

Epidemiology & Health Bulletin

- 167 Investigation of a Food
Poisoning Outbreak in
Four Schools in Tainan
City and Tainan County
177 Cases of Notifiable and
Reportable Diseases,
Taiwan-Fukien Area
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Investigation of a Food Poisoning Outbreak in Four Schools in Tainan City and Tainan County

Introduction

On 19 May 1995, the Bureau of Food Sanitation, Department of Health was notified that, on 18 May, after consuming boxed lunches from a local food preparation factory (Hao-hsiang-hsiang), many students of Chiachi and Kuanghua Girls' High Schools in Tainan City, as well as the Kangming, and the Chengkuang High Schools in Tainan County, had developed symptoms of abdominal pain, diarrhea, nausea and vomiting for which they were treated. Since four different schools were concurrently involved, the number of persons affected was large; all had consumed the same foods from a single supplier. An investigation of the outbreak was conducted, in the hope of preventing any recurrences.

Methods and Materials

1. Subjects surveyed:

All four high schools' students and teachers who had consumed the boxed lunches supplied by the Hao-hsiang-hsiang Food Factory (HhhFF): a total of 1,273 persons including 145 in Chiachi, 202 in Kuanghua, 726 in Changning, and 200 in Chengkuang.

2. Survey tool:

The self-administered, structured Questionnaire used included such items as name of school, age, sex, status (student/teacher), symptoms, time of onset, medical care, time symptoms disappeared, food items eaten (the box lunch content included egg boiled in tea, boiled dumplings, fried noodles, sushi, fish rolls, dried beancurd). Because the boxed lunches ordered by the four schools were the same, the same Questionnaire was used for all.

3. Definition of a case:

An individual who, on 18 May 1995, had eaten the boxed lunch supplied by the HhhFF and who had suffered either one of the following two conditions:

- a) had more than two episodes of diarrhea in one day and/or
- b) had more than two of the following symptoms:
 - nausea
 - vomiting
 - fever
 - abdominal pain

4. Laboratory testing:

Thirty-eight rectal swabbed specimens were collected by the Tainan City and County Health Bureaus for testing by the Southern Branch Laboratory of the National Institute of Preventive Medicine. Items tested for were: *Vibrio parahaemolyticus*, *Bacillus cereus*, *Salmonella*, *Staphylococcus aureus*, *Bacillus dysenteriae*, *Vibrio cholerae* and *Bacillus typhi*.

Four food specimens were also tested by the Southern Branch Laboratory for *Vibrio parahaemolyticus*, *Bacillus cereus*, Pathogenic *Escherichia coli*, *Staphylococcus aureus*, toxin types of *Staphylococcus aureus* in foods and enterotoxin of *Staphylococcus aureus*.

Six environmental and food specimens from the Food Factory were collected by the Tainan County Health Bureau from knives, chopping boards, water, dried beancurd, minced meat and fish rolls. All were tested for *Vibrio parahaemolyticus*, *Bacillus cereus*, Pathogenic *Escherichia coli*, *Staphylococcus aureus*, toxin types of *Staphylococcus aureus* in foods and enterotoxin of *Staphylococcus aureus*.

5. Inspection of environment:

Together with the staff of the Tainan County Health Bureau, the Hao-Hsiang-Hsiang Food Factory itself was inspected to examine its food preparation process sources of foods and their storage, sanitary conditions of the kitchen, areas where raw and cooked foods were stored, sanitary control of kitchen workers, the time the boxed lunches were packed and transportation methods from site to consumers. As some of the foods used in the boxed lunches of the day were semi-products purchased from the local market, that market was also visited to better understand any sanitary conditions which might have been likely sources of contamination.

6. Analysis of data:

Returned Questionnaires were processed and analyzed with Epi-info and SAS.

Findings

A total 1,273 copies of the questionnaire were distributed: 145 to Chaichi, 200 to Kuanghua, 726 to Changing, and 200 to Cheng-kuang. Of those, 1,188 copies returned (return rate 93.3%): 145 in Chiachi (return rate 100.0%), 196 in Kuanghua (97.0%), 712 in Kangming (98.1%), and 135 in Chengkuang (67.5%).

