

Investigation of a Food Poisoning Outbreak at a Welcoming Camp for Freshmen of University

Introduction

A welcoming camp was held at the Fairy World in Kuanhsi of Hsinchu County on 18-19 October 1997 for 180 freshmen of the day class of the Business Administration Department of a university in Taoyuan. Upon arrival at noon, each was given a picnic lunch containing one piece of chocolate-covered bread, one piece of onion-flavored bread and a bottle of mineral water (staff members had juice instead). The picnic lunch was bought from Bakery A near the university. Dinner of five dishes, soup and rice was served at the Fairy World restaurant. The snack of sandwiches (in three kinds: dried meat floss, chopped meat and cheese) served at 10:00 pm was bought from Store B near the university. At around 2 am, some students began to complain of symptoms of acute entero-gastritis such as abdominal pain, diarrhea, fever and chill and were medically cared for. As the number of students infected was large, and the local health bureau was not certain as to which meal or what food items induced the incident, the assistance of the FETP (Field Epidemiology Training Program) of the National Institute of Preventive Medicine, the Department of Health, was sought for. The FETP team began the investigation on 21 October.

Materials and Methods

1. Interview of Cases at Hospital

Students either kept at the emergency for observation or admitted for care at the Army 804 Hospital were visited. As the number admitted for hospital care was not many, a structured questionnaire was used for direct interview as to the onset of the incident, symptoms and food items consumed. The purpose was to understand the onset of the illness and the care process and also to pretest the usefulness of the questionnaire. A total of 18 patients was visited; of them, two declined the interview for tiredness.

According to the doctor, the major symptoms of these students were chill, high fever (38.0 °C and above), watery diarrhea (at least 7-8 times a day), abdominal pain (some were in deep abdominal pain though without diarrhea) and weakness. After treatment with anti-biotics (Ampicillin and Gentramycin) and fluid supplement, fever dropped though could go up again. After 1-2 days, fever of some dropped, though some still maintained it at 37.5 °C. Diarrhea and abdominal pain continued. All hospitalized students were put on fasting, tested for stools and blood, and also tested by Widal test and for serum antibodies of rotavirus.

2. Questionnaire Interviewing

The retrospective cohort study method was used. All students of the day class of the Business Administration Department of the university who had been to the camp were interviewed with a structured questionnaire as to their background information, whether being to the camp, time of onset and symptoms, food items consumed at lunch, dinner and snack, and medical care if any. About 100 students of the evening class of the same department had also been to the Fairy World for their own welcoming party. According to the teacher, they took care of their own lunch, had the same dinner (cod, stewed pork, chicken, pickled Chinese cabbage soup, squid cooked with chives, cabbage and rice) with the day class students at the same restaurant, and had for snack sweet bean paste bun and green bean soup prepared by the restaurant. Other than some of the students who got car-sickness and cold in the morning from heavy card-playing the night before, none had any problem. The food eaten at dinner was then initially rejected as the causal agents of the incident. The bread for lunch or the sandwiches for snack were suspected to be associated with the food poisoning.

The definition of a case was: one who had attended the welcoming camp on 18 October, had had the same lunch and snack, and developed diarrhea (more than twice a day) or fever 38 °C and above, and one of the following symptoms, nausea, vomiting, abdominal pain, weakness, chill, dizziness, bloody stool, rash and others.

Data Processing and Analysis

Data were stored with Epi-Info v6.02 software. The accuracy of each variable was verified. The incubation period was drawn with Excel 5.0 of the Microsoft Windows 3.1. Information was treated with Epi-Info χ^2 -test was used for analysis of the relationship between food poisoning and food items consumed by meal. When the data failed to meet the hypothetical conditions of χ^2 -test (when an expected value was lower than 5), the Fisher's Exact Test was used. Risk difference was used to indicate the difference in the onset of food poisoning between those who had and who had not consumed a certain food item. The 95%

confidence intervals of risk difference were calculated from: $RD \pm \frac{1.96}{\sqrt{x^2}}$.

