthy pigs are frequently colonized with serogroups that cause human illness; pathogenic serogroups have also been isolated from cows, sheep, goats, dogs, and cats [13–16]. Pathogenic *Y. enterocolitica* strains have been isolated from unpasteurized milk and cow faeces, although the prevalence in cows is typically lower than in pigs and the association between veterinary isolates and human disease is unclear [17, 18].

Y. enterocolitica is a ubiquitous microorganism; however, most isolates recovered from asymptomatic carriers, food, and the environment are not pathogenic [19]. Isolates are rarely recovered from food or environmental samples because a limited number of *Y. enterocolitica* organisms might be present, compared to other microflora in food and environmental samples [19].

Yersiniosis is not mandatorily reportable in Pennsylvania, but case reports are sporadically received by health departments. On 22 July 2011, a physician notified the Allegheny County Health Department (ACHD) of a patient with *Y. enterocolitica* infection. An examination of the Pennsylvania Department of Health's database of routinely submitted case reports identified four additional yersiniosis patients in Allegheny and adjacent Beaver County with onset of illness in June–July 2011. During 2008–2010, a total of 11 *Y. enterocolitica* cases had been reported in these two counties. Because of the recent cases, an investigation was launched to identify a potential common source, assess scope of illnesses, and prevent additional infections.

METHODS

Initial investigation

Using a standardized questionnaire, interviewers initially queried patients about traditional *Y. enterocolitica*

risk factors including exposure to raw pork, chitterlings, and unpasteurized milk. During these interviews, one patient was identified as a consumer of pasteurized milk that was home-delivered from a farm-based southwestern Pennsylvania dairy (dairy A). After further questioning, the other four additional patients also reported consumption of dairy A pasteurized milk. Thereafter, the investigation focused on dairy A, which on 27 July voluntarily stopped production after joint notification of the illnesses by the Pennsylvania Department of Health (PADOH) and the Pennsylvania Department of Agriculture (PDA). Dairy A also notified all home delivery customers and retail stores about the illnesses and recommended that they discard any remaining milk.

Case ascertainment

We classified a case as culture-confirmed *Y. enterocolitica* infection during 1 January 2011–31 August 2011, occurring in a resident of any of the four southwestern Pennsylvania counties (Allegheny, Beaver, Butler, Lawrence) served by dairy A. To identify cases, we reviewed state surveillance data, used shopper card information from a retail store to contact purchasers of dairy A milk, issued a press release, notified clinicians, and contacted hospital-based and commercial laboratories in southwestern Pennsylvania to identify clinical isolates of *Y. enterocolitica*. All identified patients, or their guardians, or next of kin were interviewed about potential exposures, including consuming dairy A products.

Environmental investigation

Samples of dairy A product were collected from a retail store and from the homes of dairy A consumers where available. Dairy A was certified by PDA to pasteurize on site and sell non-grade A cow's milk (milk for pasteurization that is not certified under the Interstate Milk Shippers Program) within Pennsylvania from a herd of about 150–200 cows. The dairy produced skim, 1%, 2%, whole, chocolate- and strawberry-flavoured milk, cream, buttermilk, and ice cream, and distributed ~4500 US gallons of milk (10 000 containers) weekly to ~645 households and 40 retail outlets and restaurants in southwestern Pennsylvania; ~85% of the milk was distributed in returnable glass bottles, which were washed and sanitized by the dairy's mechanical bottle washer. Milk was also sold in plastic jugs. Milk flavourings and

ice cream mix were added before pasteurization. Ice cream was packaged in 56-oz paperboard containers.

