

Gastroenteritis at a University in Texas

STUDENT'S VERSION

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NOTE: This case study is based on a real-life outbreak investigation undertaken in Texas in 1998. Some aspects of the original outbreak and investigation have been altered, however, to assist in meeting the desired teaching objectives and allow completion of the case study in less than 3 hours.

Students should be aware that this case study describes and promotes one particular approach to foodborne disease outbreak investigation. Procedures and policies in outbreak investigations, however, can vary from country to country, state to state, and outbreak to outbreak.

It is anticipated that the epidemiologist investigating a foodborne disease outbreak will work within the framework of an "investigation team" which includes persons with expertise in epidemiology, microbiology, sanitation, food science, and environmental health. It is through the collaborative efforts of this team, with each member playing a critical role, that outbreak investigations are successfully completed.

We invite you to send us your comments about the case study by visiting our website at <http://www.phppo.cdc.gov/phtn/casestudies>. Please include the name of the case study with your comments.

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Learning objectives:

After completing this case study, the student should be able to:

1. list categories and examples of questions that should be asked of key informants who report a suspected outbreak of foodborne disease
2. list four criteria for prioritizing the investigation of suspected foodborne disease outbreaks
3. list three common pitfalls in the collection of clinical specimens for the investigation of suspected foodborne diseases
4. determine the most efficient epidemiologic study design to test a hypothesis (including the case definition and the appropriate comparison group)
5. describe the advantages and disadvantages of different forms of questionnaire administration (e.g., self-administered, telephone, in-person)
6. list key areas of focus in interviewing foodhandlers and observing kitchen practices in a foodborne disease outbreak

PART I - OUTBREAK DETECTION

On the morning of March 11, the Texas Department of Health (TDH) in Austin received a telephone call from a student at a university in south-central Texas. The student reported that he and his roommate, a fraternity brother, were suffering from nausea, vomiting, and diarrhea. Both had become ill during the night. The roommate had taken an over-the-counter medication with some relief of his symptoms. Neither the student nor his roommate had seen a physician or gone to the emergency room.

The students believed their illness was due to food they had eaten at a local pizzeria the previous night. They asked if they should attend classes and take a biology mid-term exam that was scheduled that afternoon.

Question 1: What questions (or types of questions) would you ask the student?

Question 2: What would you advise the student about attending classes that day?

The "Foodborne Illness Complaint Worksheet" (Appendix 1) was completed based on the call. The student refused to give his name or provide a telephone number or address at which he or his roommate could be reached.

Question 3: Do you think this complaint should be investigated further?

TDH staff were skeptical of the student's report but felt that a minimal amount of exploration was necessary. They began by making a few telephone calls to establish the facts and determine if other persons were similarly affected. The pizzeria, where the student and his roommate had eaten, was closed until 11:00 A.M. There was no answer at the University Student Health Center, so a message was left on its answering machine.

A call to the emergency room at a local hospital (Hospital A) revealed that 23 university students had been seen for acute gastroenteritis in the last 24 hours. In contrast, only three patients had been seen at the emergency room for similar symptoms from March 5-9, none of whom were associated with the university.

At 10:30 A.M., the physician from the University Student Health Center returned the call from TDH and reported that 20 students with vomiting and diarrhea had been seen the previous day. He believed only 1-2 students typically would have been seen for these symptoms in a week. The Health Center had not collected stool specimens from any of the ill students.

Question 4: Do you think these cases of gastroenteritis represent an outbreak at the university? Why or why not?

PART II - INITIAL MICROBIOLOGIC INVESTIGATION

On the afternoon of March 11, TDH staff visited the emergency room at Hospital A and reviewed medical records of patients seen at the facility for vomiting and/or diarrhea since March 5. Based on these records, symptoms among the 23 students included vomiting (91%), diarrhea (85%), abdominal cramping (68%), headache (66%), muscle aches (49%), and bloody diarrhea (5%). Oral temperatures ranged from 98.8°F (37.1°C) to 102.4°F (39.1°C) (median: 100°F [37.8°C]). Complete blood counts, performed on 10 students, showed an increase in white blood cells (median count: 13.7 per cubic mm with 82% polymorphonuclear cells, 6% lymphocytes, and 7% bands). Stool specimens had been submitted for routine bacterial pathogens, but no results were available.

