A Food Poisoning Outbreak Involving University Students at the Hohuan Campsite in Pinglin Township, Taipei County

Abstract

The purpose of the epidemiological investigation was to identify the possible pathogen and food items related to the food poisoning outbreak on September 30 and October 1, 2000. The outbreak involved with several hundreds of students from four universities on the occasions of either welcoming new students or field training activities at the Hohuan Campsite in Pinglin Township, Taipei County. The investigation applied the case-control method with a semi-structured questionnaire. The questionnaire contained questions on the background information, major complaints, onset time of symptoms, medication, time of recovery, time of food intake, and food items eaten. A total of 317 copies of the students of the questionnaire were collected from Business Administration Department of the National Taiwan University of Science and Technology (NTUST), the Life Science Department of the National Yangming University, the International Trade Department of the Mingchuan University, the Spots and Recreation Club of the Mingchuan University and the Huafan University. Analysis was made primarily on the 86 copies of the questionnaire collected from students of the NTUST.

The food poisoning attack rate of the NTUST students was 39.5% (34/86). Major symptoms included watery diarrhea at least twice a day and

abdominal pain. No cases had bloody feces. Incubation period ranged from 7 to 68 hours, averaging 38 hours, with a standard error of 15 hours. Approximately 70% of the food poisoning cases (24/34) had been medically cared. They recovered within 1-6 days, with a median of 3 days. Results of food items analysis showed that the lemon tea, which the NTUST students had at the barbecue at noon of October 1, was significantly associated with the food poisoning outbreak (odds ratio: 5.19, 95% confidence limits: 1.02-50.33). According to the symptoms, incubation periods, and findings from environmental inspections, it was speculated that the present incident was most likely associated with the lemon tea contaminated by enterotoxigenic *Escherichia coli*.

Introduction

Pathogenic *Escherichia coli* induces various kinds of Gastroenteritis through five different mechanisms. Of them, the enterotoxigenic strains (ETEC) can, by means of plasmid, produce either heat-labile (LT) or heat-stable (ST) enterotoxin to cause cholera-like diarrhea⁽¹⁾. The ETEC infection can bring about symptoms such as watery diarrhea, nausea, abdominal pain, and mild fever. It is the major cause of diarrhea in developing countries and among tourists⁽¹⁾ and is generally induced by the intake of contaminated food or water. Since the routine laboratory examination for food poisoning does not include the ETEC testing, incidents of pathogenic *E. coli* infection are often underreported.

From September 30 to October 1, the Business Administration Department of the National Taiwan University of Science and Technology (NTUST) had welcoming parties for new students at the Hohuan campsite of Pinglin Township, Taipei County. On the early morning of October 2, some students began to show symptoms of diarrhea, vomiting, abdominal angina, fever, and prostration. They were sent to hospitals for care. At the same time, students from the Yangming, Mingchuan and Huafan universities were also having either welcoming parties or field training activities at the same place. By telephone inquiries, it was noted that some of their students also appeared similar symptoms. Because a large number of students were involved, the epidemiologists of Center for Disease Control conducted a field investigation for the purpose of finding the pathogenic agent and food items responsible for the poisoning.

Materials and Methods

The investigation applied the case-control method⁽²⁾. Cases included students who met the definition of food poisoning. Controls included non-food-poisoning cases.

Subjects for Investigation

The study subjects included students who attended activities at the Hohuan campsite on September 30 and October 1, 2000. A total of 455 students attended the study. They were 94 students of the Department of Business Administration of NTUST, 69 students of the Department of Life Science of the National Yangming University (YMU), 240 students of the Department of International Business of Mingchuan University (MCU), 24 students of the MCU Sports and Recreation Club, and 28 students of the Sports and Recreation Club of Huafan University (HFU).

Questionnaire Survey

A semi-structured questionnaire was distributed for filling out by students. Since the ways and the food items that students of the four universities consumed were different (Table 1), the contents of their questionnaires were not the same. They included, however, basically questions on the university they attended, department, name, age, gender, major symptoms, onset time of symptoms, medication, time of recovery, time of food intake, and food items eaten.

Definition of Case

A case was defined as any students who attended activities at the Hohuan campsite of Pinglin Township, Taipei County, at any time during September 30 and October 1, 2000 and had any one of the three following conditions: 1) diarrhea at least three times a day, or 2) diarrhea at least twice a day and abdominal pain, or 3) either diarrhea at least twice a day or abdominal pain, adding two of the following symptoms, nausea, vomiting and fever. One who did not meet the definition was defined as the "non-case".

