

SIXTH EPIDEMIOLOGICAL PROBLEM SET

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ILLNESSES ASSOCIATED WITH ROASTED EGGPLANT IN OIL — ITALY, 1993

In August and October 1993, public health officials in Italy were notified of seven cases of illness from two apparently unrelated outbreaks in different communities. Investigations were initiated by the Regional Health Observatory of Campania and the Italian National Institute of Health. This report summarizes the outbreak investigations, which indicated that illness was associated with eating commercially prepared roasted eggplant in oil.

Outbreak 1

On August 14, two waitresses working in a sandwich bar in Santa Maria di Castellabate were admitted to a local hospital with dysphagia, diplopia, and constipation; a clinical diagnosis of botulism was made. On August 12, the waitresses had prepared and eaten ham, cheese, and eggplant sandwiches. A third waitress also ate the sandwiches and developed dyspepsia for which vomiting was induced; she did not have neurologic symptoms. The owner of the bar, who had tasted a small piece of eggplant from the same jar later on August 12, remained asymptomatic. The cook had initially opened the jar of commercially prepared sliced roasted eggplant in oil and had tasted its contents on August 11 and developed diarrhea. Both the cook and the owner reported that the eggplant tasted spoiled.

Botulism was presumptively diagnosed in the two hospitalized patients; both were treated with trivalent botulism antitoxin and gradually improved. No food samples were available for testing. No botulism toxin was detected in the serum of the two hospitalized patients. However, cultures of their stools subsequently yielded proteolytic _____.

Outbreak 2

During October 5-6, four of nine members of an extended family who had dined together on October 2 were hospitalized in Naples with suspected botulism. The meal consisted of green olives, prosciutto, bean salad, green salad, mozzarella cheese, sausages, and commercially prepared roasted eggplant in oil. Based on an investigation and analysis of food histories, the eggplant was implicated as the probable source (relative risk = undefined; p less than 0.01). All of the patients were treated with trivalent antitoxin and gradually improved. Investigation indicated that on September 27, another family member had opened and dipped a fork into the implicated jar of eggplant; although he did not eat any eggplant, he used the fork for other food items. On September 28, he had developed vomiting, dysphagia, and double vision but was not hospitalized; his symptoms resolved spontaneously. On October 8, he was asymptomatic but was hospitalized and treated with trivalent antitoxin after _____ was diagnosed in other family members.

One of the hospitalized patients developed respiratory muscle weakness and required

mechanical ventilation. A serum specimen from one patient was negative for the toxin. Cultures of stool specimens from three patients yielded spores. No eggplant was available for testing.

Follow-Up

The commercially prepared eggplant suspected of causing both outbreaks was produced by one company and sold only in Italy. The company reported preparing the eggplant in the following manner: eggplant slices were washed and soaked overnight in a solution of water, vinegar, and salt; roasted in an oven; and subsequently placed in glass jars. Garlic, peppers, oregano, and citric acid were added. The mixtures then were covered with sunflower oil and sealed with screw-on lids; after being filled, the jars were boiled in water for 30 minutes. The pH of the product was not consistently monitored. A total of 119 jars of eggplant from the same lot that caused the outbreaks was tested; neither spores nor toxin were detected. The pH of the product varied from 3.9 to 5.1. Public health officials issued a national warning and recalled unused jars of eggplant. No additional cases associated with this product were reported.

1. What is the diagnostic significance of finding spores in a patient's stool?
2. What does it matter that the agent was proteolytic?
3. Why didn't everyone that ate the baked eggplant get sick?
4. How could this product be made consistently safe — is there a critical control point?

**PHR 150
Problem #22**

OYSTERS, BRITISH COLUMBIA

Between 1 July and 21 August 1997, 43 laboratory-confirmed cases of gastrointestinal infection were reported to the British Columbia Centre for Disease Control (BCCDC). Surveillance for clinical cases started on 5 August, and by 21 August, 57 clinical cases had been reported. Onset dates ranged from 19 June to 10 August. The number of cases peaked during the week beginning 28 July and then declined sharply.

Thirty-nine of the 43 confirmed cases were available for interview. Sixty-seven percent (26/39) were male. The ages ranged from 21 to 79 years with a mean age of 42 years. All interviewed cases (39/39) reported diarrhea, 87% (34/39) had abdominal pain, 38% (15/39) had nausea, 36% (14/39) had vomiting, 33% (13/39) had fever, and 5% (2/39) had blood in their stools. One case was hospitalized. A food history was obtained from 39 laboratory-confirmed cases. Thirty-four had eaten raw or undercooked oysters prior to onset of symptoms. Of the five cases who had not eaten oysters, two had eaten crabs, one had eaten clams, one had swum extensively in the ocean, and one had attended a banquet where raw oysters were served. Twenty-eight of the 34 cases who had eaten oysters had purchased them at restaurants or other food establishments in BC, and six cases had eaten oysters which they had harvested from BC beaches.