Milk sanitarians from PDA inspected dairy A and reviewed pasteurization and equipment cleaning logs. The dairy owners and employees were asked about illness in themselves and in family members. On 4 August, microbiological samples from the pasteurizer, ice cream machine, drainage collectors, milk bottle washer, bottle filler, crate washer, unwashed crates, door handles, water delivery truck, chocolate powdered milk flavouring, and sweetwater (cold water passed through piping to cool pasteurized milk) tank were obtained during a site visit by ACHD, PADOH, and PDA. During the visit, unpasteurized milk and water samples from the pasteurization plant (a mixture of chlorinated municipal and well water), cow herd water, standing water from two floor drains, municipal water from a water delivery truck, and sweetwater were also obtained. Environmental samples were collected in Cary–Blair transport media, refrigerated, and delivered to the state public health laboratory in <24 h.

On 1 September, dead-end ultrafiltration was used to filter 100-litre chlorine quenched water samples from each of the following dairy A locations: pasteurization plant, cow herd water, sweetwater, and municipal (truck) water [20]; 2-litre grab samples were also collected from the same locations. Filters and water samples were shipped in <24 h to the Centers for Disease Control and Prevention (CDC) for extraction and testing. In October 2011, a third party, separate from the health department investigation, collected manure samples from the dairy herd for *Y. enterocolitica* testing.

Laboratory investigation

All available *Y. enterocolitica* patients' isolates were shipped from clinical laboratories to PADOH or ACHD public health laboratory for confirmation.

Laboratories at PADOH, ACHD, PDA, and CDC tested environmental or dairy A products for *Y. enterocolitica* by using variations of cold temperature enrichment methods that are typically used to provide *Y. enterocolitica* with a competitive advantage (Table 1). Dairy A product samples sent to the ACHD laboratory were also tested by using a novel room temperature enrichment method developed specifically for this investigation based upon aspects of published research methods [21–25] and theory. In this method, dairy A product was added to 225 ml

peptone sorbitol bile broth and refrigerated at 5 °C for 1 day. On day 2, the broth was alkalinized by using 1 ml of 0·25% potassium hydroxide (KOH) and incubated at room temperature (25 °C) for 2 days. MacConkey and cefsulodin-irgasan-novobiocin agar plates were inoculated with the room temperature broth on days 3 and 4, and incubated at ± 25 °C for 1–2 days.

Available *Y. enterocolitica* isolates were subtyped by pulsed-field gel electrophoresis (PFGE) and restricted with *Not*I and *Apa*I at PADOH. *Y. enterocolitica* isolates were biotyped and serogrouped at the National *Yersinia* Reference Laboratory (CDC, Atlanta, GA) [26–28] and also at the WHO Collaborating Center for *Yersinia* (Pasteur Institute, Paris) for confirmation of unusual results (L. Martin and E. Carniel, unpublished data).

Cohort study

To determine outbreak extent and identify particular dairy A products or other exposures associated with illness, we conducted a cohort study of dairy A home delivery customers by using a list provided by dairy A. Information was obtained regarding illness, food consumption, types and quantity of dairy A product consumption, and animal exposures during 2011 for all household members. A suspected case of yersiniosis was defined as a household member receiving dairy A products during 2011 and experiencing ≥2 of the following: abdominal cramping, diarrhoea (>3 loose stools during a 24-h period), or fever (measured temperature >38·05 °C) since 1 January 2011.

On the basis of review by the PADOH Institutional Review Board and the CDC Scientific Education and Professional Development Program Office Human Subjects' Protection Coordinator, this investigation was considered a public health response and therefore non-research.

Statistical analysis

Relative risks (RRs) and 95% confidence intervals (CIs) were calculated by using generalized estimating equations and Poisson regression with multilevel modelling to account for household clustering. All analyses were conducted using SAS version 9.2 (SAS Institute Inc., USA).