Question 5: List the broad categories of diseases that must be considered in the differential diagnosis of an outbreak of acute gastrointestinal illness.

Question 6: How might you narrow the range of agents suspected of causing the gastrointestinal illness?

TDH staff asked health care providers from the University Student Health Center, the Hospital A emergency room, and the emergency departments at six other hospitals located in the general vicinity to report cases of vomiting or diarrhea seen since March 5. A TDH staff person was designated to help the facilities identify and report cases. The health care providers were also asked to collect stool specimens from any new cases. Bacterial cultures from patients seen in the emergency rooms were to be performed at the hospital at which they were collected and confirmed at the TDH Laboratory. Specimens collected by the Student Health Center were to be cultured at the TDH Laboratory.

Question 7: What information should be provided with each stool specimen submitted to the laboratory? How will the information be used?

Question 8: How should specimens be transported from the University Health Center to the TDH laboratory?

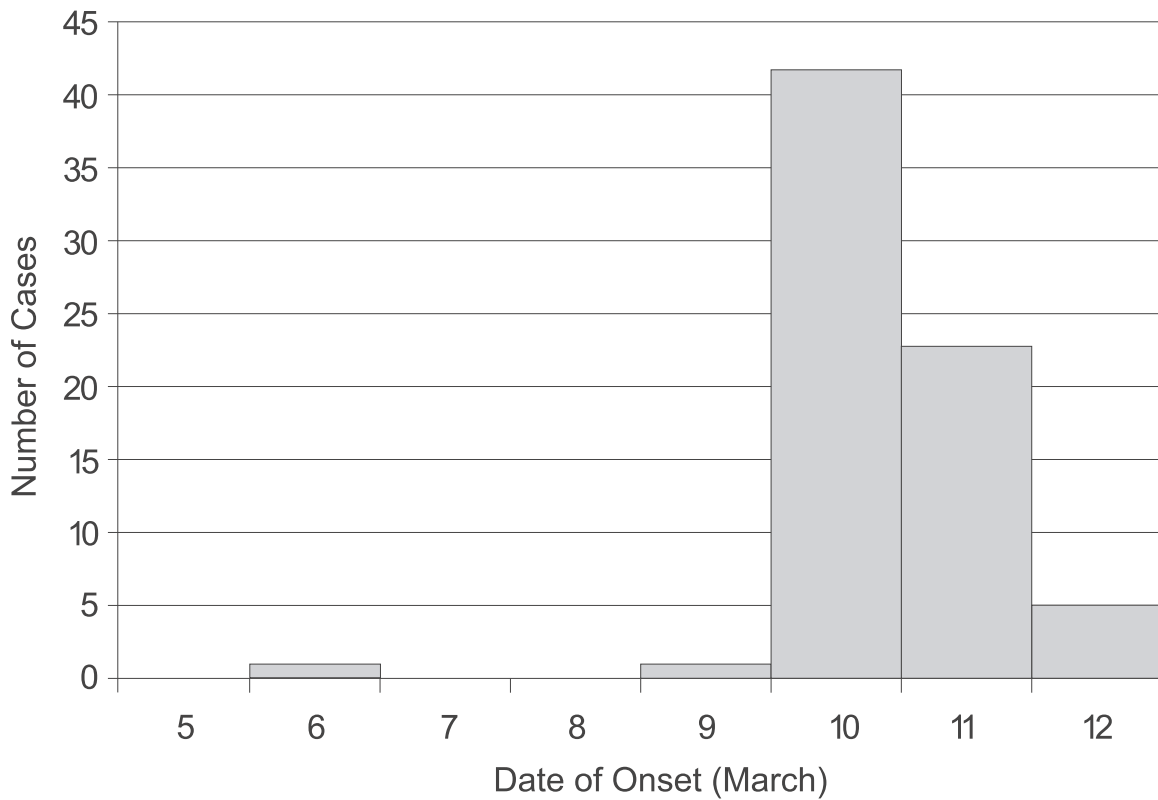
Later that afternoon, preliminary culture results from 17 ill students became available. The specimens, collected primarily from the emergency room at Hospital A on March 10, did not identify *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, *Listeria*, *Yersinia*, *Escherichia coli* O157:H7, *Bacillus cereus*, or *Staphylococcus aureus*. Some specimens were positive for fecal leukocytes and fecal occult blood.