3. Laboratory Testings for Human and Food Remains Specimens

Human Specimens

Ten rectal swabs of students admitted to the Army 804 Hospital, rectal and hand swabs of the two cooks at the Sandwich Store B, and rectal swabs of the four cooks at Bakery A were collected by the Taoyuan County Health Bureau and sent to the Bacteriology Division of the National Institute of Preventive Medicine, the Department of Health, for laboratory testings for: *Staphylococcus aureus* (including enterotoxin), *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella*, *Salmonella typhi* and *paratyphi*, and shigella.

Food Remains Specimens

There was only the leftover of the sandwiches. The specimens were sent to the National Institute of Preventive Medicine for laboratory testings. As there was no more leftover of the lunch, some bread specimens were collected at random from Bakery A for laboratory testings at the National Institute of Preventive Medicine. Specimens of materials for the sandwiches such as frozen cheese and ham, dried pork floss and frozen chopped pork were also collected from Store B for laboratory testings. They were tested for: *Staphylococcus aureus* (including enterotoxin), *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella*, Pathogenic *Escherichia coli*, and shigelkL.

4. Site Observation of Manufacturing Process

Making of sandwiches at Store B: Work began at around 3:00 pm of the day to prepare sandwiches by husband and wife of the Store. The sandwiches came in three kinds: dried pork floss, cheese and chopped pork. Each also contained ham and egg. Hams and eggs were fried first by the husband, and sandwiches were then made by the wife in the following order: dried meat, cheese and chopped pork. The chopped pork was the hamburger meat supplied by the Hanlin Food. They were taken out for defrosting at 1:00 pm and fried at around 4:00 pm. The work was completed at around 6:00 pm, and the sandwiches contained in carton boxes were collected and brought to the Fairy World for consumption at 10:00 pm.

Store B is in an alley near the rear gate of the university. The Store was dusty for being close to the road. Flies were everywhere. Though the temperature of the freezers met the requirements (below 6 °C), the open kitchen tables could have been easily contaminated by flies. The same worker prepared and handled food and collected payments. He was not in the habit of washing hands. The food could have been contaminated.

The Bakery A: Bakery A is located near the front gate of the university. The store was clean, though the two workshops were dirty with the floors slippery and the walls covered with dirt. The stainless table of the first workshop was covered with bread crumbs; and the baking plates with bread crumbs and chocolate crumbs. The manager said that the baking plates should not be washed with water but scraped off and oiled, they had therefore never been washed. They were piled up. In the second workshop, there were mixers for cream, eggs and dough, a water faucet, a washing basin and a toilet. Flies were everywhere. A mousetrap was placed outside the toilet. The sewer was not covered; the toilet was without door. The knob of the flush toilet was broken, the water tank was

not covered, and the toilet could only be flushed manually by putting the hand in the water tank.

Eggs for bread and shells of used eggs were put together in boxes. Chocolate was placed on the bread after the bread was baked. Bread was baked at above 200°C. The bread for the occasion was baked at 5:00 pm the day before. No storing facilities for bread were noted.

The water bills of the Bakery were a mere NT\$500 a month. Though the manager insisted that no underground water was used, and there was only one water faucet, it was likely that underground water was in fact used. It also indicated that cooks were not in the habit of washing hands frequently. The manager should be supervised to improve the sanitation of the workshops.

Results

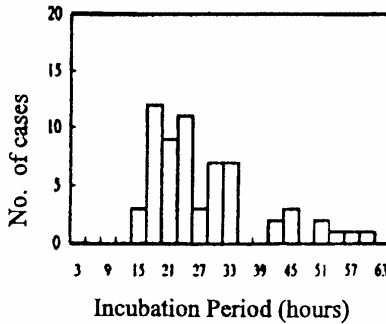
1. The Questionnaire Survey

Of the 116 copies of the questionnaire distributed, 115 had been returned, giving a return rate of 99.1%. They were: 59 students of Class A (5 1.3%), 51 students of Class B (44.3%), 2 teaching assistants (1.7%), 3 second- and third- year students (2.6%). 42 of them were male (36.5%), and 73 female (63.5%).