Table 1. *Yersinia enterocolitica isolation methods used by local, state, and federal laboratories during an investigation of* Y. enterocolitica *infections associated with improperly pasteurized milk products – southwest Pennsylvania, March–August, 2011*

| Laboratory | Samples | Enrichment | | | | Incubation | | | |
		Broth or Buffer	Temperature	Time (days)	Potassium hydroxide	Inoculation day	Agar plates	Temperature	Time (days)
ACHD									
Method 1	Dairy A products	PSBB	5 °C	21	0·5% to agar plates	10, 21	MacConkey, CIN	±25 °C	1–2
Method 2*	Dairy A products	PSBB	5 °C, 25 °C	1, 2	0·25% to broth after 1 day cold enrichment	3, 4	MacConkey, CIN	±25 °C	1–2
PDA									
Method 1 (FDA BAM) [27]	Dairy A products and environmental	PSBB	10 °C	10	0·5% to agar plates†	10	MacConkey, CIN	30 °C	1–2
PADOH									
Method 1 (FDA BAM)	Dairy A products and environmental	PSBB	10 °C	10	0·5% to agar plates†	10	MacConkey, CIN	30 °C	1–2
Method 2	Dairy A products and environmental	PSBB	4 °C	21	0·5% to agar plates†	7, 14, 21	MacConkey, CIN	30 °C	1–2
CDC									
Method 1	Environmental	n.a.	n.a.	n.a.	n.a.	Direct plating	MacConkey, CIN	25 °C, 30 °C	2
Method 2	Environmental	PBS	4 °C	21	Broth treated with 0·25% for 2 min, then 0·5% for 15 s before plating	21	MacConkey, CIN	25 °C, 30 °C	2
Method 3	Environmental	n.a.	25 °C	2	Broth treated with 0·25% for 2 min, then 0·5% for 15 s before plating	1, 2	MacConkey, CIN	25 °C, 30 °C	2

ACHD, Allegheny County Health Department; PSBB, peptone sorbitol bile broth; CIN, cefsulodin-irgasan-novobiocin agar; PDA, Pennsylvania Department of Agriculture; FDA, Food and Drug Administration; BAM, Bacterial Analytical Manual [27]; PADOH, Pennsylvania Department of Health; CDC, Centers for Disease Control and Prevention; PBS, phosphate buffered saline; n.a., not applicable.

* In this method, dairy A product was added to 225 ml peptone sorbitol bile broth and refrigerated at 5 °C for 1 day. On day 2, the broth was alkalinized by using 1 ml of 0·25% potassium hydroxide (KOH) and incubated at room temperature (25 °C) for 2 days. MacConkey and cefsulodin-Irgasan-novobiocin agar plates were inoculated with the room temperature broth on days 3 and 4, and incubated at ±25 °C for 1–2 days.

† 1:10 dilution of enrichment without potassium hydroxide also plated on agar per BAM guidelines.
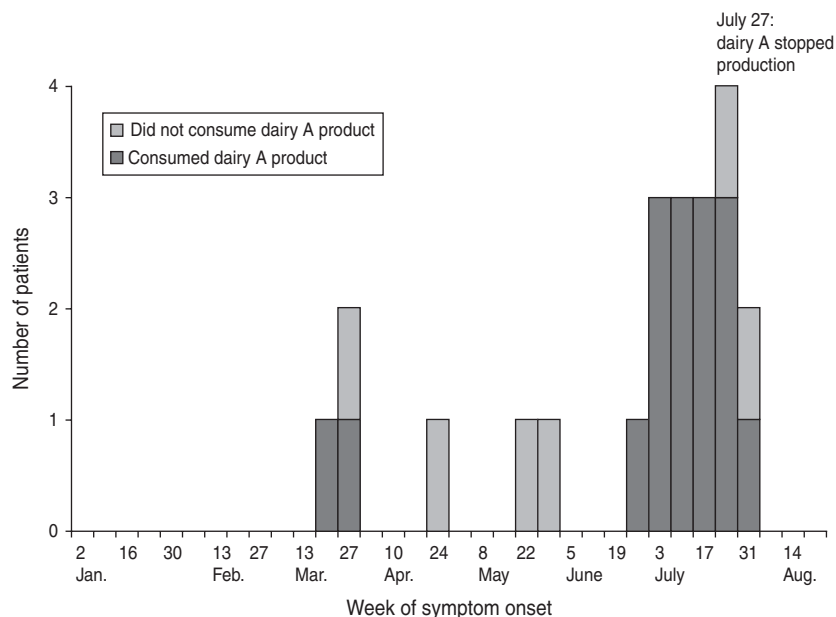
Fig. 1. Patients with culture-confirmed *Yersinia enterocolitica* infection ($n = 22$), by week of onset, southwest Pennsylvania, March–August, 2011.