1. Characteristics of cases

Distribution and Demographic Characteristics: Of the 196 persons in Kuanghua Girl's High School, 158 (80.6%) met the case criteria; all students were female with an average age of 15 years. Of the 145 potentials in Chiachi Girl's High School, 101 (69.7%) were cases, all students were female, averaging 17 year age. Of 712 in Kangming High School, 441 (61.9%) were cases; 292 were male (66.2%) and 149, females (33.8%) with an overall average age of 16 years. Of 135 in Cheng-kuang High School, there were 79 (58.5%) cases of whom 70 were male (88.6%) and 9, females (11.4%); their average age was 15 years. The total number of cases was 779 (65.6%), including 362 males (46.5%) and 417 females (53.5%) (Table 1).

Table 1. Distribution and Demographic Characteristics of Cases

School	No. Lunches Eaten	No. of Cases	Male	Female
Kuanghua	196	158 (80.6%)	0	158 (100.0%)
Chiachi	145	101 (69.7%)	0	101 (100.0%)
Kangming	712	441 (61.9%)	292 (66.2%)	149 (33.8%)
Chengkuang	135	79 (58.5%)	70 (88.6%)	9 (11.4%)
Total	1,188	779 (65.6%)	362 (46.5%)	417 (53.5%)

2. Distribution of symptoms

Of the 779 cases, 654 (84.0%) developed abdominal pains; 740 (95.0%), diarrhea; 253 (32.5%), nausea; 153 (19.6%), vomiting and 73 (9.4%), fever. No difference was noted in the distribution of symptoms between schools (Table 2).

3. Incubation period

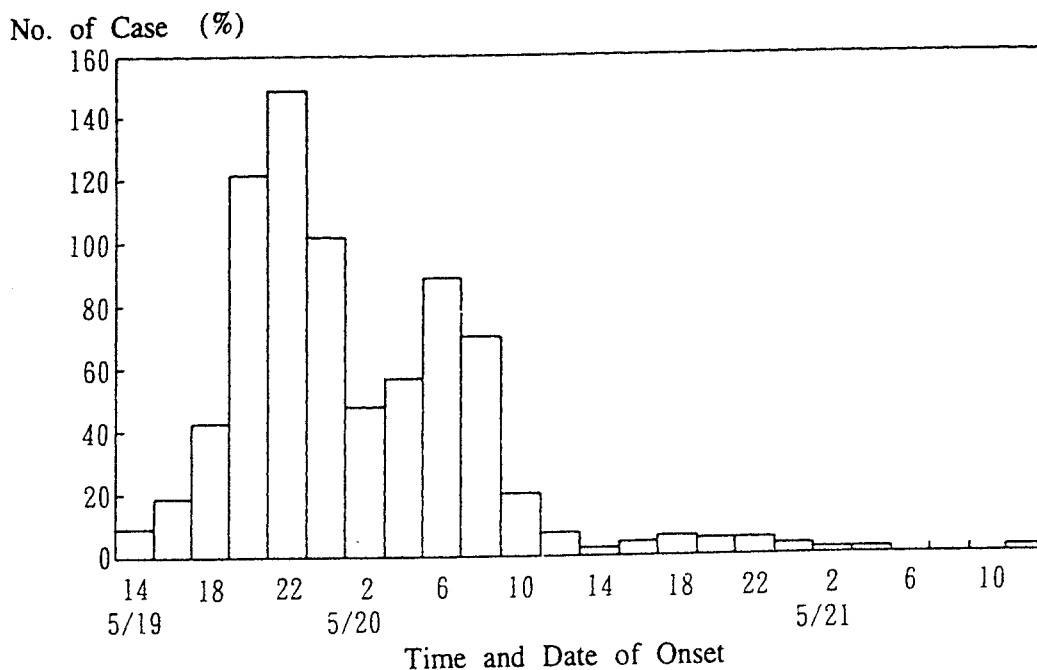
The average and median incubation periods were, respectively 13 hours and 11 hours for Chiachi; 11 hours and 9 hours for Kuanghua; 13 hours and 11.5 hours for Kangming; and 11 hours and 9 hours for Chengkuang.

all above 0.05, showing no difference in the incubation periods among schools. Of the total, the average incubation period was 12 hours and the median was 10.5 hours. The epidemic curve is shown in Figure 1.

