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Epidemiological Investigation of a Food Poisoning Outbreak at a National Conference

Introduction

Convenience is the major concern of food supply at large-scale meetings and group gatherings. Often boxed lunch in large quantity is ordered from restaurants or food factories. Food poisoning incidents often occur when any human errors on the part of the restaurants or food factories happen in the process of procurement, storage, handling and packing of food.

A national conference was convened at the International Conference Hall of the National Library by the Ministry of Justice and other related organizations on 3 June 1997. The lunch for the day was ordered from a restaurant in Taipei City. Cooks and food handlers of the restaurant gathered at the cafteria of the National Library to prepare for the boxed lunch. Around 12 at noon on 3 June 1997, participants, staff and securities, after eating the boxed lunch, developed one after another food poisoning-like symptoms such as vomiting, abdominal pain and diarrhea. Some seriously ill patients were treated at the National Taiwan University Hospital and the Taipei Municipal Ho-Ping Hospital. Upon report, a team from the Chung-Chen District Health Center visited the site immediately to collect rectal swabs for laboratory testings by the National Institute of Preventive Medicine of the Department of Health. The remaining lunch boxes kept at the police station were forwarded to the Taipei City Health Bureau for laboratory testings. Preliminary information collected by the Chung-Chen Health Center showed that a total of 650 lunch boxes had been ordered and 38 of those who had eaten the lunch had developed food poisoning-like symptoms. The incident was suspected to be associated with the consumption of the leftovers of the lunch. FETP was then requested by the Bureau of Food Sanitation of the Department of Health, in

collaboration with the Chung-Chen Health Center, for an epidemiological investigation to identify the causes of the incident, the food items responsible and the pathogenic agents concerned.

Materials and Method

1. Supply of Boxed Lunch by the Restaurant

At a preliminary count, 38 persons eating the boxed lunch prepared by a restaurant were affected. By the definition given in the “Operational Manual for the Collection of Specimens for Food Poisoning….”, a “food poisoning incident is one in which two or more persons consuming the same food develop similar symptoms...”, the incident was confirmed a food poisoning outbreak. Table 1 gives the number of participants by organization, the number of lunch boxes consumed by type, and the number of suspected cases on 3 June 1997.

Table 1. No. of Participants and Cases by Organizations

Organization	No.	No. and Type of Lunch Boxes	No. of Suspected Cases
Participants:			
Office of the President	16	16 (Japanese type)	0
The Executive Yuan	25	25 (Japanese type)	1
Legislative Yuan	5	5 (Japanese type)	0
Ministry of Interior	11	11 (Japanese type)	0
Ministry of Foreign Affairs	1	1 (Japanese type)	0
Ministry of Defence	11	11 (Japanese type)	1
Ministry of Finance	4	4 (Japanese type)	0
Ministry of Education	12	12 (Japanese type)	0
Ministry of Justice	31	31 (Japanese type)	5
Ministry of Communications	6	6 (Japanese type)	0
Ministry of Economic Affairs	3	3 (Japanese type)	0
Department of Health	14	14 (Japanese type)	2
Representatives of county/city	52	52 (Japanese type)	8
Staff:			
Ministry of Justice	170	170 (paper box)	56
Ministry of Education	25	25 (paper box)	7
Department of Health	8	8 (paper box)	4
Other ministries	125	125 (paper box)	6
Securities:			
Chung-Chen Squad 1	41	41 (paper box)	41
Others:			
Reporters	70	70 (paper box)	15
Total:	630	191 (Japanese type) 439 (paper box)	146

2. Questionnaire Survey

The “retrospective cohort study” method was used in the present investigation.

Subjects studied:

Participants of the conference came from all parts of the country, especially the northern part. The staffs and securities came from different ministries and departments such as the Ministries of Justice and Education, Department of Health, and other ministries and the Chung-Chen First Squad of the police department. By the distribution of suspected cases, more were found among participants and staffs of the Ministry of Justice, the Department of Health and the First Squad. The Ministry of Justice and the Department of Health had both participants and staffs at the conference, they had lunch of either the Japanese type or in paper boxes. By comparing the two groups, the association between the incident and the kinds of lunch consumed could be understood. Human specimens from members of the Ministry of Justice and the First Squad were collected by the Chung-Chen Health Center; specimens of food leftovers were supplied by the Ministry of

Justice. As incidence was not necessarily related to organizations, members involved in the conference from the Ministry of Justice, the Department of Health and the First Squad were selected for the questionnaire survey.

Contents of the questionnaire:

A structured questionnaire was used in the survey. The questionnaire contained such questions as the background information, time of onset, symptoms, medical care, food items consumed, and whether leftovers were consumed.