RESULTS

Patients' characteristics

A total of 22 patients with culture-confirmed yersiniosis were identified; 16 (73%) had consumed dairy A products. Of the 16 patients with dairy A exposure, symptom onset occurred during 24 March–5 August 2011 (Fig. 1). Median age was 27 years (range 1–76 years); 63% were female (Table 2). Clinical information was available for 14 patients and included diarrhoea (71%), fever (50%), abdominal cramping (43%), nausea (21%), sore throat (14%), and rash (14%). Seven patients (44%) were hospitalized; three (19%) required intensive care; one (6%) had abdominal surgery to rule out lymphoma and one (6%) patient died. *Y. enterocolitica* was isolated from the stool of 14 dairy A-associated patients and the blood of two other patients. All 16 reported having consumed dairy A's glass-bottled pasteurized milk; three patients also had eaten dairy A ice cream. The patients had consumed multiple types of dairy A milk and ice cream. The six patients who did not consume dairy A products had no recognized common exposures.

Patients' specimens

Antimicrobial susceptibility testing was performed on isolates of 6/7 hospitalized patients; five isolates demonstrated resistance to ampicillin/sulbactam,

Table 2. *Characteristics of patients with culture-confirmed* Yersinia enterocolitica *who reported exposure to dairy A products* ($n = 16$)*, southwest Pennsylvania, January–August 2011*

Characteristics	No. (%)
Age, median (range)	27 (1–76) years
Female	10 (63)
Time from symptom onset to diagnosis, mean (range)	14·5 (3–38) days
Hospitalized	7 (44)
Intensive care unit	3 (19)
Died	1 (6)
Ate dairy A ice cream	3 (19)
Consumed dairy A milk from glass bottles	16 (100)
Consumed 2% milk only	7 (44)
Consumed skim milk only	1 (6)
Consumed chocolate milk only	1 (6)
Consumed multiple types of milk	4 (25)
Consumed 2% milk and ice cream	3 (19)
Received home delivery	8 (50)
Purchased from dairy A onsite store	4 (25)
Purchased from retail store	4 (25)

ampicillin, and cefazolin and one isolate demonstrated intermediate sensitivity to ampicillin (ampicillin/sulbactam and cefazolin susceptibility testing was not performed). *Y. enterocolitica* isolates from nine patients who consumed dairy A products were indistinguishable by PFGE (no bands of difference) and

were confirmed as bioserotype 1B/O:8, a highly pathogenic bioserotype of *Y. enterocolitica*. Available isolates from the two patients who did not consume dairy A products with *Y. enterocolitica* infection had PFGE patterns and biotype/serogroup combinations that differed from each other and from the outbreak strain. Isolates were not available for the remaining four patients who did not consume dairy A products.

Environmental investigation

Temperature and time logs indicated that standards for adequate pasteurization (72 °C for 15 s) [29] were met, but other deficiencies were noted including worn and broken pasteurizer gaskets. A sanitizer spray pump used to clean the pasteurization equipment was underpowered. Water temperatures used during cleaning were too hot (>93 °C). Milk soils (residue) were observed in the pasteurization equipment and multiple items were inadequately cleaned. An employee reported that the mechanical bottle washer's iodine spray pump had been underpowered for an undetermined period, probably resulting in inadequately cleaned glass bottles. Additionally, iodine concentrations and caustic soak tank concentrations were measured incorrectly (dairy A had been using pH strips rather than per cent causticity). Milk soils were observed in the rubber filler gaskets and stainless nozzles of the bottle filler.