Inspection of Environment

The investigation team, accompanied by staff of the Taipei County Health Bureau, inspected the kitchen environment of the campsite and understood the process of food preparation and storage. The sources of food materials, drinking water, and the health conditions of the kitchen staff were also investigated.

Laboratory Testing

A total of 95 rectal swabs were collected by the Taan and Peitou District Health Centers of the Taipei City from students of the NTUST Business Administration Department (71 swabs) and the YMU Life Science Department (24 swabs). The Taipei County Health Bureau also collected two hand swabs from the kitchen staff. All specimens were sent to the Division of Bacterial Diseases (now the Division of Laboratory Research and Development) of the Center for Disease Control and tested for: *Vibrio parahaemolyticus, Bacillus cereus, Salmonella, Staphylococcus aureus, Vibrio cholerae, Bacillus dysenteriae,* and *Bacillus typhi.* In addition, the Taipei County Health Bureau collected four environmental specimens from knives, chopping boards, and water from the kitchen for testing. The tests were conducted by the National Laboratories of Food and Drugs for: *Staphylococcus aureus, Salmonella, Bacillus cereus,* pathogenic *E. coli,* and *Vibrio parahaemolyticus*

Data Processing and Analysis

Data were keyed in with the EPI-Info 6.04 software ⁽³⁾ and verified record by record to ensure their correctness. Descriptive and statistical analyses of the data were also made with the EPI-Info software. The distribution curve of the incubation periods was drawn with Excel 2000[®] software. Odds ratios and their 95% confidence limits (CL) were used to indicate the intensity of the relationships between food items eaten and the food poisoning outbreak. Either χ^2 or the Fisher's exact test was used to test the relationships between meals taken and the food poisoning outbreak. The association between food items eaten and the food poisoning outbreak were also tested.

Results

Of the 455 copies of the questionnaire distributed, a total of 317 (at a return rate of 69.7%) were collected, 86 from the NTUST (91.5%), 47 from the YMU (68.1%), 139 from the MCU (57.9%), and 45 from the MCU and HFU Sports and Recreation Clubs (86.5%). Students of the NTUST returned the questionnaires more and faster. They also had a higher rectal

swab collection rate of 75.5% (71/94). Analysis was, therefore, made primarily on the returned questionnaires of the NTUST students. Other questionnaires were used at times for comparison.

Of the 86 NTUST students interviewed, 31 were males (36.0%) and 55 females (64.0%), aged between 18 and 25, with a median of 19 years. Of them, 34 met the definition of case, giving an attack rate of 39.5% (34/86). Of the 34 cases, 17 were males (50.0%) and 17 females (50.0%), aged between 18 and 25, with a median of 20 years. Their symptoms were, by frequency, diarrhea 88.2%, abdominal pain 70.6%, nausea 52.9%, dizziness 44.1%, vomiting 41.2%, fever 29.4%, and chill 26.5%. Among the 30 diarrhea cases, 83.3% had watery stool and 16.7% had mucous stool. No bloody feces were reported. Of them, 24 had been medically cared, giving a medication rate of 70.6% (24/34). They recovered within 1-6 days, with a median of 3 days.

The meals or the food items that was most likely associated with the incident should be first identified before to calculate the incubation period of the food poisoning outbreak. The results of food intake analysis indicated that 84 students had dinner on the evening of September 30. The food items they ate are shown in Table 2. The odds ratios and their 95% confidence limits related to each eaten food item were, respectively, 2.25 (0.71-7.89) for curried chicken, 1.38 (0.42-5.06) for sweet and sour fish, 1.55 (0.50-4.92) for fried egg with onion, 0.91 (0.30-2.76) for fried water convolvulus, 0.88 (0.32-2.42) for fried cabbage, 0.77 (0.28-2.11) for the bamboo shoots and chicken soup, 1.78 (0.65-4.89) for shredded pork and dry beancurd, 1.30 (0.48-3.56) for fried mustard, 0.99 (0.33-2.94) for white gourd and pork rib soup, and 2.72 (0.25-138.24) for steamed rice. Since every 95% confidence limits covered 1.0, all dinner dishes on September 30

were not statistically related to the food poisoning incident.