Question 9: How might you interpret the bacterial culture results? What questions do these results raise?

PART III - DESCRIPTIVE EPIDEMIOLOGY AND HYPOTHESIS GENERATION

By March 12, seventy-five persons with vomiting or diarrhea had been reported to TDH. All were students who lived on the university campus. No cases were identified among university faculty or staff or from the local community. Except for one case, the dates of illness onset were March 9-12. (Figure 1) The median age of patients was 19 years (range: 18-22 years), 69% were freshman, and 62% were female.

Figure 1. Onset of gastroenteritis among students, University X, Texas, March 1998. (N=72) (Date of onset was not known for three ill students.)



TDH staff met with the Student Health Center physician and nurse, and several university administrators including the Pro vost. City health department staff participated in the meeting.

Question 10: What topics would you include in discussions with university officials?

TDH and City Health Department staff gathered the following information:

The university is located in a small Texas town with a population of 27,354. For the spring semester, the university had an enrollment of approximately 12,000 students; 2,386 students live on campus at one of the 36 residential halls scattered across the 200+ acres of the main campus. About 75% of the students are Texas residents.

The university uses municipal water and sewage services. There have been no breaks or work on water or sewage lines in the past year. There has been no recent road work or digging around campus.

The campus dining service includes two cafeterias managed by the same company and about half a dozen fast food establishments; about 2,000 students belong to the university meal plan which is limited to persons living on campus. Most on-campus students dine at the main cafeteria which serves hot entrees, as well as items from the grill, deli bar, and a salad bar. A second smaller cafeteria on campus offers menu selections with a per item cost and is also accessible to meal plan members. In contrast to the main cafeteria, the smaller cafeteria tends to be used by students who live off campus and university staff. The smaller cafeteria also offers hot entrees, grilled foods, and a salad bar, but has no deli bar.

Spring break is to begin on March 13 at which time all dining services will cease until March 23. Although many students will leave town during the break, it is anticipated that about a quarter of those living on campus will remain.

Hypothesis generating interviews were undertaken with seven of the earliest cases reported by the emergency rooms and the Student Health Center; all of the cases had onset of illness on March 10. Four were male and three were female; all but one was a freshman. Two students were psychology majors; one each was majoring in English and animal husbandry. Three students were undecided about their major.

The students were from five different residential halls and all reported eating most of their meals at the university's main cafeteria. During the past week, all but one student had eaten food from the deli bar; two had eaten food from the salad bar, and three from the grill. Seven-day food histories revealed no particular food item that was common to all or most of the students.

Except for the psychology majors, none of the other students shared any classes; only one student had a roommate with a similar illness. Five students belonged to a sorority or a

fraternity. Three students had attended an all school mixer on March 6, the Friday before the outbreak began; two students went to an all night science fiction film festival at one of the dorms on March 7. Students reported attendance at no other special events; most had been studying for midterm exams for most of the weekend.

Question 11: Using information available to you at this point, state your leading hypothesis(es) on the pathogen, mode of transmission, source of the outbreak, and period of interest.

Question 12: What actions would you take?

PART IV - ENVIRONMENTAL INVESTIGATION

Based on clinical findings, the descriptive epidemiology of early cases, and hypothesis-generating interviews, investigators hypothesized that the source of the outbreak was a viral pathogen spread by a food or beverage served at the main cafeteria at the university between March 5 and 10. As a result, TDH environmental sanitarians inspected the main cafeteria and interviewed staff on March 12.

Thirty-one staff members were employed at the cafeteria of whom 24 (77%) were foodhandlers. Except for one employee who worked at the deli bar and declined to be interviewed, all dining service personnel were interviewed.

Question 13: What key areas should be explored during interviews with the cafeteria foodhandlers?

Cafeteria staff were questioned about their responsibilities in the cafeteria such as the foods they handled, which meals they served, and where they usually worked (e.g., deli bar, grill). They were also asked about use of gloves, handwashing practices, their work schedule during the week before the outbreak, and if they had been ill at that time.