Water sources for the herd and pasteurization plant varied. When well-water levels were sufficient, well water was pumped into an underground concrete reservoir. During dry periods, the dairy obtained municipal water by using its own tanker trunk. This water was gravity-fed into the reservoir where it mixed with well water. The water was then pumped into the pasteurization plant where it was used for multiple purposes including pasteurizer cleaning and to supply the bottle washer. During 2011, the dairy began using municipal water in mid-June. The inside of the water tanker truck was not cleaned between its last use in November 2010 and first use in June 2011. Drinking water for the cow herd was obtained from a cistern containing a mixture of rain water and municipal water (when necessary).

New calves were born onsite and housed in a separate barn. The milking barn was adjacent to the processing plant. One milk processing room adjoined both the bottle-washing room and a cooling room. Used glass bottles and crates were stored in the same room as the bottle washer.

The farmhand who tended cows did not process milk or ice cream, but did deliver milk. This farmhand reported a 2-week history of watery, non-bloody diarrhoea without abdominal cramping during late July. He reportedly did not consume dairy A milk, but did drink the dairy's well water. A stool specimen obtained on 8 August did not yield *Y. enterocolitica* or any other enteric pathogen. No other dairy personnel reported illness in themselves or their family members during June–July 2011; asymptomatic dairy personnel were not tested. Dogs and cats but not pigs were observed on dairy premises. No pig farms were located in the vicinity of dairy A, and employees denied delivering milk to any pig farms.

Food and environmental specimens

Standard plate counts, coliform counts, somatic cell counts, and phosphatase (indicative of inadequate pasteurization) in routine regulatory samples of dairy A milk collected during January–June 2011 met PDA requirements. Ice cream testing during February–June 2011 detected intermittently elevated coliforms (>10 coliforms/ml).

Y. enterocolitica was not cultured from any of the 31 environmental or product samples tested at PADOH, the 16 environmental or 18 product samples tested at PDA, or the eight environmental samples tested at CDC. All manure samples collect from the dairy herd in October 2011 were reported as negative for *Y. enterocolitica*. None of the 19 food samples tested at the ACHD laboratory yielded *Y. enterocolitica* by using cold enrichment; however, two samples (unopened ice cream from a patient's home and homemade yogurt made by using dairy A milk from an asymptomatic customer) tested by using the room temperature enrichment method yielded *Y. enterocolitica*. Both food isolates were confirmed as bioserotype 1B/O:8 and were indistinguishable by PFGE (no bands of difference) from dairy A-associated patients' isolates.

Cohort study

Of 645 dairy A home delivery households, interviews were completed for 356 (55%). The 356 participating households included 992 persons. Dairy A products were consumed by 849 (86%) household members. Twenty (2%) respondents reported direct contact with pigs. Symptoms consistent with the case definition for suspected yersiniosis were reported by

Table 3. *Symptom prevalence in cohort study participants who reported a* Yersinia-*like illness, southwest Pennsylvania, January–August 2011*

Symptom	No.	%
Diarrhoea	64	100
Abdominal cramping or pain	62	97
Fatigue	35	55
Headache	24	38
Fever	23	36
Chills	19	30
Sore throat	16	25
Joint pain	13	20
Vomiting	12	19
Swollen lymph nodes	11	17
Bloody diarrhoea	6	9
Rash	3	5

64 (6%) household members (Table 3). Ten (16%) respondents had stool specimens tested before interview; one yielded *Y. enterocolitica* and this person's illness was included as a confirmed case.

Drinking any milk was associated with illness (RR 4·0, 95% CI 0·5–28·6) (Table 4), although not significantly. Those consuming ⩾3 cups of milk/day (10%) were more likely to be ill, compared to those who drank <1 cup/day (5%, RR 2·0, 95% CI 1·0–4·1). Drinking only glass-bottled milk, compared to drinking only milk distributed in plastic jugs was not significantly associated with illness (RR 1·8, 95% CI 0·2–12·8). No specific milk type was associated with illness, nor were traditional *Y. enterocolitica* risk factors.

