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Investigation of A *Vibrio parahaemolyticus* Poisoning at An Elementary School in Yuanli Township, Miaoli County

Abstract

At 10 PM of 11 December 1997, 77 of the 144 students of the Yuanli elementary school in Yuanli township, Miaoli county, started to develop symptoms such as: watery diarrhea (42.4%), abdominal pain (42.4%), vomiting (35.4%), headache/dizziness (22.2%), chilliness (16.7%), nausea (12.5%), and fever (6.3%). About 50 of them visited the Lee's and Chiu's General Hospital of the township for treatment. Of them, 11 were hospitalized for care. All these students ate the lunch ordered by the school. The purpose of the investigation was to find out the causes and pathogenic agents of the poisoning.

The investigators applied a questionnaire to collect the following information: the demographic characteristics of the cases, food items eaten on 11 December, symptoms and signs, time of onset, medical care, and duration taken for recovery. By analyzing previously collected information, the results indicated that the incubation period ranged from 2-40 hours, with a median and a mode of 15 hours respectively; and the cases took approximately three days to recover. These epidemiological findings and the clinical symptoms led to the conclusion of food poisoning possibility induced by *Vibrio parahaemolyticus*. In addition, the National Institute of Preventive Medicine had isolated 13 cases of *Vibrio parahaernolyticus* type K6 from 15 rectal swabs. *Vibrio parahaernolyticus* was also isolated from specimens collected from the hospitalized students by the Lee's General Hospital laboratory and from the residue specimens of the fried pork chop by the National Laboratories of Foods and Drugs. It was therefore concluded that the pathogenic agent of the food poisoning was *Vibrio parahaernolyticus*.

Statistical analysis showed that the fried pork chop was significantly related to the incident ($p<0.0l$) with a relative risk of 2.08 (95% confidence interval, 1.34~3.23). Since *Vibrio parahaernolyticus* was isolated from the residue specimen of the fried pork chop, it was considered as the food item responsible for the poisoning.

Introduction

On 11 December 1997, tens of students from the Yuanli elementary school in Yuanli township of Miaoli county, after eating the lunch ordered by the school, started to develop symptoms such as stomach upset, abdominal pain, vomiting, diarrhea, and nausea around 10 o'clock of that evening. They were later treated by the Lee's and the Chiu's General Hospitals, some were even hospitalized for care. The occurrence of food poisoning incident in a cold weather day was relatively unusual. An investigation was thus proceeded to determine if the incident was in fact food poisoning, and if so, what are the causes, the food items responsible, and the pathogenic agents of the poisoning. A team from the Field Epidemiology Training Program (FETP) was then dispatched for an epidemiological investigation.

Materials and Methods

The investigation was divided into in several parts: confirming the incident as being food poisoning; visiting the hospitalized students and understanding their medical care process by reviewing their medical records; inspecting the food manufacturing plan; interviewing the students with a questionnaire; and laboratory testing.

1) confirmation of the incident

From the sick leave records of the school, no unusual increase in the number of sick leaves prior to 11 December was noted. The school health diary showed only a few injury cases prior to 11 December, no cases of either diarrhea or common cold were recorded. With respect to the school water supply, both the underground water and the tap water were used. The latter was installed recently. Most of the diarrhea or vomiting students mainly concentrated on the fifth and the sixth grades and not distributed through the whole school. On the incident day, students had consumed different kinds of food for breakfast. Therefore, the water supply and the breakfast were then excluded from the list of likely causes related to the poisoning incident. Consequently, the lunch ordered by the school was suspected as the common source of infection. The incident therefore was recognized a food poisoning outbreak.

2) visiting the hospitalized students

There are two large general hospitals in Miaoli Township, the Lee's and the Chiu's. The former is a district teaching hospital of larger size and better facilities and the latter is a long-time hospital in the local area. A total of 43 students visited the Lee's, and 9 stayed for hospital care. Interview was completed to the 3 remaining students at the Lee's and I at the Chiu's. Their medical records and those of other hospitalized students were also reviewed. Physicians of the Lee's General Hospital said that, in addition to the students,

there had been some adult food poisoning cases seeking for treatment at the hospital. They all had the food from the same source.