In the cafeteria, the deli bar had its own preparation area and refrigerator. During mealtimes, sandwiches were made to order by a foodhandler. Each day, newly prepared deli meats, cheeses, and condiments were added to partially depleted deli bar items from the day before (i.e., without discarding leftover food items). While the deli was open for service, sandwich ingredients were not kept refrigerated or on ice. The deli bar containers were not routinely cleaned. Samples of leftover food, water, and ice were collected.

None of the foodhandlers interviewed reported being ill in the last two weeks. Stool cultures were requested from all cafeteria staff.

Before dinner on March 12, the City Health Department closed the deli bar.

Question 14: Do you agree with the decision to close the deli bar? What actions would you take now?

**PART V - DESIGNING AN EPIDEMIOLOGIC STUDY TO TEST THE HYPOTHESIS
(STUDY #1)**

On the evening of March 12, about 36 hours after the initial call to the health department, TDH staff conducted a matched case-control study among students at the university. Ill students (reported from emergency rooms and the Student Health Center) who could be reached at their dormitory rooms were enrolled as cases. Dormitory roommates who had not become ill were asked to serve as matched control subjects. Investigators inquired about meals the students might have eaten during March 5-10 and where the foods were eaten. All information was collected over the telephone.

Question 15: What are the advantages and disadvantages of undertaking a case-control study instead of a cohort study at this point in the investigation?

Question 16: How would you define a case for this study?

Twenty-nine cases and controls were interviewed over the telephone. Investigators tabulated the most notable results in Table 1.

Table 1. Risk factors for illness, matched case-control study, main cafeteria, University X, Texas, March 1998.

Exposure	Ill exposed/ Total ill* (%)	Well exposed/ Total well* (%)	Matched Odds Ratio**	95% Confidence Interval	p-value
Ate at deli bar - lunch on March 9	11/28 (39)	1/29 (3)	11.0	1.6-473	<0.01
Ate at deli bar - dinner on March 9	7/27 (26)	2/29 (7)	6.0	0.73-275	0.06
Ate at deli bar - lunch on March 10	8/29 (28)	1/28 (4)	8.0	1.1-354	0.02
Ate at deli bar - dinner on March 10	2/29 (7)	2/28 (7)	1.0	0.01-79	0.75
Ate at deli bar - lunch or dinner on March 9 or lunch on March 10	15/27 (56)	3/28 (11)	7.0	1.61-63.5	<0.01

*Denominator does not always total to 29 because several subjects could not remember where they ate the indicated meal.

**The data provided for cases and controls cannot be used to calculate the matched odds ratio which is based on an analysis of discordant pairs.

Question 17A: How do you interpret these data?

Question 17B: What elements of this case-control study might affect the validity of the measured association?

Eating at the main cafeteria, in general, was not associated with illness; however eating from the deli bar during lunch on March 9 or March 10 was significantly associated with illness. Because such a small number of controls ate at the deli bar, individual food items from the deli bar could not be examined.

PART VI - DESIGNING AN EPIDEMIOLOGIC STUDY TO REFINE THE HYPOTHESIS (STUDY #2)

By March 13, one hundred and twenty-five persons with vomiting or diarrhea had been reported to TDH. TDH invited staff from the Centers for Disease Control and Prevention (CDC) to participate in the ongoing investigation. CDC staff suggested submission of fresh stool specimens from ill students for viral studies including reverse transcriptase-polymerase chain reaction (RT-PCR). TDH and CDC staff decided to undertake an unmatched case-control study to further explore the source of the outbreak.

Question 18: Who should be enrolled as subjects for this study?

The case-control study was undertaken among students who ate at the main cafeteria. A case was defined as vomiting or diarrhea (≥ 3 loose bowel movements during a 24-hour period) with onset on or after March 5, 1998, in a student who was a member of the university meal plan. Cases were selected from those reported to TDH by one of the local emergency rooms or the Student Health Center. Controls were students enrolled in the university meal plan who did not have nausea, vomiting, or diarrhea since March 5.

Forty cases were randomly selected from the 125 reported through March 13. One hundred and sixty controls were randomly selected from the university meal plan database.