DISCUSSION

This outbreak of yersiniosis was associated with consumption of pasteurized milk products from a dairy in southwestern Pennsylvania. The dairy was a family-run, farm-based operation that performed on-site pasteurization and distributed the majority of its products through home delivery. The dairy was inspected annually by PDA. Multiple lines of epidemiological evidence implicated the dairy, including 16 culture-confirmed illnesses in consumers of products from the dairy, high relative risk and dose–response observed in the dairy's home delivery customers, and abrupt decline in illnesses when the dairy ceased production. Isolation of *Y. enterocolitica* from unopened ice cream and from yogurt made with the dairy's milk that were indistinguishable by PFGE from patients' isolates confirmed the dairy as

the outbreak source, although the specific mechanism for contamination was not identified.

The extent of the outbreak was probably greater than the 16 culture-confirmed cases. Yersiniosis is not reportable in Pennsylvania; many clinicians are unfamiliar with this infection, and a limited number of laboratories routinely culture clinical isolates for *Yersinia* species. Although the total number of illnesses cannot be estimated, the 6% rate of *Yersinia*-like illness in the dairy's interviewed home delivery customers indicates additional illnesses had occurred. The closure of dairy A after the likely source was identified probably prevented additional cases.

Yersiniosis outbreaks in the USA are rare. However, this is the fifth reported outbreak of yersiniosis associated with pasteurized milk since 1976 [6–10]. The most recently reported milk outbreak [6] in New England also involved bioserotype 1B/O:8 and occurred in a similar setting to our investigation. That outbreak occurred at a farm-based dairy that pasteurized glass-bottled milk on site. A specific cause or mechanism for product contamination was not determined, and similar types of deficiencies in pasteurization and bottle-washing were identified. This indicates that transmission opportunities for *Y. enterocolitica* in farm-based dairy operations are possible.

Although *Y. enterocolitica* was isolated from dairy products left over in patients' households, we were unable to isolate *Y. enterocolitica* from environmental samples. Pathogenic strains of *Y. enterocolitica* are often difficult to isolate from the environment, possibly because of the fastidious enrichment requirements of the organism, combined with the limited number of pathogenic strains present in correlation to a substantial burden of background flora [19]. In each of the four previously published outbreaks regarding *Y. enterocolitica* associated with pasteurized milk, multiple environmental samples were collected and few yielded *Y. enterocolitica*, and similar to our investigation, the exact mechanism of contamination was difficult to determine [6–8, 10]. The long incubation period for *Y. enterocolitica* (3–7 days) is another reason why conditions at the dairy at the time of inspection might not reflect the conditions at the time of the outbreak. Our investigation was further hindered by the fact that dairy A began extensive cleaning of the pasteurization plant and dairy processing and packaging equipment as soon as they were notified of illnesses; samples were not obtained until cleaning was completed. The dairy subsequently took additional corrective actions including replacement of worn

Table 4. *Risk factors for* Yersinia-*like illness in dairy a cohort study survey respondents, southwest Pennsylvania, January–August 2011*