3) inspecting the food manufacturer plan

The lunch of the incident day was supplied by a cafeteria. It was located at a two-story apartment house. The first floor serves as the cafeteria, and the second floor is designed for residential use. The cafeteria catered for workers of the nearby factories, students of nine vocational, junior high, and elementary schools in boxed lunch or group feeding, as well as the general public. On 11 December, lunch boxes had been prepared for about 1,130 persons. The Yuanli elementary school had ordered 152 boxes (24 for class A, 24 for class B, and 21 for class C, all of the fifth grade; and 27 for class A, 28 for class B, and 28 for class C, all of the sixth grade). Students of some other classes had also ordered the lunch and none became sick. The number of boxed lunch ordered was not reported. Every morning at 11 o'clock, workers of the cafeteria brought to the school food and steamed rice in bulk. The food was portioned into each class according to the number of students, and then distributed to the students who had ordered the lunch. Disposable paper boxes, cups, spoons, and chopsticks were supplied by the cafeteria.

The manager of the cafeteria was questioned regarding the sources of the raw materials, their storage and processing, supply and transportation of the boxed lunch. The Miaoli County Health Bureau was requested to join the team to - inspect the cafeteria environment, health conditions of the employees, and to collect human, food residue, and environment specimens.

4) questionnaire interview at the school

Cases came primarily from classes A, B, and C of the fifth grade, and classes B, and C of the sixth grade. Students of these two grades were then selected for interviewing with a questionnaire. The questionnaire contained items on personal demographic information, food taken at breakfast and lunch on 11 December, food items eaten at lunch, any uncomfortable feeling, time of onset and symptoms, history of medical care, time needed for recovery. A total of 173 students were interviewed.

5) laboratory testing

The Miaoli County Health Bureau had collected rectal swabs from 15 students and specimens from eight employees' hands of the cafeteria. These specimens were sent to the Central Branch Laboratory of the National Institute of Preventive Medicine for laboratory testing. The testing items included *Vibrio parahaemolyticus*, *Bacillus cereus, Staphylococcus aureus* and *toxigenic types, Salmonella, Vibrio cholerae, Salmonella typhi* and *paratyphi,* and *Bacillus dysenteriae.* Food specimens were also collected from fried pork chop, scrambled egg with dried radish, fried sesame balls, fried fish, steamed rice, stewed pork chop, and pineapple juice kept by the school. They were sent to the

National Laboratories for Foods and Drugs and tested for laboratory testing for: *Staphylococcus aureus and toxigenic types, Salmonella, Bacillus cereus, pathogenic Escherchia coli,* and *Vibrio parahaemolyticus*.

6) data processing and analysis

All collected information was keyed-in with Epi-Info 6.0. Each variable was checked for its accuracy and database was then established. Descriptive and multi-variable analyses of the date were then conducted.

Symptoms and their frequency of the hospitalized cases and questionnaire- interviewed students were taken into consideration in defining as a "case" of the incident. The attack rate and the relative risk for each food item were calculated [1, 2]. In the process of statistical analysis, χ^2 was used to study the relationship between breakfast/lunch and the uncomfortable feeling, and also the relationship between each food item and the poisoning. Those food items with statistical significance were then analyzed by the method of categorical data analysis. Results of statistical analyses and findings of laboratory testing of human specimens and food residues were referred to determine the likely food items and pathogenic agents attributed to the present incident.

The epidemiological figure was prepared with Word ^{R} 7.0 and Excel ^{R} 7.0 software in Microsoft ^{R} Windows 95 environment. SAS^R 6.12 was also used for the descriptive statistical analyses (frequencies of symptoms, percentage, relative risks, etc.) and hypothesis testing.

Results

1) visiting of hospitalized cases

Patients under hospital care at the two hospitals had been bed-ridden for three days. Their white blood cells ranged from 12,000 to 20,000/rnL, with an increase in neutrophil, though not lymph cells, indicating acute bacterial infection. Stools of some patients showed occult blood reaction. Antibiotics such as cefazoline and gentamicin were used for treatment with success. The pathogenic agents of the incident were therefore not viruses such as *rotavirus*.

2) Inspecting the food manufacturing plan

Food items for the lunch of the incident day included fried pork chop, scrambled egg with dried radish, stir-fried cabbage, pork bloods soup, fried sesame balls, steamed rice, and pineapple juice. Teachers had stewed pork chop instead of the fried one, and fried fish in their lunch. With the exception of the scrambled egg with dried radish, which was bought from elsewhere, the cafeteria processed each dish by itself. Raw materials used for cooking above dishes were bought from the Kangchang market and placed under refrigeration or in the freezer. Temperatures of the refrigerator and the freezer used to store the raw materials met the requirement.