Of the 57 clinical cases, 95% (54/59) had eaten raw or undercooked oysters prior to illness onset. Twenty-one of the 54 had harvested the oysters themselves. Oysters related to cases were traced back to numerous different harvesting areas on the BC coast.

Samples of oysters from these areas were tested and found to contain _____ [see question 1, below]. However, the organism was present in the range of less than 100 to 200 colony forming units (CFU) per gram of oyster tissue, a level which is lower than is thought necessary to cause human illness. One confirmed case had eaten oysters from Prince Edward Island as well as oysters from BC. An initial investigation of oyster processing and distribution did not reveal deficiencies that could account for the outbreak.

1. What was the probable etiological agent in this outbreak?
2. This outbreak was very unexpected in the area where it occurred. How might “El Niño” (warm Pacific waters) have played a role?

OUTBREAK AMONG ATTENDEES OF A HIGH SCHOOL DRILL TEAM CAMP

Background

In mid-June 1999, the Texas Department of Health (TDH) was notified by the Tarrant County Health Department (TCHD) of a cluster of illnesses among persons who had attended a summer camp from June 9-11 on the campus of a state university. Ill persons reported nausea, vomiting, severe abdominal cramping, and diarrhea, some of which was bloody. Two persons were hospitalized with hemolytic uremic syndrome (HUS) and two others underwent appendectomies.

Investigation by TDH indicated that approximately 750 persons, mostly adolescent women from 33 different schools, had attended the summer camp, a 3-day training session for high school drill teams; there were reports of similar illnesses among additional camp attendees from other counties. All persons at the drill camp resided in one dormitory, Dormitory A, and ate at the cafeteria located in this building. Since May 19, the beginning of the university's summer session, many other groups resided at Dormitory A; there were anecdotal reports of diarrheal illness among persons in some of these groups. There had been no notable increase in reports of undiagnosed bloody diarrheas in the community where the university was located or elsewhere in Texas.

TDH and the TCHD conducted independent, parallel, cohort studies of persons who had attended the drill team camp at the university. Questionnaires focused on exposure to food items served to the campers at the Dormitory A cafeteria from breakfast on June 9 through dinner on June 11. TDH interviewed 142 of the 754 total drill team camp attendees (18.8%). Interviews were conducted as names and phone numbers became available, without randomization. Statistical analysis demonstrated an increased relative risk (RR) associated with having reported eating beef pot pie, beef lasagna, and beef barbecue, exposures which did not adequately explain a majority of illnesses or that occurred after illness developed.

Beginning July 14, CDC staff attempted to interview all persons who had attended the drill camp. Using a revised questionnaire, drill team camp attendees were interviewed in person or by telephone. Only teaching staff, whoever could not be located, and persons age 11 and under were excluded. Illness was defined as either diarrhea (≥ 3 loose stools in any 24-hour period) accompanied by abdominal cramps or bloody diarrhea alone, occurring within 14 days after the start of the drill camp.

Environmental Investigation

On July 3, and July 15-16 the Dormitory A cafeteria was inspected and kitchen staff were interviewed regarding preparation and service of foods and ice. From July 17-18, walk-throughs

of Dormitory A and a gym where the drill teams practiced were conducted with university staff and engineers; maintenance records and plumbing diagrams were reviewed.

Laboratory Investigation

TDH collected rectal swabs and/or stool samples from staff who prepared or served food and ice at the Dormitory A cafeteria anytime during the drill camp. These samples were cultured. Specimens of beef barbecue, beef tips used in the pot pie, and ground beef used in the lasagna were also collected and cultured. On July 13, serum from persons in two school groups that attended the drill camp were collected and sent to CDC for antibody testing.

Statistical Analysis

Data were analyzed for relative risks; associations that had p-values < 0.05 and 95% confidence intervals (95% CI) that did not include 1 were considered significant. A multivariate model was sequentially constructed using those significant associations that explained $> 25\%$ of illnesses in univariate analysis.