Of them, 63 met the definition of a case, giving an attack rate of 58.3%. Their symptoms were: diarrhea (more than twice a day) 84.1% (53/63), fever 81.0% (51/63), abdominal pain 81.0% (51/63), weakness 60.3% (38/63), chill 39.7% (25/63), nausea 14.3% (9/63), vomiting 15.9% (10/63), dizziness 63.5% (40/63), others 6.3% (4/63), bloody stool 0% (0/63), rash 1.6% (1/63), sore throat 9.5% (6/63). Those with sore throat were found to have caught cold before departure.

The outbreak was suspected to be the result of either the lunch or the snack being contaminated. If it was the lunch, the incubation periods (one did not fill out) ranged from 14 to 59 hours, with a mean of 29 hours, a median of 25 hours and a mode of 18 hours. If it was the snack, the incubation periods ranged from 4 to 49 hours, with a mean of 17 hours, a median of 14 hours and a mode of 8 hours. The epidemiologic curve based on the lunch is shown in Figure 1.

Figure 1. Epidemiologic Curve of the Outbreak (n=62)



In the univariate analysis, both the lunch and the snack were found to be significantly associated with the food poisoning ($p < 0.05$). In a group activity such as this, most students would have had both meals. Through cross examination, it was found, however, that the outcomes of those students who had lunch only (did not have the snack) and those who had snack only were not significantly related to the food poisoning. The risk difference of 66% between the attack rate of those who had had both meals (65.6%) and those who did not have any (0%) was statistically significant (Table 1).

Table 1. Interaction of Lunch and Snack on Attack Rate

Lunch	Snack	Ill	Not Ill	Attack Rate (%)	Risk Difference	95% CI
No	No	0	4	0	0	
Yes	No	1	3	25	0.25	-0.174-0.674
No	Yes	1	6	14.3	0.143	-0.116-0.402
Yes	Yes	61	32	65.6	0.656*	0.559-0.753

* $p < 0.05$

Further analysis by food items showed that only the onion-flavored bread for lunch was significantly related to the food poisoning. The risk difference was 58% (95% confidence intervals at 41.3%-74.9%) (Tables 2 and 3).

Table 2. Two Kinds of Bread for Lunch and Food Poisoning

	Ill		Not Ill		Risk Difference	95% CI
	Eaten	Not Eaten	Eaten	Not Eaten		
Chocolate-	53	10	32	11	0.15	-0.09-0.38
Onion-	58	5	19	24	0.58*	0.41-0.75

* $p < 0.05$

Table 3. Three Kinds of Sandwiches for Snack and Food Poisoning

Sandwiches	Ill		Not Ill		Risk Difference	95% CI
	Eaten	Not Eaten	Eaten	Not Eaten		
All flavor	59	4	38	7	0.25	-0.06-0.55
Cheese	16	31	18	23	0.18	-0.25-0.46
Floss	15	32	9	32	0.13	-0.10-0.35
Pork	20	27	12	29	0.14	-0.07-0.36

2 Findings of Laboratory Testings

Human Specimens

Salmonella enteritidis was isolated from the ten rectal swabs of patients. Rectal swabs of four cooks of Bakery A and rectal swabs and hand swabs of two cooks (husband and wife) of Store B were all negative.

Food Specimens

Only Bacillus cereus was isolated from the leftover of the sandwiches for snack, the number of colonies was 1.0×10^6 CFU/g. Materials randomly selected from Store B, including hams, cheese and dried pork floss, were all negative. From the frozen chopped pork, however, pathogenic Escherichia coli of serotype O1 l2ac was isolated, the number of colonies being 1.5×10^2 MPN/g. The specimens were also positive of Salmonella enteritidis (serotype O4).

The bread collected from Bakery A was not the leftover. The bread collected the next day was positive of Salmonella enteritidis (serotype O9, same as the human specimens collected from patients). No further confirmation was made.

3. Worry of Community Outbreak

From the chopped pork for sandwiches of Store B, pathogenic E. coli and Salmonella enteritidis were isolated. The bread specimens collected from Bakery A though were bread prepared two days after the incident, Salmonella enteritidis of the same serotype was isolated. The two shops were still in business. The worry that the community people could have also been infected made the team to visit hospitals and clinics around the university for any suspected cases. Visits were made to the New Citizen's General Hospital and the Huayang Hospital for names and telephone numbers of any patients of acute gastro-enteritis. They were telephone-interviewed. It was found that with the exception of two students of the university who had been to the camp and had been initially treated at these hospitals and two more students (not of the Business Administration Department) who had developed acute gastro-enteritis from eating bread of Bakery A, no other individuals had developed diarrhea from eating either bread or sandwiches from these two shops. The worry of these two shops inducing a community-wide food poisoning outbreak was eliminated. These two shops seem to cater more for the students of the university.