Table 3 shows the results of analyzing food items of the breakfast eaten by 80 students on October 1. The odds ratio of each eaten food item and it 95% confidence limits are: 0.69 (0.05-10.02) for gruel, 1.27 (0.39-4.20) for steamed bread, 1.22 (0.35-4.59) for fried pork flakes, 0.88 (0.26-3.05) for peanuts and gluten puff, 0.84 (0.26-2.76) for preserved beancurd, and 0.79 (0.26-2.38) for pickles. None of them was statistically associated with the food poisoning incident.

A total of 83 students had barbecue for lunch on October 1. The results of analyzing food items they ate shown in Table 4. The odds ratios and their 95% confidence limits are, respectively, 2.15 (0.16-116.35) for salted pork, 0.54 (0.20-1.44) for corns, 1.11 (0.37-3.32) for tempura, 2.24 (0.77-6.69) for sausage, 0.89 (0.32-2.47) for dry beancurd, 0.87 (0.25-2.99) for toast, 0.75 (0.28-1.98) for pork blood square, 5.19 (1.02-50.33) for lemon tea, 0.46 (0.16-1.28) for mushroom, and 0.52 (0.18-1.49) for assorted vegetable soup. Of them, the lemon tea seemed to have a high and statistically significant odds ratio. By subtracting the onset time of symptoms from the time of drinking the lemon tea at lunch, the incubation periods of the 32 cases range from 7 to 68 hours, averaging 38 hours, with a standard deviation of 15 hours (Figure 1).

For the 71 rectal swabs of the NTUST students, *Staphylococcus aureus* was isolated in three. Two of them had identified with *Staphylococcus aureus* type B enterotoxin and one had type D enterotoxin. No pathogenic agents had been isolated from the two hand swabs of the kitchen staff. Of the environmental specimens, *Bacillus cereus* was isolated from the kitchen knives. No pathogenic agents had been isolated from either the chopping boards or water specimens.

Inspection of the environment found that the campsite is in a remote

mountain area for either camping or lodging. There is no public water supply. Water for cooking, drinking, washing, bathing and toilet use comes from the spring water. In the kitchen, cooking articles and utensils are randomly discarded. The kitchen staff is temporarily employed pending on business conditions. No aprons, masks and caps were worn at the time of cooking.

Discussion

The present incident had the following special features: 1) longer incubation period, ranging from 7 to 68 hours, averaging 38 hours; 2) longer period of illness lasting 1-6 days, with a median of 3 days; 3) younger victims of 18 to 25 years, averaging 19 years; and 4) non-invasive gastroenteritis of primarily watery diarrhea. Pathogenic agents commonly isolated in rectal swabs of food poisoning cases such as *Bacillus cereus*, *Vibrio parahaemolyticus, Salmonella, Bacillus dysenteriae, Vibrio cholerae*, and *Bacillus typhi* were all negative. Incubation periods of these pathogenic agents, their symptoms and epidemiological characteristics would not correspond with the features of the present incident⁽⁴⁾. Therefore, these pathogenic agents were not related to the food poisoning outbreak.

In three of the 71 rectal swabs collected from students of the NTUST, *Staphylococcus aureus* was isolated. Its enterotoxins are either type B or type D. The incubation periods of the three cases ranged from 41 to 52 hours, averaging 47 hours. Their symptoms were abdominal pain (3/3), nausea (3/3), diarrhea (2/3), vomiting (1/3) and dizziness (1/3). These features did not correspond with the special features of *Staphylococcus aureus* infection: vomiting and incubation period being 30 minutes to 8 hours (usually 2-4 hours)^(4,5). In one of the 24 rectal swabs collected from

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students of the YMU, *Staphylococcus aureus* was isolated, the enterotoxin being type A. *Bacillus cereus* was also isolated from kitchen knives, indicating that the sanitary conditions of the kitchen were relatively poor. Generally speaking, the primary symptom of *Bacillus cereus* infection with an incubation period ranging from 1 to 6 hours was vomiting. The primary symptom of *Bacillus cereus* infection with an incubation period of 6 to 24 hours was watery diarrhea^(4,5). Again, these special features did not correspond with the special features of the present food poisoning incident, the likelihood of *Staphylococcus aureus* and *Bacillus cereus* involvement in the present incident was ruled out.