Question 19: Investigators considered collecting information for the case-control study through face-to-face interviews, telephone interviews, or self-administered questionnaires. What are the advantages and disadvantages of each method of data collection? Which method would you recommend given the circumstances around the outbreak?

The investigators administered the study questionnaire by telephone from March 15-23. Students selected for participation were called at their dormitory room or their home telephone number as recorded in university records. If the student was not present at either location but information on his/her whereabouts was available, additional phone calls were made to contact the student. Students not reached during spring break were interviewed on their return to the university.

Thirty-six cases and 144 controls were contacted. Cases included in the study were similar to all cases with respect to gender, age, and year in college. Their dates of onset of illness had a distribution similar to that seen in Figure 1.

Results from the unmatched case-control study were tabulated by TDH and CDC epidemiologists. Only persons who ate at the main cafeteria for the specified period were included in the meal-specific analyses. (Table 2)

Table 2. Risk factors for illness among persons eating at the main cafeteria, unmatched case-control study, University X, Texas, March 9-10, 1998.

Exposure	Ill exposed/ Total ill	Well exposed/ Total well	Measure of association	p-value
Ate at salad bar - lunch March 9	9/30	36/100		
Ate at salad bar - dinner March 9	5/18	15/59		
Ate at salad bar - lunch March 10	6/28	23/96		
Ate at salad bar - dinner March 10	6/15	12/44		
Ate at salad bar*	13/19	49/69		
Ate at deli bar - lunch March 9	18/30	12/101		
Ate at deli bar - dinner March 9	7/18	5/61		
Ate at deli bar - lunch March 10	13/29	12/96		
Ate at deli bar - dinner March 10	4/16	4/44		

Ate at deli bar*	28/36	20/116		
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*for lunch or dinner March 9 or lunch March 10

Question 20: Calculate the appropriate measure of association for these exposures. Interpret the results.

To identify the specific item(s) at the deli bar causing the outbreak, investigators reanalyzed study data from only cases and controls who ate at the deli bar during March 9-10. (Table 3)

Table 3. Food items eaten by students who ate at deli bar during implicated meals, unmatched case-control study, University X, Texas, March 9-10, 1998.*

Exposure	Ill exposed/ Total ill (%)	Well exposed/ Total well (%)	Odds Ratio	95% Confidence Interval	p-value
American cheese	13/28 (46)	4/20 (20)	3.4	0.80-17.5	0.06
Swiss cheese	8/28 (29)	8/20 (40)	0.61	0.15-2.4	0.30
Ham	11/28 (39)	6/20 (30)	1.5	0.38-6.3	0.36
Turkey	15/28 (54)	11/20 (55)	0.95	0.26-3.5	0.57
Shredded lettuce	13/28 (46)	10/20 (50)	0.87	0.24-3.2	0.52
Tomato	7/28 (25)	6/20 (30)	0.78	0.18-3.5	0.50
Pickles	7/28 (25)	7/20 (35)	0.63	0.15-2.6	0.63
Mayonnaise	20/28 (71)	9/20 (45)	3.1	0.78-12.4	0.06
Mustard	10/28 (36)	9/20 (45)	0.68	0.18-2.6	0.52

*includes lunch and dinner on March 9 and lunch on March 10

Question 21: Interpret the results in Table 3.

PART VII - ADDITIONAL INVESTIGATIONS

Water and ice samples obtained from the cafeteria on March 12 were negative for fecal coliforms. Stool cultures and rectal swabs from the 23 foodhandlers were negative for bacteria.

The staff member who initially refused to be interviewed worked primarily at the deli bar. When she finally agreed to be interviewed on March 23, she reported slicing ham on March 9, for use at the deli bar during lunch and dinner that day, and lunch the following day. She also prepared and served sandwiches for these same meals. She reported that she had worn gloves while slicing the ham and while serving sandwiches at the deli bar. She denied any gastrointestinal illness during the outbreak period but reported that her infant had been sick with watery diarrhea since March 7, two days before she prepared items for the implicated meals. Because the foodhandler wore gloves during food preparation and serving, she did not feel that handwashing was an important activity.