For the cooking process, fish and pork were defrosted and prepared one

day before cooking. They were dipped in soy sauce first, added flour and pepper, and deep-fried. Fish, pork and the rest food items were cooked in the following order: pork bloods soup, fried pork chop, fried sesame halls, stir-fried cabbage, and fried fish. All dishes were possessed in a large plate after the processing, then replaced into steel containers according to the class and grade for delivery to the Yuanli elementary school. These dishes was also served in the cafeteria for other customers. Because the school was located near the food-manufacturing plan, the food was delivered the last. The manager admitted that fish fluid could have contaminated other food items such as pork chop.

3) findings of the questionnaire interviewing

All 173 copies of the questionnaire were collected. Of them, 25 students (14.5%) had breakfast only; 13 (7.5%) had only the lunch ordered by the school; and 131 (75.7%) had both. Four students (2.3%) had neither breakfast nor the lunch. Seventy-seven (44.5%) had stomach upset on 11 December. χ^2 test did not indicate any relationship between breakfast and stomach upset ($p>0.05$); though showed a significant relationship between lunch eaten and stomach upset ($p<01$). In the later analyses, only information of the 144 persons (13+131) who ate the lunch was used. Of them, 77 (53.5%) had stomach upset, their symptoms contained watery diarrhea (at least twice a day; 42.4%, 61/144), abdominal pain (42.4%, 61/144), vomiting (35.4%, 5 1/144), headache/dizziness (22.2%, 32/144), chilliness (16.7%, 24/144), nausea (12.5%, 18/144), and fever (6.3%, 9/144).

The distribution of symptoms and signs of the hospitalized cases then defined a case of food poisoning defined as one who had the lunch supplied by the cafeteria on 11 December and had one of the following conditions:

(1) diarrhea and abdominal pain; or
(2) diarrhea and two of the following: vomiting, headache/dizziness, chilliness, nausea, and fever; or
(3) abdominal pain and two of the following: vomiting, headache/dizziness, chilliness, nausea, and fever.

Sixty-one students met the criteria of a case. The attack rate therefore was 42.4% (6 1/144). The number of lunch boxes ordered, number of cases, and attack rate by class is shown in Table 1. The incubation period ranged 2-40 hours, with a median and a mode of 15 hours individually. Its distribution curve is shown in Figure 1.

The results of single food item analysis (Table 2) showed that: fried pork chop, scrambled egg with dried radish, pork bloods soup, and fried sesame balls were each significantly related to the poisoning (p value smaller than either 0.01 or 0.05, respectively). The relative risks of fried pork chop, pork bloods soup, and fried sesame balls reached as high as 2.08, 2.42, and 2.24 respectively. Multiple food items analysis (Table 3) also showed that fried pork chop, pork bloods soup, and fried sesame balls were significantly related to the poisoning ($p < 0.001$, individually).

4) findings of laboratory testing

Vibrio parahaemolyricus type K6 was isolated from 13 of the 15 rectal swabs collected from students. In 1 of the 8 hand specimens collected from employees of the cafeteria, non-toxigenic Staphylococcus aureus was isolated. The Lee's General Hospital laboratory also isolated *Vibrio parahaemolyticus* from specimens collected from the students. Table 4 shows that, with the exception of the pineapple juice, Bacillus cereus had been isolated from all seven specimens of food residues. *Vibrio parahaemolyticus* of unknown type was also isolated from fried pork chop, rice, and stewed pork chop. However, neither the rectal swabs of the employees, nor environmental specimens of the cafeteria had been collected for laboratory testing.

Discussion and Conclusion

The incubation period of *Vibrio parahaemolyticus* infection usually ranged from 12 to 24 hours, though can be as short as four hours, and as long as 30 hours. The infection can last from one to seven days [3]. Diarrhea and abdominal pain were the earlier symptoms [4, 5], and almost all cases appeared these two symptoms. Diarrhea looked watery and occasionally bloody. Some cases even developed other symptoms such as vomiting, nausea, fever, chilliness, and dizziness. Fatality was low. The incubation period of the present poisoning outbreak ranged from 2 to 40 hours. Symptoms were primarily diarrhea, with some patients having complications such as dizziness, vomiting, fever, chilliness. The infection lasted for about 3 days. These epidemiological findings indicated that the food poisoning induced by *Vibrio parahaernolyticus*. Furthermore, *Vibrio parahaemolyticus* was isolated in both human and food specimens. The pathogenic agent of the incident therefore was determined to be *Vibrio parahaemolyticus*.