Since no sufficient laboratory testing evidence was available, we then used epidemiological findings to explore possible pathogenic agents, such as Norwalk virus and enterotoxigenic E. coli (ETEC), related to the present food poisoning incident. Norwalk virus infection has an incubation period of 24-48 hours, and onset of infection is often within 60 hours⁽⁶⁾. The ETEC infection has an incubation period of 21-68 hours, and onset of infection is often beyond 72 hours⁽⁷⁾. Symptoms of the Norwalk virus infection are, by frequency, nausea 51-100% (with a median of 79%), vomiting 25-100% (69%), diarrhea 21-100% (66%), abdominal pain 17-90% (71%), and fever 13-71% (37%). In children and young adults, vomiting and diarrhea are more often $(^{(7,8)})$. Symptoms of the ETEC infection are, by frequency, diarrhea 83-100% (with a median of 99%), abdominal pain 37-100% (82%), nausea 33-100% (48%), vomiting 2-80% (14%), and fever 5-50% (22%). Diarrhea occurs more often than vomiting, the ratio being 1.3 to 45.5 (with a median of 7.1)⁽⁷⁾. Above findings seem to suggest that the ETEC was likely to be the pathogenic agent of the present food poisoning episode.

In the Taiwan Area, there had been 36 outbreaks of pathogenic E. coli

infection in the period between 1986 and 1995, the fourth food-borne infectious disease next in frequency to the Vibrio parahaemolvticus, Staphylococcus aureus, and Bacillus cereus infections. In all infected cases, the ETEC was isolated more often⁽⁹⁾. ETEC is a common cause of diarrhea in developing countries and among tourists. The infection is caused primarily by the intake of food or water contaminated by feces. In volunteers, infection occurs after the intake of 100 million or more microorganisms⁽¹⁰⁾. Reports are that ETEC outbreaks can occur by contaminated water sources⁽¹¹⁾, sick kitchen staff⁽¹²⁾, and contaminated agricultural produces $^{(13)}$.

By the epidemiological curve of incubation periods projected from the NTUST students, there was a peak in the range of 41 to 44 hours after the suspected lunch meal on October 1. It indicates that the present food poisoning outbreak had a common source of infection. Comparing the meals taken by students of different universities on September 30 and October 1 (Table 1), a common factor of barbecue was noted. Also, all students at the barbecue had the lemon tea. Analysis of food and beverage items taken by the NTUST students at noon of October 1 also showed that the lemon tea was significantly associated with the food poisoning outbreak (odds ratio is 5.19 with a 95% confidence limits of 1.02-50.33). The lemon tea could have been the cause of the food poisoning outbreak, and students could have become sick by taking it at the barbecue.

The owner of the campsite said that the lemon tea was prepared with boiled water and lemon tea powder and then mixed with cold water. For a long time period, the campsite used the spring water from the near mountain area for cooking, drinking, washing, bathing and toilet use. The kitchen was disordered, Bacillus cereus was isolated from kitchen utensils. and no

facilities were there to boil water. All these evidence indicated that, under the busy and confusing conditions of September 30 and October 1, spring water was definitely not boiled and used directly to prepare lemon tea for four to five hundred people. In the recent few years, the construction of a highway between Taipei and Ilan counties destructed the environment around the Hohuan campsite. Along with the large number of incoming workers, living quarters and toilet facilities have been built to accommodate them. These facilities and human activities may affect the sanitary conditions of water sources. Whether the water used for the lemon tea was contaminated by the ETEC in this way remains to be studied further.

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Table 1. Food Items Eaten by Students in Each Meal from September 30to October 1, 2000.

Meals Group	9/30Lunch	9/30Dinner	10/1Breakfast	10/1Lunch
NTUST	None	Prepared ^a	Prepared ^b	Barbecue ^c
YMU	Pork noodle	Self-cooked ^d	Prepared ^b	Barbecue ^c
MCU	None	Barbecue ^e	Prepared ^f	Prepared ^g
Clubs HFU,MCU	Pork noodle	Barbecue ^c	Prepared ^f	Self-cooked ^h

prepared: meals prepared by the kitchen of Hohuan campsite; self-cooked and barbecue: meals prepared by the students.

- a : curried chicken, sweet and sour fish, fried egg and onion, fried water convolvulus, fried cabbage, bamboo shoots and chicken soup, shredded pork and dry beancurd, fried mustard, white gourd and pork rib soup, and steamed rice.
- b: gruel, steamed bread, fried pork flakes, peanut and gluten puff, preserved beancurd, and pickles.
- c: salted pork, corn, tempura, sausage, dry beancurd, toast, pork blood square, <u>lemon tea</u>, mushroom, assorted vegetable soup.
- d: potato, squid, fish, chicken, water convolvulus, celery, assorted vegetable soup, and steamed rice.
- e: sorted pork, chicken wing, chicken drumstick, sausage, pork blood square, mushroom, dry beancurd, <u>lemon tea</u>, corn, green pepper, tempura, assorted vegetable soup.
- f: gruel, steamed bread, fried meat flakes, peanut and gluten puff, preserved beancurd, pickles, peanuts, fried cabbage, sweet and dry beancurd.
- g: curried pork, fried fish, fried egg and onion, fried water convolvulus, fried cabbage, shredded pork and Szechuan pickles, white gourd with chicken claw soup, <u>lemon tea</u>, and steamed rice.
- h: tomato, egg, beancurd, dry beancurd, fish, water convolvulus, assorted vegetable soup, <u>lemon tea</u>, and steamed rice.