Of the 18 fresh stool specimens sent on ill students to CDC, 9 (50%) had evidence of Norwalk-like virus (NLV) by RT-PCR. Of the four deli foods available from the implicated meals, only the ham sample, from March 9, was positive by RT-PCR for the presence of NLV RNA. NLV was also detected by RT-PCR in a stool sample from the ill infant of the foodhandler who prepared the deli sandwiches on March 9. The sequence of the amplified product was identical to those products from the ill students and the deli ham.

Question 22: Do you think the evidence implicates the foodhandler as the source of the outbreak? Explain.

PART VIII - CONTROL

Spring break at the university ended on March 23. The chief of the campus food service called TDH to find out what must be done to reopen the deli bar.

Question 23: Which of the following actions would you recommend? What are the pros and cons of each?

- A) throw away all leftover deli bar foods and ingredients
- B) clean and disinfect all equipment and surfaces in the deli bar
- C) require all foodhandlers to submit a stool specimen before allowing them to return to work
- D) educate foodhandlers on proper foodhandling procedures including handwashing and appropriate hot-holding and cold-holding temperatures
- E) develop a sick foodhandlers policy

Question 24: Who might you consult in developing actions/policies for the campus food service to prevent a recurrence of this problem in the future? Why?

FOOD HISTORY

⇒ Obtain history **back 72 hours** prior to symptoms, or, if organism identified, **use min and max incubation** periods (see p.3)

⇒ If ≥2 ill, follow above time frame for common meals (foods) only

Date & Time²	# Exp³	Food(s) consumed	Restaurant / store where purchased (name, town)	Place consumed
March 8 <input type="checkbox"/> B <input type="checkbox"/> L <input type="checkbox"/> D		university cafeteria		<input type="checkbox"/> Same (as left) <input type="checkbox"/> Home <input type="checkbox"/> Other (specify):
March 9 <input type="checkbox"/> B <input type="checkbox"/> L <input type="checkbox"/>		university cafeteria		<input type="checkbox"/> Same (as left) <input type="checkbox"/> Home <input type="checkbox"/> Other (specify):
March 10 <input type="checkbox"/> B <input type="checkbox"/> L <input checked="" type="checkbox"/>		Anchovy pizza and beer	Local pizzeria	<input type="checkbox"/> Same (as left) <input type="checkbox"/> Home <input checked="" type="checkbox"/> Other (specify):
<input type="checkbox"/> B <input type="checkbox"/> L <input type="checkbox"/>				<input type="checkbox"/> Same (as left) <input type="checkbox"/> Home <input type="checkbox"/> Other (specify):
<input type="checkbox"/> B <input type="checkbox"/> L <input type="checkbox"/> D				<input type="checkbox"/> Same (as left) <input type="checkbox"/> Home <input type="checkbox"/> Other (specify):
<input type="checkbox"/> B <input type="checkbox"/> L <input type="checkbox"/> D				<input type="checkbox"/> Same (as left) <input type="checkbox"/> Home <input type="checkbox"/> Other (specify):

NOTES

Student refused to provide food history beyond foods eaten at local pizzeria. He reported that he and his roommate shared no other meals in the last 72 hours; they ate separately at the university cafeteria.

FOOD TESTING

Food(s) available for testing? Yes No Unknown Sent to SLI ¹? Yes No Unknown
If Yes, specify food(s) & sources:

Product and Manufacturer Information for Commercially-Processed Food(s)

Product name: _____ Code/lot # _____
 Expiration date: ____ / ____ / ____ Package size/type: _____
 Manufacturer: _____ ☎: () _____ - _____
 Address: _____

Incubation Periods for Selected Organisms

	<i>Min</i>	<i>Max</i>		<i>Min</i>	<i>Max</i>		<i>Min</i>	<i>Max</i>
B. cereus (short)	1 hr	6 hrs	E. coli O157:H7	3 days	8	Staph. aureus	30 min	8 hrs
B. cereus (long)	6 hrs	24 hrs	Hepatitis A	15 days	50 days	Shigella	12 hrs	96 hrs
Campylobacter	1 day	10 days	Salmonella (non-typhi)	6 hrs	5 days	Vibrio cholerae	few hrs	5 days
Cyclospora	1 day	14 days	Salmonella typhi	1 wk	3 wks	Viral GI	12 hrs	48 hrs
C. perfringens	6 hrs	24 hrs	Shellfish poisoning	min	few hrs	Yersinia	3 days	7 days