Statistical analyses showed that fried pork chop, pork bloods soup, and fried sesame balls were all related to the poisoning. No *Vibrio parahaemolyticus*, however, was isolated from the sesame balls, its involvement in the incident was excluded. As no specimen of the pork bloods soup was collected, there was not any laboratory evidence to indicate its involvement in the incident. Generally speaking, contaminated fishery products such as fish, shrimps, crabs, oysters, and their products are more likely to induce *Vibrio parahaemolyticus* food poisoning. No studies have ever reported any relationship between pork bloods soup and *Vibrio parahaemolyticus* food poisoning. As *Vibrio parahemolyticus* was found in the fried pork chop, it was the most likely cause of the incident. The fried pork chop could have been contaminated accidentally at the end of the processing by the water that came from the fish defrosting process. A bulk of the contaminated fried pork chops was then supplied to the students of the fifth and sixth grades and to customers of the cafeteria. This was why only students of the fifth and sixth grades of the Yuanli school and some customers of the cafeteria had developed food poisoning.

The stewed pork chop was fried pork chop dressed with gravy. The amount was

small and it was prepared the last. *Vibrio parahemolyticus* was also isolated from it, as some of the contaminated fried pork chop could have been made stewed. It was probably by cross infection that *Vibrio paralzaemolyticus* was also isolated from the rice. *Bacillus cereus* is commonly found in the environment. Bacterial multiplication under room temperature could have been the reason that *Bacillus cereus* was isolated in all six food specimens (Table 4). Although the amount of Bacillus cereus in scrambled egg and stewed pork chop exceeded the pathogenic amount of 10^5 CFU/g[3], the symptoms, the conditions of the hospitalized cases, laboratory findings, and treatment did not correspond to the characteristics of Bacillus cereus infection[3,10]. No *Bacillus cereus* was isolated in the human specimens. Therefore, *Bacillus cereus* was not considered the pathogenic agent of the incident.

According to the statistics reported by the Department of Health, *Vibrio parahemolyticus* is one of the major pathogenic agents of food poisoning in Taiwan. *Vibrio parahaernolyticus* had always been the first leading cause of food poisonings from 1990 to 1996 in, the Taiwan area, accounting for 49.0% of total (245/497) [11-17]. The number of *Vibrio parahaemolyticus* infection has increased in the recent years. The infection occurs more often between April and November, and sporadically in other months [11-17]. In the US and Japan, the infection occurs more often in the warm months between May and October [4]. An epidemic involving 279 victims occurred in Peru in February (summertime) 1994[18]. Even in countries with cold weather such as Sweden, gastrointestinal enteritis induced by *Vibrio parahemolyticus* also occurs [19]. The present incident occurred on a cold day in early December. In other words, *Vibrio parahaemolyticus* can occur in any season of the year. More precautions should be taken.

Some researchers [20] maintained that only *Vibrio parahaernolyticus* containing hemolysin was pathogenic. In the environment, only 1.0% of *Vibrio parahaemolyticus* that can induce infections of the gastrointestinal system is hemolytic. Substances that can induce hemolysis contain phospholipase A, lysophospholipase, and glycerophosphorylcholine diesterase [21]. These substances destroy the epidermic cells of the small intestines to cause diarrhea [20], and bloody stools in some patients. This was why occult blood reaction was noticed in the stools of some hospitalized patients.

With adequate amount of electrolyte and water, patients of *Vibrio parahaernolyticus* poisoning recover in 2-4 days. In the present incident, antibiotics such as cefazoline and gentamicin were used for treatment with success. Some reports [22] maintained that *Vibrio parahaemolyticus* was resistant to penicillin, but sensitive to choloromycetin, aztreonam, cefotaime, imipenam, tetracycline, ciprofloxacin, trimethoprim/ sulfamethoxazol, and aminoglycosides. There is a range of antibiotics available for doctors to choose from following the conditions of patients.

Recommendations

1. Health bureaus should intensify health education of restaurant owners to avoid

the occurrence of further food poisoning incidents. Food safety should be monitored all year round, regardless of the seasons and the weather.