Results

Epidemiologic Investigation

There were 37 other groups in attendance at the university between May 19 and June 30. Ten were selected for screening, including the group on-campus during the investigation; contact information for three groups was not available. Groups which had resided at Dormitory A immediately before the drill team camp were not able to be contacted. No remarkable illness was noted among six of the seven groups interviewed (Table 1). Two groups that were on-campus on the same dates as the drill team camp had eaten their meals at another cafeteria with a different menu and staffed by different persons.

In one group (Group V), which resided and ate at Dormitory A 2 weeks before the drill team camp, 15 of 52 (29%) attendees reported one or more gastrointestinal symptoms (Table 1). However, this group's symptom profile differed from that of the drill team camp attendees in two ways: abdominal cramping was mild and no one reported bloody diarrhea.

Table 1. Results of screening surveys for illness in other group eating and residing at university

<u>Group</u> <u>(Dorm A</u> <u>except</u> <u>Group I)</u>	Dates	Number in cohort	Number selected for survey (%) of cohort	Number interviewed (% completed interviews)	Abdominal cramping (%), non- menstrual	Any diarrhea (%)	Bloody diarrhea (%)	Vomiting (%)
Group I	6/9-6/11	10	5(50)	3 (30)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)
Group II	6/11-6/12	194	39 (20)	24 (12)	2/24 (8)	1/24 (4)	0/24 (0)	0/24 (0)
Group III	6/12-6/15	200	4 (20)	39 (19)	4/39 (10)	5/39 (13)	1/39 (3)	1/39 (3)
Group IV	6/13-7/2	83	17 (20)	17 (20)	2/17 (12)	2/17 (12)	1/17 (6)	0/17 (12)
Group V	5/24-5/27	300	60 (20)	52 (17)	10/52 (19)	9/52 (17)	0/52 (0)	7/52 (14)
Group VI	5/25-5/28	79	16 (20)	12 (15)	0/12 (0)	1/12 (1)	0/12 (0)	0/12 (0)
On campus group	6/27-7/3	48	48 (100)	32 (67)	9/32 (28)	4/32 (12)	0/32 (0)	3/32 (9)

There were 650 drill camp attendees available for interview; 521 (80.2%) were reached for interview. An epidemic curve is shown in Figure 1. Demographic and symptom profiles are shown in Tables 2 and 3. There were 58 ill persons, providing an attack rate of 11.1%, although the attack rate among the 43 attending school groups varied from 0 to 100% (Table 4, *not reproduced*). The median age of ill persons was 16 years (range: 12-53) and 94.8% were female, which was similar to the age and sex distribution for the entire cohort. Among ill persons, 62% reported nausea, 56% headache, 38% vomiting, 37% bloody diarrhea, and 29% fever. Among 16 persons reporting a measured fever, the median temperature was 100°F (range: 99-103°F). The median number of stools in any 24-hour period was 5 (range: 3-20) and the median duration of illness was 5 days (range: 1-37).

Univariate analysis showed that dinner on Wednesday, 6/9/99 was statistically associated with risk of illness (Table 5). Statistically significant risk of illness was also associated with the 21 food exposures shown in Table 6.