1 State Laboratory Institute
 2 Always record **Time** if possible; otherwise, choose **B**=breakfast, **L**=lunch, **D**=dinner
 3 Total # persons (both ill and well) who consumed indicated food(s)

APPENDIX 2. Causative agents for acute enteric illness

INFECTIOUS

Bacteria

Aeromonas (not proven)

Bacillus cereus

Campylobacter

Clostridium perfringens

Escherichia coli

Shiga Toxin producing *E. coli* (STEC)

Enterotoxin producing *E. coli* (ETEC)

Enteroinvasive *E. coli* (EIEC)

Enteropathogenic *E. coli* (EPEC)

Plesiomonas (not proven)

Salmonella, non-typhoid

Salmonella Typhi

Shigella

Vibrio

Yersinia enterocolitica

Viruses

Norwalk and Norwalk-like agents (caliciviruses)

Rotavirus

Hepatitis A

Parasites

Cryptosporidium parvum

Cyclospora

Entamoeba histolytica

Giardia lamblia

TOXINS

Bacillus cereus

Staphylococcus aureus

Clostridium perfringens

Clostridium botulinum

heavy metals (cadmium, copper, zinc, tin)

mushroom toxins

fish and shellfish toxins (scombroid,

ciguatera)

insecticides

drugs

boric acid

OTHER

psychogenic

radiation

APPENDIX 3. Recommendations for Collection of Stool Specimens for Laboratory Examination (*from the Morbidity and Mortality Weekly Report: Recommendations and Reports 1990;30 [No. RR-14]*)

Specimen collection is critical in identifying the causative agent in an outbreak of gastroenteritis. Bacteria, viruses, and parasites each require different specimens and methods of storage and transport for optimal diagnosis. When the causative agent is unknown, one should consider plausible pathogens based on predominant signs and symptoms and other outbreak information.

For bacterial pathogens

Rectal swabs or swabs of fresh stools should be placed in refrigerated Cary-Blair transport medium. If the specimens are likely to be examined within 48 hours after collection, they can be refrigerated at 4°C until shipping. Specimens should be enclosed in a secure container and placed in a waterproof bag. Specimens should be packed with ice or frozen refrigerant packs in an insulated box.

If specimens must be held longer than 48 hours, they should be frozen as soon as possible after collection. Although storage in an ultra-low freezer (-70°C) is preferable, storage in a home-type freezer (if it is properly set at -20°C) is acceptable for short periods. So that the specimens remain frozen, they should be shipped on dry ice. Sufficient dry ice should be used to keep specimens frozen until the laboratory processes them (i.e., enough dry ice to fill one-third to one-half of the shipping container). Glass tubes should not be in direct contact with the dry ice; a layer of paper or other material should be placed between the tubes and the dry ice. To prevent excess exposure to carbon dioxide (from the dry ice), screw caps should be tightened and sealed with electrical tape or specimens should be sealed in a plastic bag within the container of dry ice.

For viral pathogens

Collect as large a quantity of diarrheal stool as can be obtained (at least 10 cc). Place in a leak-proof, clean, dry container, and refrigerate immediately at 4°C. **DO NOT FREEZE SPECIMENS IF ELECTRON MICROSCOPY EXAMINATION IS ANTICIPATED.** The use of rectal swabs to detect viral causes of gastroenteritis is discouraged because the sensitivity of detection compared to bulk stool is suspected to be low.

For parasites

Mix fresh bulk stool specimens thoroughly with each of two preservatives, 10% formalin and polyvinyl alcohol fixative, at a ratio of one part of stool to 3 parts of preservative. If there is any delay in obtaining the preservatives, refrigerate untreated stool specimens at 4°C for up to 48 hours. For routine microscopy, **DO NOT FREEZE.** Once preserved, the specimens can be stored and transported at room temperature or refrigerated. Note, it is now possible to do genotyping on many parasites, but this may require different preservatives. If parasites are considered a likely etiology, contact a lab that has the capacity to conduct genetic testing and ask for specific instructions. Currently recommended preservatives for genetic analysis include freezing the specimen or preserving it in ethanol or potassium dichromate.