Discussion and Conclusion

Salmonella, by its somatic antigen O and flagella antigen H, can be classified into 2,000 some serotypes. Of them, *S. typhimurium*, *S. choleraesuis* and *S. enteritidis* more often induce food poisoning incidents⁽¹⁾. In Taiwan, though *Salmonella*-induced food poisoning incidents have been relatively few, they have been increasing, from 3 in 1991 to 9 in 1996^(2,3). In the last ten years, the incidence of salmonellosis in man has shown a significant trend of increase in many countries, developing or developed⁴. In the United States for instance, salmonellosis infections caused by *S. enteritidis* had increased from 9.9% in 1976 to 26.1% in 1994. *S. enteritidis* is now the most common serotype of salmonellosis infections in the US^(5,6).

S. enteritidis has a wide distribution. They can be found in soil, snow, insects, marine products, poultry, food, and intestinal tracts of healthy men. It can be isolated in 64.5% of slaughtered sick pigs; and in 0.5-3% of eggs of poultry. Experiments show that *S. enteritidis* can stay through the egg, maggot and pupa stages of flies to survive lifelong in adult flies. *S. enteritidis* can survive under difficult conditions. It grows better under 20-37°C. and hence more infections occur in summertime. It can survive in milk and meat products for weeks and months; in human stools for 1-2 months; and even longer in chicken and duck eggs and egg powder. It is heat-tolerant, can only be killed by boiling for 5 minutes under 70°C or 15-30 minutes under 60°C^(7,8).

S. enteritidis multiplies in vast number in contaminated food. Through the intake of contaminated food, *S. enteritidis* enters human body, multiplies quickly in the intestines, stays on the intestinal mucosa to cause inflammation (edema, hyperemia and hemorrhage). It then enters the lymphatic system and blood circulation to cause general bacteriemia to release endotoxin in large quantity to induce poisoning. Clinical symptoms of patients of gastro-enteritis are initially nausea, dizziness, headache, weakness, loss and even lack of appetite, paleness, cold sweat and then, vomiting, diarrhea (watery or gruel-like stool), abdominal distension, abdominal pain, chill, shivering, fever (38-39°C), increase in white blood cells, lowering of blood pressure, depression and somnolence. The incubation periods range from 2-8 hours to 48-72 hours, more often between 12-24 hours. The duration of infection depends on the amount of pathogenic agents absorbed, the physical conditions of the patients and the seriousness of the infection. Under normal circumstances, patient recovers in 3-5 days, even 1-2 days, though may last for three weeks in serious cases. Prognosis generally is good^(7,8).

The major source of *S. enteritidis* poisoning is the contaminated animal food in which *S. enteritidis* multiplies in large quantity. When the food is not heat-sterilized or well-cooked, or re-contaminated after heat treatment and then

placed under optimum conditions for long, *S. enteritidis* will multiply in large quantity again. Food not heat-treated before eating may also cause poisoning^(9,10).

A food poisoning is defined by the Department of Health as: by epidemiologic definition and definition adopted by the US CDC, a food poisoning is one that two or more persons take the same food and develop similar symptoms; and further that from the suspected food remains specimens, and human specimens such as stools, vomitus and blood of patients, or other relevant environmental specimens (such as air, water and soil), pathogenic agents of the same type (serotype, phagotype) are isolated⁽¹¹⁾. The information collected and analyzed of this outbreak that the clinical expressions of gastro-enteritis symptoms such as vomiting, diarrhea, abdominal pain, chill and shivering with high fever, and the incubation period of 14 to 59 hours based on the time of the intake of lunch, with a mode of 18 hours, corresponded to the characteristics of *S. enteritidis* food poisoning. Bacteriological testings also showed that *S. enteritidis* was isolated from the rectal swabs of student patients; *S. enteritidis* of the same serotype, 09, was isolated from the bread of Bakery A; and *S. enteritidis* of the same serotype was also isolated from suspected food, and vomitus or stools of patients. It was, therefore, decided that the outbreak was caused by *S. enteritidis*.