Table 2. Distribution of Symptoms (N=799)

School	Abdominal Pain	Diarrhea	Nausea	Vomiting	Fever
Kuanghua	143 (90.5%)	155 (98.1%)	48 (30.4%)	30 (19.1%)	21 (13.3%)
Chaichi	84 (83.2%)	95 (94.1%)	52 (51.5%)	21 (20.8%)	13 (12.9%)
Kangming	367 (83.2%)	414 (93.9%)	140 (31.7%)	89 (20.2%)	36 (8.2%)
Chengkuang	60 (75.9%)	76 (96.2%)	13 (16.5%)	13 (16.5%)	3 (3.8%)
Total	654 (84.0%)	740 (95.0%)	253 (32.5%)	153 (19.6%)	73 (9.4%)

Figure 1. Cases by Time and Date of Onset of Outbreak (N=779)



4. Medical care

The number of cases medically treated in each school was: 31 out of 100 (31.0%) in Chiachi; 19 out of 158 (12.0%) in Kuanghua; 50 out of 431 (11.6%) in Kangming; and 2 out of 79 (2.5%) in Cheng-kuang. The total number treated was 102.

5. Analysis of food items

The four schools did not differ in their demographic distributions nor in distribution of symptoms and incubation periods; the foods consumed at the four schools were the same, hence they were treated as one group in the analysis of attack rates by food items in order to explore the major causes of this outbreak. Findings showed that food item was associated with this food poisoning incident ($p < 0.05$) (see Table 4). Further analysis by logistic regression model showed, however, that only the "egg in tea" and the "fish roll" were associated, and that both also interacted (Table 5). A significant difference ($p = 0.0001$) was noted when individuals who did not eat either or both of the two food items were analyzed. From the distribution of symptoms, those who had both food items seemed to have more symptoms than those who had only one. (Table 5).

Table 3. Relative Risk by Food Items (N=1,118)

Food	Eaten			Not Eaten			Relative Risk	95% Confidence Interval	P-Value
	Ill	Not Ill	Attack Rate (%)	Ill	Not Ill	Attack Rate (%)			
Egg in tea	537	206	72.3	242	203	54.4	2.19	1.70-2.82	<0.001
Dumpling	713	357	66.6	66	52	55.9	1.57	1.05-2.35	0.02
Fried noodle	748	368	67.0	31	41	43.1	2.69	1.62-4.48	<0.001
Sushi	715	354	66.9	64	55	53.8	1.74	1.16-2.59	0.005
Fish roll	487	161	75.2	292	248	54.1	2.57	1.99-3.31	<0.001
Beancurd	575	260	68.9	204	149	57.8	1.62	1.24-2.11	<0.001

* $p < 0.05$

6. Laboratory findings

Of the 38 rectal swabs tested, 24 were positive: 23 were serotype K18, the other was serotype K56.

Of the four food specimens, the dried beancurd and the egg in tea from Chia were found positive for *Vibrio parahaemolyticus* K63; the dumpling from Kuangh contained a small amount of *Vibrio parahaemolyticus*; the fish roll, minced meat and dried beancurd from Kangming were found positive for *Vibrio parahaemolyticus* K63; the fish roll, egg in tea, sushi and fried noodles from Chengkung were positive for *Vibrio parahaemolyticus* K56.

Table 4. Logistic Regression Analysis by Food Items (N=1,188)

Food	Logistic Regression	Standard Error	Odd Ratio	95 % Confidence Interval	P-Value
Egg in tea	0.570	0.136	1.767	1.519-2.056	0.0001*
Dumpling	0.040	0.221	1.040	1.023-1.058	0.0319
Fried noodle	0.708	0.267	2.030	1.403-2.929	0.0079*
Sushi	0.116	0.214	1.123	1.070-1.170	0.588
Fish roll	0.820	0.145	2.269	1.797-2.866	0.0001*
Beancurd	-0.150	0.156	0.860	0.901-0.822	0.336
Egg-Noodle	-0.191	0.512	0.826	0.682-1.001	0.709
Noodle-Fish	0.529	0.580	1.697	0.931-3.093	0.362
Egg-Fish	0.899	0.268	2.458	1.534-3.940	0.0008*