	Са	ases		No	on-		Odds Ratio
		I		Cases			
Food Items	E*	NE*	Exposure	Е	NE	Exposure	(95%CL)
		(2)	Odds	(4)	(5)	Odds	(7)=(3)/(6)
	(1)		(3)=(1)/(2)			(6)=(4)/(5)	
Curried chicken	27	6	4.50	34	17	2.00	2.25(0.71~7.89)
Sweet and sour fish	27	6	4.50	39	12	3.25	1.38(0.42~5.06)
Fried egg and onion	26	7	3.71	36	15	2.40	1.55(0.50~4.92)
Fried water convolvulus	24	9	2.67	38	13	2.92	0.91(0.30~2.76)
Fried Cabbage	21	12	1.75	34	17	2.00	0.88(0.32~2.42)
Bamboo shoots and	20	13	1.54	34	17	2.00	0.77(0.28~2.11)
chicken soup							
Shredded pork and dry	22	11	2.00	27	24	1.13	1.78(0.65~4.89)
beancurd							
fried mustard	13	20	0.65	17	34	0.50	1.30(0.48~3.56)
White gourd and pork	9	24	0.38	14	37	0.38	0.99(0.33~2.94)
rib soup							
Steamed rice	32	1	32.00	47	4	11.75	2.72(0.25~138.24)

Table 2. The Results of Analyzing Food Items Eaten by NTUSTStudents for Dinner on September 30, 2000.

E* : Eaten

 $\ensuremath{\mathsf{NE}^*}$: Not Eaten

	Cases			Non			Odds Ratio
				Cases			
Food Item	Е*	NE*	Exposure	Е	NE	Exposure	(95%CL)
			Odds			Odds	(7)=(3)/(6)
	(1)	(2)	(3)=(1)/(2)	(4)	(5)	(6)=(4)/(5)	
Gruel	31	2	15.50	45	2	22.50	0.69(0.05~10.02)
Steamed bread	26	7	3.71	35	12	2.92	1.27(0.39~4.20)
Fried meat flakes	27	6	4.50	37	10	3.70	1.22(0.35~4.59)
Peanut and gluten	26	7	3.71	38	9	4.22	0.88(0.26~3.05)
puff							
Preserved beancurd	25	8	3.13	37	10	3.70	0.84(0.26~2.76)
Pickles	23	10	2.30	35	12	2.92	0.79(0.26~2.38)

Table 3. The Results of Analyzing Food Items Eaten by NTUST Students for Breakfast on October 1, 2000

	Ca	ses		No	on-		Odds Ratio
				Ca	ses		
Food Item	E*	NE	Exposure	Е	NE	Exposure	(95%CL)
			Odds	(4)	(5)	Odds	(7)=(3)/(6)
	(1)	(2)	(3)=(1)/(2)			(6)=(4)/(5)	
Salted pork	33	1	33.00	46	3	15.33	2.15(0.16~116.35)
Corn	15	19	0.79	29	20	1.45	0.54(0.20~1.44)
Tempura	25	9	2.78	35	14	2.50	1.11(0.37~3.32)
Sausage	26	8	3.25	29	20	1.45	2.24(0.77~6.69)
Dried beancurd	22	12	1.83	33	16	2.06	0.89(0.32~2.47)
Toast	27	7	3.86	40	9	4.44	0.87(0.25~2.99)
Pork blood square	17	17	1.00	28	21	1.33	0.75(0.28~1.98)
Lemon Tea [*]	32	2	16.00	37	12	3.08	5.19(1.02~50.33)
Mushroom	19	15	1.27	36	13	2.77	0.46(0.16~1.28)
Assorted vegetables soup	9	25	0.36	20	29	0.69	0.52 (0.18~1.49)

Table 4.	The Results of Analyzing Food Items Eaten by NTUST
	Students for Lunch on October 1, 2000

*Statistically significant < 0.05 °