Exposure	Exposed		Unexposed		RR	95% CI
	Ill	AR (%)	Ill	AR (%)		
Drank any dairy A milk (ref = never drinks milk)	61/910	7	1/59	2	4·0	0·5–28·6
Amount of milk consumed per day*						
<1 glass per day (reference)	18/340	5			Reference	
1–2 glasses per day	30/476	6			1·2	0·6–2·3
≥3 glasses per day	14/134	10			2·0	1·0–4·1
Ate any dairy A ice cream (ref = no ice cream)	8/168	5	56/824	7	0·7	0·3–1·4
Drank dairy A milk from glass bottles only (ref = drink only plastic)	54/787	7	1/26	4	1·8	0·2–12·8
Drank dairy A milk varieties						
Any fat free milk	26/288	9	38/704	5	1·7	0·9–3·0
Any 1% milk	13/135	10	51/857	6	1·6	0·8–3·5
Any 2% milk	28/429	7	36/527	6	1·0	0·6–1·8
Any whole milk	18/213	8	46/779	6	1·4	0·8–2·7
Any chocolate-flavoured milk	25/370	7	39/622	6	1·1	0·6–2·0
Any strawberry-flavoured milk	3/64	5	61/928	7	0·7	0·2–3·0
Any buttermilk	5/43	12	59/949	6	1·9	0·8–4·4
Any cream	2/34	6	62/960	6	0·9	0·3–3·7
Only fat free milk	13/150	9	51/842	6	1·4	0·7–2·8
Only 1% milk	2/45	4	62/947	7	0·7	0·2–2·7
Only 2% milk	8/168	5	56/768	7	0·7	0·3–1·8
Only whole milk	3/75	4	61/917	7	0·6	0·1–2·5
Only chocolate-flavoured milk	0/15	0	64/977	7	n.a.	n.a.
Only strawberry-flavoured milk	0/0	n.a.	64/992	6	n.a.	n.a.
Only buttermilk	0/0	n.a.	64/992	6	n.a.	n.a.
Only cream	0/2	0	64/990	6	n.a.	n.a.
Drank raw milk	0/5	0	64/987	6	n.a.	n.a.
Contact with pigs	3/20	15	61/972	6	2·4	0·6–8·8
Contact with cows	4/42	10	60/950	6	1·5	0·6–4·0
Ate raw or undercooked pork	0/5	0	64/987	6	n.a.	n.a.
Chitterlings	0/1	0	64/991	6	n.a.	n.a.
Well water	10/136	7	54/855	6	1·2	0·5–2·7

AR, Attack rate; RR, relative risk; CI, confidence interval; n.a., not applicable.

* χ^2 test for trend = 3·4, P = 0·07.

pasteurizer gaskets, installation of a new crate washer in a room separated from sanitized bottles, and use of titration to measure the bottle washer's causticity and iodine concentration.

Post-pasteurization contamination was postulated in all outbreaks of *Y. enterocolitica* associated with pasteurized milk; potential mechanisms included flavouring ingredients added post-pasteurization [7] and contamination of a bottle-filling valve, milk bottles, or crates [7–10]. Although pigs were not identified on the dairy and swabs from milk crates tested negative, our cohort study identified several home-delivery customers who reported direct contact with pigs; therefore, cross-contamination from workers who handled crates or bottles potentially contamin-ated by other persons who cared for pigs cannot be ruled out.

Because all 16 patients in our investigation had consumed glass-bottled milk, we focused our initial investigation on the mechanical bottle washer. However, after the unopened container of ice cream, which would not have been affected by the bottle washer, yielded *Y. enterocolitica*, additional contamination hypotheses were considered.

The results of our investigation and cohort study indicate that no single product or milk type could explain illness in the majority of persons with either confirmed or suspected yersiniosis. Therefore, the likely contamination source either from pasteurization or post-pasteurization deficiencies resulted from a

mechanism common to production pathways for all dairy A products such as dairy personnel. Although the single ill farmhand tested negative for *Y. enterocolitica*, asymptomatic dairy personnel were not tested and cannot be excluded as possible sources of contamination.