The incubation periods of *S. enteritidis* infections range from 2-8 hours to 48-72 hours, more around 12-24 hours. The incubation periods of the present outbreak, either calculated from the lunch or the snack, corresponded to the incubation period of *S. enteritidis* infection. Although *S. enteritidis* of the same type was isolated from human specimens and the food specimens collected the day after, the epidemiologic curve showed that there were two peaks. The incubation period of the one person who had the lunch but not the snack was 24 hours; that of the one person who had the snack but not the lunch was 56 hours. From the materials for the sandwiches, *S. enteritidis* of serotype 09 and pathogenic *E. coli* were also isolated. Statistical analysis also indicated that the risk of infection was significantly higher for those who had both meals. It was, therefore, reasoned that both the lunch and the snack were responsible for the present food poisoning outbreak.

From the sandwich specimens, *Bacillus cereus* was isolated. *B. cereus* can survive in many cooked food items such as meat, soy sauce, pudding, rice, potato and vegetables. They can still grow in cooked food not kept above 60°C or cold-stored under 4°C and cause food poisoning. However, *B. cereus* was not isolated from the rectal swabs of student patients. The leftovers of the sandwiches were not cold-stored. They were already decayed when the specimens were collected. The *B. cereus* isolated from the sandwiches was the result of the sandwiches being left too long.

The store area of Bakery A is rented. The Bakery is operated at a low budget. The workshops have never been cleaned, the baking plates never washed, the floor is slippery, and walls covered with dirt. Flies are everywhere. Employees are not in the habit of washing hands regularly. The mousetrap suggests that there are rats.

On a day, 270 pieces of bread are baked by the evening, 60 of them will be left over. Though the manufacturing process is not considered sanitary, baking under 200 °C for 8-10 minutes should kill any organisms. However, when the bread are placed in the shop for sale, they could be contaminated by Salmonella through flies and others. The 60 leftovers of the day are wrapped in plastic bags. When the shop is closed and the air-conditioners turned off, the room temperature in summertime should be ideal for the rapid multiplication of Salmonella. The bread that the students had for lunch must have been cross-contaminated by the hands of the cooks or flies and rats.

Sandwiches of Store B are prepared at 3:00 pm. Husband and wife prepare sandwiches and at the same time attend to customers. They don't wash hands after they have collected payments. The shop is next to the street. Working tables and ovens are placed outside the shop. The sandwiches already prepared can be easily contaminated by the dust caused by cars and motorcycles, the cooking oil and the flies. About 140 sandwiches are prepared in two hours and packed while still warm. In the process between 6 and 10:00 pm, any organism can grow in vast number.

Recommendations

For the prevention of Salmonella-induced food poisoning, the following are suggested:

1. Food firms should be licensed. They should be supervised of sanitation by health bureaus. The two shops are not licensed.
2. The sanitary conditions should be improved. Utensil and cooking facilities should be kept clean. Flies and rats should be eliminated. Workshops should be cleaned regularly. Baking plates should be washed. Through telephone interview, the Bakery has painted the shop and improved its sanitation. The Health Bureau has been told to continue the supervision.
3. From the onion-flavored bread prepared by the Bakery that supplied the lunch, Salmonella of serotype 09 was isolated. The shop should be further spot-checked for several times to see if it was an accident or there are sources of infection. The shop should be told to prepare just enough number of bread for the day. The leftovers should not be sold again but be cold-stored or discarded to avoid any further infection.
4. The Hanlin Food should be told by the Health Bureau that Salmonella of serotype 04 was isolated from the hamburger meat supplied by them. The source of meat should be checked and the sterilization of meat strengthened.
5. Food handlers should receive regular health examination to make sure they do not carry Salmonella.
6. In the case of food poisoning, the stores in question should be penalized according to law, and the employees given lectures in food sanitation to improve

their knowledge and to protect the rights of consumers.

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