* $p < 0.01$

Table 5. Consumption of Egg and/or Fish Roll and Number of Symptoms

Food	No. Symptoms	1	2	3	4	5	No.
Not eaten	106 (34.6%)	45 (24.1%)	81 (20.9%)	40 (18.8%)	12 (15.0%)	1 (16.7%)	285 (24.07%)
Only egg	79 (25.8%)	55 (29.4%)	73 (18.8%)	33 (15.5%)	12 (15.0%)	3 (30.0%)	255 (21.54%)
Only fish roll	53 (17.3%)	21 (11.2%)	45 (11.6%)	27 (12.7%)	13 (16.3%)	0 (0.0%)	159 (13.43%)
Both eaten	68 (22.2%)	66 (35.2%)	189 (48.7%)	113 (53.1%)	43 (53.8%)	6 (60.0%)	485 (40.96%)
Total	306 (100.0%)	187 (100.0%)	388 (100.0%)	213 (100.0%)	80 (100.0%)	10 (100.0%)	1,184 (100.00%)

In the six environmental and food specimens collected from the HhhFF, only a small amount of *Vibrio parahaemolyticus* was identified.

7. Environmental inspection findings

The Food Factory source had previously been graded "A" in assessments made both the Tainan City, and the County, Health Bureaus. The Factory is located between the City and the County, but is under the jurisdiction of the Tainan County Health Bureau. The Factory owner said that on 18 May, only the dried beancurd and the fish noodles were prepared by the Factory. On that day, the other food items (sushi, egg in tea) were bought from the nearby market; the dumplings were purchased, frozen from a supermarket and then boiled; the fish rolls came from a market in Yungka. The dried beancurd was prepared at around 3:00 am of that same day, and the fish noodles were prepared with gravy left over from the day before. Boxed lunches were packed including the food items purchased from outside at around 09:00 hours and delivered to the schools between 10:30 and 11:00 hours.

The major pathogen identified was *Vibrio parahaemolyticus*. This is most often found in sea foods or on foods, containers and chopping boards contaminated by sea food. The owner said the Factory seldom supplied sea food, though one of the food items provided on the outbreak day had been fish rolls which were used without further treatment after purchase.

The fish rolls were purchased from a food store in a market in Yungka. The wholesaler often begins preparation at 0200 hours by defrosting deep sea fish such as shark and mincing them into paste. The fish rolls are then made of shark paste and pork, and fried. They are placed in sifters to drain before packing and delivery at around 0730 hours. Placed in the boxed lunches directly, without further handling, they were consumed by the students at 1200 hours.

Discussion

Vibrio parahaemolyticus are Gram-negative, aerobic, salt-loving bacteria. They grow under 37°C, and are found ubiquitously in off-shore sea water. In the spring and summertime, they live on shell-fish; in winter, they float with sea water sediments and start the cycle again⁽¹⁾. They fission and multiply rapidly and, under optimum environment at 30-37°C, the pathogenic *Vibrio parahaemolyticus* can double in number in 12 to 18 minutes. When foods are contaminated by even a small amount of *Vibrio parahaemolyticus*, under optimum circumstances, the amount of bacteria can reach pathogenic level in a very short period of time⁽²⁾. There was an outbreak of gastroenteritis in Osaka in 1950 and, in 1953, Fujino et al. of Japan isolated *Vibrio parahaemolyticus* from feces of patients and from cooked sardines. The name was given in 1963 by Sakazaki et al.^(3,4,5). By antibodies, the pathogen can be classified into 13 O antibody groups and 65 K sub-groups. The pathogens can induce Kanagawa phenomenon through their hemolytic activity which causes red blood cells to produce β -hemolysis⁽⁶⁾.

The incubation period of *Vibrio parahaemolyticus* is from 2 to 48 hours, averaging from 15 to 17 hours. Patients are sick for an average of two days, and 95% of them will have diarrhea and abdominal pain. Sudden onset of watery diarrhea recurring more than 15 times in one day is common^(7,8). Other accompanying symptoms include fever, headache, nausea, vomiting and dehydration. Some patients may have bloody or mucous feces. Death is rare⁽⁹⁾. Consumption of inadequate frozen, raw or insufficiently cooked sea foods or foods contaminated by sea water, foods not properly stored after cooking, may bring about diseases of the gastro-enteric system. Reports also show that food poisoning may occur through foods contaminated indirectly by media such as kitchen knives, chop boards, dish cloths, utensils, containers and fingers^(10,11).