Some duty officers of the First Squad collected lunch boxes at 11 and some at 3:00. The duty officers reported that about 120 lunch boxes similar to the ones distributed at noon were supplied by the restaurant at 3:00. They thought these lunch boxes were for duty officers of the afternoon. The lunch boxes were then taken back to the squad office for other duty officers to share. Many officers, soon after the lunch at noon, had developed symptoms such as abdominal pain and diarrhea. Some officers took the lunch boxes home for family members to share. The family members had also developed such food poisoning-like symptoms as nausea, abdominal pain and diarrhea. An additional questionnaire was given to the duty officers of the First Squad to understand the outcomes of either the lunch or the dinner.

Definition of case:

A case was defined as one who had eaten the boxed lunch supplied by the restaurant at noon of 3 June and had developed diarrhea (more than twice a day) or two of the following symptoms: nausea, vomiting, abdominal pain, weakness, chillness, fever, dizziness, bloody stool, rashes and others.

Data processing and analysis:

The information was processed with Epi-Info v6.02. Each variable was confirmed of its accuracy after input. The incubation period was drawn with Excel 5.0 of Microsoft Windows 3.1. Other information was treated with Epi-Info and SAS 6.04. X^2-test was used to test the relationship between the incident and either the Japanese type lunch or lunch in paper box, and also the food items consumed. When an expected number was lower than 5 and the use of X^2-test was not appropriate, the Fisher's Exact Test was used instead. The 95% confidence intervals of the relative odds ratios were calculated by the Exact Method. The stepwise multiple logistic regression was used for multi- variable analysis by controlling relevant factors.

3. Survey of the Environment

The boxed lunch suspected of the outbreak was supplied by the restaurant located at the basement of the National Library with its kitchen on B2. The lunch was packed at B 1 at the rear of the restaurant. Facilities were sufficient. There were four cooks, six other employees and some additional helpers hired on the

occasion for food handling and packing. On 3 June, a total of 650 lunch boxes was supplied for the national conference; 250 in the Japanese style for participants, and 400 in paper boxes for staffs and securities. With the exception of packing, and times of food handling and supply, the food and the processing of food were the same for the two types of boxed lunch.

Food items included: shrimps cooked with carrot, steamed egg, sliced meat in vegetable, fried fish with pineapple, pork in black pepper, leaf mustard, corns and rice. The pork was added soy sauce and black pepper the day before and kept in the freezer (in the same freezer with the sea food). The rest food items were prepared in the morning of the day. Preparation started at 5:00. Sea food and pork were taken out of the freezer first. Sea food included shrimps, fish cakes and cod, all vacuum packed and frozen at -18℃.

The first food item, the steamed egg, was prepared at 6:00. Eggs, fish cakes and salt were mixed and put together in the molds in the steamer. Each steamer could steam 150 eggs in 10 minutes. After steaming, the eggs were placed elsewhere for cooling. When the 650 eggs were steamed, they were carried upstairs for packing. Cods were then sliced and deep fried with pineapples. The pork in black pepper prepared overnight, after defrosting, was fried. Shrimps were cooked with carrot, and sliced meat with vegetables.

The cooking was completed at around 8:00, and the packing began. Lunch boxes for staffs and securities should be ready by 11:00, lunch for 400 in paper boxes was prepared first. Food in Japanese style for the participants was kept in the heater at 60-70℃. At 10:00, the packing of the 250 boxes of Japanese style lunch began. They were supplied to the participants at 12 at noon.

Though 250 boxes of the Japanese style lunch were ordered, only 190 were consumed. The remaining 60 were, without further heating, re-packed into paper boxes at 2:00 by the restaurant staff and distributed to the duty officers.

4. Specimen Collections

Human specimens

Of those affected, 20 were medicated (7 at the National Taiwan University Hospital, 13 at the Taipei Municipal Ho-Ping Hospital) and 5 hospitalized (3 at the National Taiwan University Hospital and 2 at the Taipei Municipal Ho-Ping Hospital). The Chung-Chen District Health Center had collected 11 rectal swabs. Specimen collection was refused by the rest. The specimens were sent to the National Institute of Preventive Medicine for laboratory testings for: Staphylococcus aureus (including enterotoxin), Bacillus cereus, Vibrio parahaemolyticus, Salmonella, Salmonella typhi and paraty phi, and Shigella.

Environment specimens

Two swabs of the hands of the food handlers were also collected. Two swabs of the kitchen table, two of the knives, and one of the chop-board were

collected and sent to the Taipei City Health Bureau for laboratory testings for: Staphylococcus aureus (including enterotoxin), Bacillus cereus, Vibrio parahaemolyticus, Salmonella, pathogenic E. coli, E coli and Shigella.