2. In addition to hand specimens of the food cooking employees, their rectal swabs as well as the environment specimens such as the chopping board, dishcloth, and knives should also be collected for laboratory testing for the purpose of identification of th pathogenic agents.
3. Carelessness is often the major cause of food poisoning. Health authorities should help food manufacturers establish, in addition to improvement in the hardware, standard operational procedures for food processing.
4. Physicians, while using antibiotics for the treatment of patients, should not use antibiotics of higher level without referring prior antibiotic sensitivity testing or relevant literature.

Prepared by: WC Huang[1, 2], DD Jiang[1], SL Chuang[3], KT Chen[1], CB Horng[4]

1. FETP, National Institute of Preventive Medicine, DOH.
2. Department of Family Practice Medicine, the Army Tsuoying Hospital.
3. Miaoli County Health Bureau.
4. National Institute of Preventive Medicine, DOH.

Correspondent: DD Jiang

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Table 1. No. of Lunch Boxes Ordered, No. of Cases, and Attack Rates

Class	No. of Students/Boxes Ordered* (No. of Questionnaire**)	No. of Cases	Attack Rate (%)
5th Grade, Class A	30/24 (24)	9	37 5 (9/ 24)
B	30/24 (22)	2	9.1 (2/ 22)
C	29/24 (22)	12	54.6 (12/ 22)
6th Grade, Class A	33/27 (27)	1	3.7 (1/ 27)
B	30/28 (27)	19	70.4 (19/ 27)
C	32/28 (22)	18	81.8 (18/ 22)
Total	184/155(144)	61	42.4 (61/144)

* the number of students who ate the lunch ordered by the school.
** the number of students who filled out the questionnaire.

Table 2. Attack Rates and Relative Risks by Food Items

Food Items	Eaten			Not Eaten			Relative Risks
	Sick	Not Sick	Attack Rate	Sick	Not Sick	Attack Rate	(95% Confidence
	(1)	(2)	(3)=(1)/(1)+(2)	(4)	(5)	(6)=(4)/(4)+(5)	Interval) (3)/(6)
Fried pork chop*	43	34	55.84	18	49	26.87	2.08(1.34-3.23)
Scrambled egg*	42	38	52.50	19	45	29.69	1.77(1.15-2.72)
Stir-fried Cabbage**	40	37	51.95	21	26	31.34	1.16(0.79-1.71)
Pork Bloods soup*	48	39	55.17	13	44	22.81	2.42(1.45-4.04)
Sesame balls*	49	44	52.69	12	39	23.53	2.24(1.32-3.81)
Steamed Rice	44	57	43.56	17	26	39.53	1.10(0.72-1.70)
Pineapple juice	49	56	46.67	12	27	30.77	1.52(0.91-2.53)

*statistically significant, $p<0.01$, by χ^2 test;
**statistically significant, $p<0.05$, by χ^2 test.

Table 3. The Results of Multiple Regression Analysis of Food Items

Food Items	Regression Coefficient	Standard Deviation	p Value	Risk Ratio*
Model 1				
Fried pork chop**	0.1079	0.0402	0.0073	1.11
Pork blood soup**	0.1298	0.0402	0.0012	1.14
Model 2				
Fried pork chop**	0.1094	0.0404	0.0068	1.12
Sesame ball**	0.1080	0.0410	0.0084	1.11
Model 3				
Fried pork chop***	0.0818	0.0372	0.0281	1.08
Porkblood soup**	0.1285	0.0382	0.0008	1.14
Sesame ball**	0.0790	0.0379	0.0370	1.08

*risk ratio=exp (regression coefficient)
**statistical significant at $p<0.001$
***statistical significant at $p<0.05$

Table 4. Findings of Laboratory Testing of Food Residues

Food Items	S. aureus	S. aureus Salmonella	Toxin	B. cereus CFU/g	E-coli	V. paarahaemolyticus CFU/g
Fried chop	(-)	x	(-)	$1.3x10^3$	(-)	70
Faried egg	(-)	x	(-)	$>1.5x10^6$	(-)	(-)
Sesame ball	(-)	x	(-)	$3.3x10^4$	(-)	(-)
Fried fish	(-)	x	(-)	$2.3x10^4$	(-)	(-)
Stewed pork	(-)	x	(-)	$2.9x10^5$	(-)	70
Pineapple juice	(-)	x	(-)	(-)	(-)	(-)

(-)· negative
x: not isolated

Figure 1. Distribution of Incubation Periods