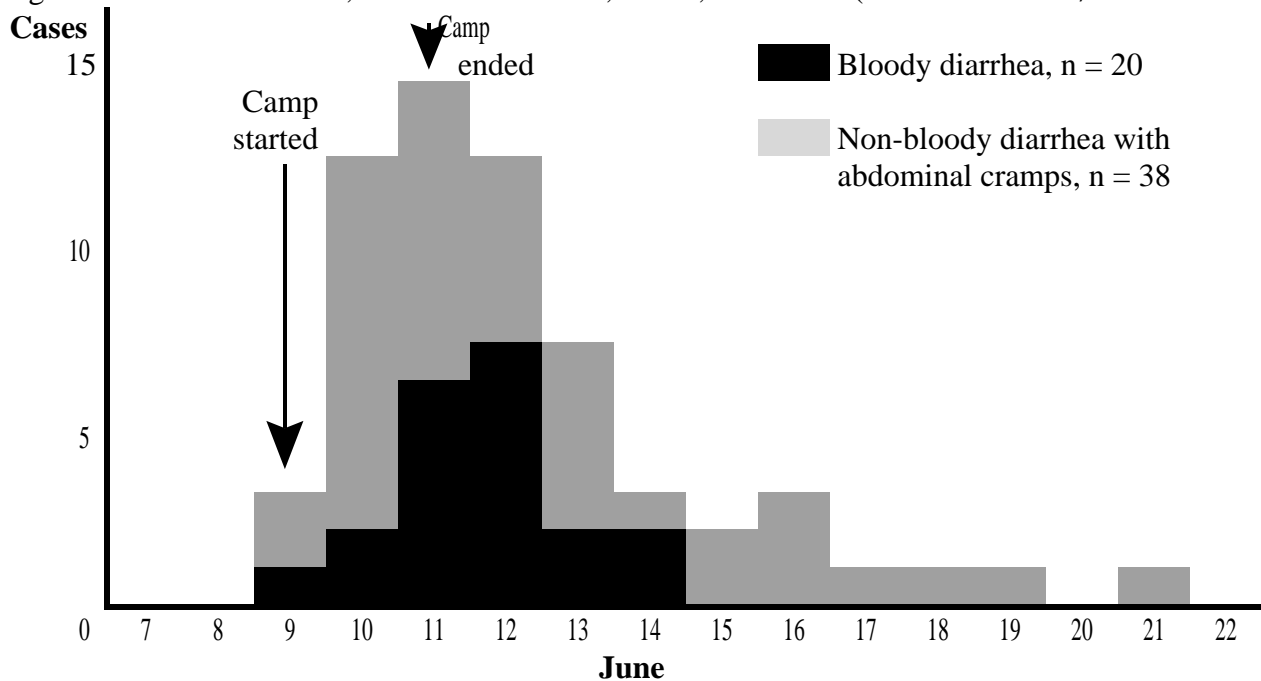
Environmental Investigation

Inspection of the kitchen and the cooking techniques practiced by the staff revealed evidence of potential temperature abuse, both undercooking and inadequate reheating of foods.

Table 2. Demographics of drill team cohort

	Entire cohort (n=518)	All ills (n=58)	Ills with bloody diarrhea (n=20)
Demographics			
Age, median (range), years	16 (12-53)	16 (12-53)	17 (14-36)
Sex % female	97.1	94.8	95.0

Figure 1. Onsets of illness, drill team outbreak, Texas, June 1999 (Total cases = 58)



Interviews with food handlers demonstrated no history of illness, consistent with the signs and symptoms, during or in the 2 months before the time period of interest.

The salad bar contained lettuce, multiple cut or chopped vegetables, cheese, eggs, dressings, and toppings. All items on the salad bar were self-serve from individual containers placed in ice. The lettuce was a commercial pre-packaged ready-to-use product of mixed-leaf greens with shredded red cabbage and carrot shavings in plastic bags. Although it was the cafeteria's policy to wash greens before serving, kitchen staff reported that this product was often transferred to serving bowls on the salad bar directly from the bags without washing. All other salad bar vegetables were washed then cut or chopped prior to serving, with the exception of bean sprouts first used during the drill team camp in a spinach salad at lunch on June 10. All dressings were purchased in pre-made commercial containers, with the exception of the ranch

dressing, which was made by-hand from a commercial dry mix combined with mayonnaise and buttermilk. Shredded cheese was available but no meats were reported by kitchen staff to be available on the salad bar.

Throughout the drill team camp, the cafeteria placed trash barrels of ice in the foyer of the dormitory. The barrels were double-lined with plastic bags, punctured at the top to allow escape of trapped air. The ice came from three ice machines in the kitchen; none of these machines had a record of failure or repair in the 30 days before the drill team camp.

Table 3. Symptoms reported by ill persons in drill team cohort, outbreak, Texas, June 1999

Symptoms	All ills (% or range)
Abdominal cramps (n=58)	58 (100)
Any diarrhea (n=58)	58 (100)
Nausea (n=58)	36 (62)
Headache (n=54)	30 (56)
Chills (n=56)	26 (46)
Vomiting (n=58)	22 (38)
Bloody diarrhea (n=54)	20 (37)
Fever (n=56)	16 (29)
Measured temperature (n=16)	9 (56)
Median temperature (°F)	100 °F (99 °F-103 °F)
Median maximum number of stools in 24 hours (n=55)	5 stools (3-30 stools)
Median number of days ill (n=55)	5 days (1-37 days)
Visited physician for illness (n=57)	21 (37)
Visited hospital for illness (n=57)	3 (5)
Took antidiarrheal medications for illness (n=57)	33 (58)
Took antibiotics for illness (n=57)	21 (37)

The trash barrels were filled with ice using a one-quart metal scoop and/or five-quart plastic buckets. However, no scoop was provided in the trash barrels; camp attendees could dip their hands or cups directly into the ice. Ice was placed out three times daily in two to three barrels for approximately 1-3 hours at a time. When the barrels were refilled, the old ice and melt water were sometimes discarded, the barrels cleaned, and the plastic liners replaced. At other times, fresh ice was added on top of the ice and water remaining in the barrel. Staff and drill team camp attendees reported seeing grass, paper trash, and chewing gum at various times in the ice.