Because dairy A added all ingredients (including flavourings and ice-cream mix ingredients) before pasteurization, the chocolate powder tested negative for *Y. enterocolitica*, and chocolate milk and ice cream were reportedly consumed by few of the patients, these were unlikely to be the source of contamination. Dairy A pasteurization temperatures were adequate, although the equipment was old and worn. No apparent post-pasteurization mechanism for cross-contamination with raw milk was identified, and the absence of phosphatase in tested samples makes the presence of raw milk in finished products unlikely. Contamination from sweetwater was possible. Thoroughly cleaned milk systems are critical in preventing product contamination; however, during this outbreak, pasteurization equipment was inadequately cleaned. Water temperatures exceeding 77 °C, which occurred in this investigation, can result in denaturation of milk proteins, allowing milk residue to adhere to surfaces [30]. Multiple areas of milk soiling were observed, providing a potential mechanism for milk contamination. The dairy only started using municipal water in June. Because onset for the earliest cases occurred in March, contamination of the tanker truck or municipal water could not explain all infections. If the bacterial load in pre-pasteurized bulk tank milk is high, some *Yersinia* might survive pasteurization [31]. Although the single sample of unpasteurized bulk tank milk tested during our investigation did not yield *Y. enterocolitica*, we cannot rule out the possibility that intermittent faecal shedding prevented detection or that the test was falsely negative given the challenges of isolating *Y. enterocolitica* from food. Although specific methods varied, all laboratories in this investigation used cold enrichment, the standard isolation method used in the USA [32]. Cold temperatures, while providing a competitive advantage for *Y. enterocolitica*, also slow its growth; as a result, long enrichment times, typically 10–21 days are required. Although the International Organization for Standardization method for the detection of *Y. enterocolitica* recommends room temperature enrichment [25], the use of room temperature enrichment after 1 day of cold enrichment and KOH treatment to inhibit growth of background flora was novel and

shortened incubation time. Isolation of *Y. enterocolitica* from yogurt and ice cream through this novel method confirmed the epidemiological association; furthermore, isolation from ice cream led to a broadened environmental investigation and a second press release. Validation studies of this method are necessary to determine its potential benefit during future *Y. enterocolitica* outbreaks.

Our investigation had limitations, including that (1) collection of environmental samples occurred weeks after the initial case was identified and after thorough equipment cleaning; (2) cow manure samples were not obtained as part of our investigation; and (3) isolates were not available for PFGE from patients with onset during spring to determine the association of these illnesses to later ones.

Nonetheless, this outbreak serves as a reminder of *Y. enterocolitica*'s importance as a foodborne pathogen in North America, severe consequences of infection, and difficulties identifying and investigating outbreaks caused by this organism. In fact, bioserotype 2/O:8 was originally suspected in this outbreak since lipase activity was not detected; however, further work indicated that lipase activity was weak and delayed. The high pathogenicity island (HPI) specific for *Y. enterocolitica* 1B was detected by polymerase chain reaction of the HPI *fyuA* gene, asserting that this outbreak was caused by a strain of 1B/O:8 [33–35; L. Martin and E. Carniel, unpublished data]. In the future, molecular methods will be useful in discriminating and identifying strains where inconsistencies in phenotype exist.

Because of the potential for *Y. enterocolitica* to cause serious illness, hospitalization, and death, clinicians should consider yersiniosis in patients with unexplained febrile diarrhoea and abdominal pain. Although most dairy-associated outbreaks in the USA are linked to raw (unpasteurized) milk [36], this outbreak also highlights that improperly pasteurized milk can also serve as a vehicle for foodborne illness. The ability of *Yersinia* species to survive and multiply at refrigeration temperatures makes milk a particularly good vehicle for human infections if the pasteurization process is insufficient or if post-pasteurization contamination occurs. The importance of thorough cleaning and maintenance of pasteurization plant equipment, as well as cross-contamination and control should be routinely emphasized to dairy owners [36, 37]. Pasteurization is widely regarded as the most effective method for decreasing the number of pathogenic organisms in milk; outbreaks associated

with pasteurized milk are rare [36]. However, because consumption of pasteurized milk is common and outbreaks have the potential to become widespread, public health interventions (e.g. consumer advisories or dairy closures) should be implemented immediately to prevent additional illnesses if epidemiological or laboratory evidence implicates pasteurized milk as the exposure source.

DECLARATION OF INTEREST

None.

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