Vibrio parahaemolyticus is a common pathogen of food-borne gastroenteritis. The disease is seasonal, occurring more often in warm weather. It is common in Taiwan, Japan, Southeast Asia and the United States⁽¹¹⁾. In 1994 in Taiwan, the 62 incidents of bacteria-induced food poisoning reported, 35 resulted from *Vibrio parahaemolyticus*; it was the primary cause, responsible for 34.3% of the total 102 food poisoning incidents for that year (data supplied by the Bureau of Food Sanitation, Department of Health). The Japanese are fond of raw sea food and shellfish. Reporting says that more than 60% of their food-borne food poisoning incidents come from *Vibrio parahaemolyticus*⁽¹²⁾.

In both food and human specimens from the outbreak reported here, *Vib. parahaemolyticus* was isolated. The median incubation period of 11.5 hours is close to the average of 13 hours as shown by the epidemic curve of the outbreak and also close to the 2- to 48-hour incubation period of *Vibrio parahaemolyticus* (averaging 15 to 17 hours). The major symptoms of abdominal pain and diarrhea exhibited were accompanied by fever, nausea and vomiting, again corresponded to those of *Vib. parahaemolyticus*. It was thus inferred that the outbreak was caused by *Vib. parahaemolyticus*. However, the serotypes of *Vibrio parahaemolyticus* identified in both the food and human specimens were not identical. There could have been other causes of outbreak, or these differences could have resulted from different testing methods used by different laboratories.

After analysis of food items, eggs in tea and fish rolls were considered the major factors. When either one was consumed, symptoms developed. The two factors interacted and, therefore, consumption of both foods also brought about poisoning. However, it was not possible to identify which of the two was more important. From the survey, the likelihood of cross-contamination of the fish rolls during processing through human error was thought to be high. *Vibrio parahaemolyticus* can be killed in a few minutes at a temperature higher than 80°C. The owner of the food store did not segregate the raw food area from the cooked food area; a food stand was selling fresh fish just outside the store; the workers handled both raw and cooked foods at the same time. All of these factors increased the likelihood of cross-contamination. The fish rolls were delivered to the HHHFF at around 07:30 hours, and were placed in boxed lunches without further treatment before being eaten by students at 12:00 hours. In that long period, the pathogens had ample time to grow into a pathogenic amount.

That the egg in tea found significant could have been because, though *V. parahaemolyticus* is a salt-loving bacterium and grows more often on sea fish and the eggs are certainly not sea foods, contamination could have occurred during the cooking of preparation or the eggs could have been contaminated by the fish rolls as they were packed together in the boxed lunches. The market where the eggs in tea were purchased was not investigated. However, since *Vibrio parahaemolyticus* was isolated from a food item, the second reason seems more likely. The pathogens found in the tea could be related to the incubation period and the number of symptoms. Analysis shows that those persons who had consumed both the egg and the fish rolls had developed more symptoms. A consumer who had consumed both food items would thus have ingested more pathogens, with consequent appearance of more, or more severe symptoms.

Recommendations

1. When products or semi-products are purchased from a market, their sanitary conditions should be checked before they are placed in boxed lunches. Food sold in markets are often neither supervised nor inspected by health authorities. If products or semi-products must be purchased from outside, they should be re-heated to prevent the multiplication of pathogens.
2. Food dealers should segregate raw from cooked foods, paying careful attention to sanitary conditions to avoid food poisoning outbreaks from cross-contamination. Schools, when signing contracts with food factories, must insist that those factories are insured. Insurance gives both the school and the factory more protection in the event of food poisoning outbreaks. Factory closures can also thus be minimized.

Prepared by: D.Y. Chao¹, P.F. Wu, S.Y. Hsu², T.M. Pan³, K.T. Chen¹

1. FETP, National Institute of Preventive Medicine, DOH
2. Southern Branch Laboratory, NIPM, DOH
3. Division of Bacteriology, NIPM, DOH

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