Verbal reports from investigators from the regional state water authority that performed a site inspection did not reveal evidence of cross connections in the Dorm A cafeteria or kitchen.

No coliforms were isolated from water samples taken at the facility, including samples from ice machines in the kitchen.

Table 5. Risk associated with eating meals at Dormitory A cafeteria by univariate analysis

Meals	RR	ill (%)	not-ill (%)	95%CI	p
Breakfast - Wednesday 6/9/99	0.62	5/57(9)	64/457(14)	0.26 – 1.50	0.28
Lunch - Wednesday 6/9/99	4.74	55/56(98)	421/461 (91)	0.67 – 33.36	0.11
Dinner - Wednesday 6/9/99	5.75	55/57(96)	371/458(81)	1.43 – 23.12	0.004
Reception - Wednesday 6/9/99	1.13	14/57 (25)	98/442 (22)	0.64 – 1.98	0.68
Breakfast - Thursday 6/10/99	1.60	50/57(88)	373/461 (81)	0.75 – 3.43	0.21
Lunch - Thursday 6/10/99	1.34	55/57 (96)	437/459 (95)	0.35 – 5.18	1.00
Dinner - Thursday 6/10/99	1.06	48/56 (86)	389/458 (85)	0.52 – 2.15	0.88
Breakfast - Friday 6/11/99	0.90	43/57 (75)	357/460 (78)	0.51 – 1.58	0.71
Lunch - Friday 6/11/99	0.96	52/57 (91)	416/454 (92)	0.40 – 2.26	0.80
Dinner - Friday 6/11/99	0.43	2/56 (4)	39/457(8)	0.11 – 1.69	0.29

Table 6. Significant exposures, by univariate analysis

Exposure	RR	ill (%)	not-ill (%)	95% CI	P
Ate ice from buckets, any day	3.16	41/56 (73)	198/459 (43)	1.79 – 5.59	0.00002
Ate any salad	3.33	54/58 (93)	364/463 (79)	1.23 – 8.97	0.009
Ate any corn-on-the-cob	2.06	27/58 (46)	128/463 (28)	1.27 – 3.33	0.0003
Salad bar, lunch, 6/9/99	2.86	48/54 (89)	293/409 (72)	1.26 – 6.52	0.007
Corn-on-the-cob, dinner, 6/9/99	1.84	23/54 (43)	95/356 (27)	1.12 – 3.01	0.02
Cheesecake, dinner, 6/9/99	1.72	17/52 (33)	73/356 (20)	1.01 – 2.92	0.05
Hard-boiled egg, breakfast, 6/10/99	5.02	7/50 (14)	6/364 (2)	2.82 – 8.94	0.0002
Biscuit, breakfast, 6/10/99	2.13	40/49 (82)	237/361 (66)	1.07 – 4.27	0.03
Beef lasagna, lunch, 6/10/99	1.85	36/54 (67)	212/424 (50)	1.08 – 3.17	0.02
Carrots, lunch, 6/10/99	2.08	13/54 (24)	50/423 (12)	1.18 – 3.67	0.01
Spinach salad, lunch 6/10/99	2.65	4/54 (7)	10/424 (2)	1.11– 6.32	0.06
Salad bar, lunch, 6/10/99	2.12	42/54 (78)	257/426 (60)	1.15 – 3.92	0.01
Dinner roll, dinner, 6/10/99	2.88	32/45 (71)	160/372 (43)	1.56 – 5.34	0.0004
Corn-on-the-cob, dinner, 6/10/99	2.23	14/46 (30)	56/381 (15)	1.26 – 3.96	0.007
Salad bar, dinner, 6/10/99	2.29	35/46 (76)	212/379 (56)	1.20 – 4.39	0.009
Hard-boiled egg, breakfast, 6/11/99	3.61	3/43 (7)	5/350 (1)	1.41– 9.25	0.05
French toast, breakfast, 6/11/99	1.95	30/43 (70)	182/348 (52)	1.05 – 3.62	0.03
Cream cheese, breakfast, 6/11/99	2.28	6/43 (14)	20/349 (6)	1.06 – 4.91	0.05
Salad bar, lunch, 6/11/99	1.72	34/51 (67)	207/397 (52)	0.99 – 2.98	0.05
Spinach, dinner, 6/11/99	38.80	1/2 (50)	0/37 (0)	5.49 – 62.86	0.05
Frozen desert novelty ("Chill") any day	2.13	21/56 (38)	92/458 (20)	1.29 – 3.51	0.003

Direct inspection of the toilet and drinking water systems at both Dormitory A and the gym revealed no evidence of cross-connections or other unusual plumbing events. Review of maintenance records demonstrated no evidence of interrupted supply or recent repairs. Interviews with custodial staff at both facilities revealed no evidence of toilet, sink, or shower overflows, or other recent plumbing system failures since May 15, 1999

Based on the information given:

1. What agent do you think was involved? Why?
2. What would you ask the laboratory to test for you? Why?
3. Can you think of any additional statistical test for evaluation of exposure and response?
4. What food item(s) do you believe was the vehicle(s) in this outbreak? Why?
5. What measures might prevent a recurrence of this outbreak?

OUTBREAK OF ACUTE GASTROENTERITIS — HELENA, MONTANA, 1994

During February-March, 1994, four persons in Helena, Montana (1995 population: 24,569), developed bloody diarrhea and severe abdominal cramps. Stool cultures for *Salmonella*, *Shigella*, *Campylobacter*, and *Escherichia coli* O157:H7 were negative; however, sorbitol-negative *E. coli* colonies were identified in stools from all four patients. Isolates from three patients were identified at CDC as a rare serotype — *E. coli* O104:H21 that produced Shiga-like toxin II. This report summarizes the epidemiologic and laboratory investigations of this outbreak by the Lewis and Clark County Department of Health and Environmental Sciences, the Montana Department of Health and Environmental Sciences (MDHES), and CDC.

A confirmed case was defined as acute infection with *E. coli* O104:H21 during February 20-May 25, 1994 — based on stool culture or serologic evidence — in a resident of or a visitor to the Helena area. A suspected case was defined onset of bloody diarrhea or abdominal cramps during the same period in a resident of or visitor to the Helena area. MDHES and county health departments contacted clinicians, laboratories, and the public through news media reports and requested that suspected cases be reported.

Eleven confirmed and seven suspected case-patients were identified. Manifestations included abdominal cramps (18 [100%]), diarrhea (17 [94%]), bloody stools (16 [89%]), vomiting (10 [56%]), and fever (6 of 15 [40%] for whom information was available). The median age was 36 years (range: 8-63 years), and 12 (67%) were female. Four (22%) persons were hospitalized.

Potential sources and risk factors for illness were assessed by a case-control study that included 17 case-patients and three age-, sex-, and neighborhood-matched controls for each case-patient. A history of milk consumption during the 7 days before illness was reported by all 17 case-patients compared with 40 (83%) of 48 controls (matched odds ratio [OR]=undefined). One brand of milk (Brand A) was significantly associated with illness: of those persons who drank milk at home, 11 (92%) of 12 case-patients compared with 17 (47%) of 36 controls reported drinking Brand A (matched OR=16.0; 95% CI=1.3-492.7). Within this brand, no specific type of milk product was associated with illness. Factors not associated with illness included consumption of other brands of milk, other foods or drinks, and dining in specific restaurants.

On May 16, the local and state health departments, the Food and Drug Administration, and CDC inspected the dairy plant where Brand A milk was produced. Based on review of the plant's records for internal microbiologic quality-control testing, on 12 days during February 1-May 13, 1994, the coliform count exceeded the state regulation limiting maximum coliform levels in milk products to less than or equal to 10 coliforms per 100 mL on at least one

ready-for-sale milk product. Cultures from selected post-pasteurization piping and equipment surfaces in contact with finished milk products yielded fecal coliforms; however, *E. coli* O104:H21 was not isolated from any culture samples obtained at the dairy. Two farms provided

raw milk for this dairy; rectal swabs obtained from a sample of cattle from these farms did not yield *E. coli* O104:H21.

1. What is the significance of “sorbitol-negative” in the second sentence of this report?
2. What is the significance of excessive coliform levels in pasteurized milk?
3. What means are available to verify that pasteurization has been done?
4. What is the significance of the finding that cultures from selected post-pasteurization piping and equipment surfaces in contact with finished milk products yielded fecal coliforms?
5. How do you interpret the finding that within this brand, no specific type of milk product was associated with illness?
6. Was rectal swabbing a reasonable way to sample cattle for *E. coli* O104:H21?