Testings of leftovers

The remaining boxed lunch kept at the Ministry of Justice was sent to the Taipei City Health Bureau for laboratory testings for: Staphylococcus aureus (including enterotoxin), Bacillus cereus, Vibrio parahaemolyticus, Salmonella, pathogenic E. coli, E coli and Shigella.

Results

1. Findings from the questionnaires

Of the 200 copies of questionnaire distributed, 197 had been returned, giving a return rate of 98.5%. They were: 112 copies from the Ministry of Justice (100% return), 18 from the Department of Health (94.4% return), 41 from the First Squad (100% return), and 26 from other ministries and departments (100% return). 69.5% (137) of the respondents were male and 30.5% (60) female.

Of those interviewed, 185 (93.9%) had had the boxed lunch of the day; of them, 103 met the criteria of a case, giving an attack rate of 55.7%. Their symptoms were: diarrhea more than twice a day, 93.2% (96/103); abdominal pain, 67.0% (69/103); weakness, 62.1% (64/103); chillness, 41.7% (43/103); fever, 36.9% (38/103); nausea, 32.0% (33/103); vomiting, 30.1% (31/103); dizziness, 28.2% (29/103); others, 4.9% (5/103); bloody stool, 1.9% (21103); rashes, 1.0% (11103); sore throat, 0% (0/103).

Table 2 gives the number of participants and their attack rates. The attack rate (64.2%) of those who had had lunch in paper boxes was significantly higher than that of those who had had the Japanese style lunch (21.6%). The lunch in paper boxes was prepared earlier and left under room temperature longer. Pathogenic micro-organism could have multiplied more during the period.

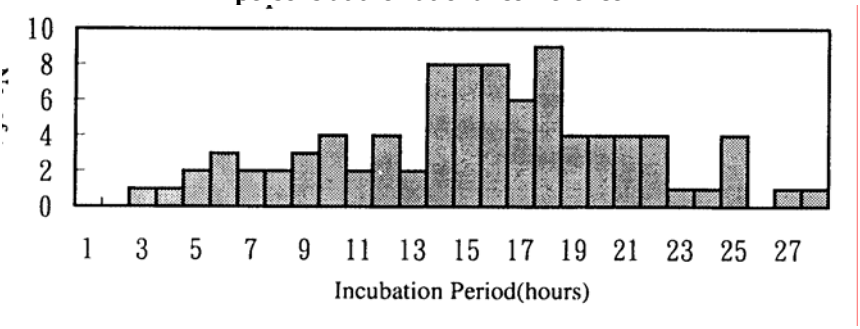
Table 2. No. of Participants and Attack Rates

Organization	No of participants	No. eaten lunch	No meeting criteria of case	Attack Rate (%)	Attack Rate (%) Japanese Style	Attack Rate (%) Paper Boxes
Ministry of Justice	112	105	48	45.7% (48/105)	25 0% (4/16)	49 4% (44/89)
Department of Health	18	15	3	20.0% (3/15)	9.1% (1/11)	50.5% (2/4)
First Squad	41	41	40	97 6% (40/41)	not eaten	97.6% (40/41)
Others	26	24	12	50 5% (12/24)	30 0% (3/10)	64 3% (9/14)
Total	197	185	103	56.0% (103/185)	21.6% (8/37)	64.2% (95/148)

The incubation period was defined as the period between the consumption of the lunch and the onset of symptoms. An epidemiological curve is shown as Figure 1. The incubation periods ranged from 3 to 28 hours, with an average of 15 hours, a median of 16 hours and a mode of 18 hours.

An additional questionnaire was distributed to 41 officers of the First Squad. Of them, 38 had had the lunch in paper boxes supplied by the restaurant, and 3 had had the same food in paper boxes for both lunch and dinner. Of the 38, 8 had, off duty later at 3:00, had the lunch in paper boxes re-packed from lunch of the Japanese style. Of the 41, with the exception of the 8 who had had lunch later in the afternoon, the rest 33 officers had developed food poisoning-like symptoms such as abdominal pain and diarrhea soon after the lunch. It was suspected that the outbreak was associated with the boxed lunch consumed at noon rather than with the leftovers of the lunch.

Figure 1. The Incubation Periods of food-poisoning persons at the national conference



The food items

The attack rate of those who had eaten the Japanese style lunch was lower (21.6%). Each food item was not found statistically significant. The attack rate of those who had had the paper box lunch was higher (64.2%). The food items contained in the paper boxes were therefore analyzed.

Table 3 shows the attack rates by food item, the attack rates of food items not consumed and their relative odds ratios. Single variate analysis found that shrimps with carrot, pork in black pepper and fried fish with pineapple were statistically related to the food poisoning outbreak ($p<0.05$). The steamed egg, sliced meat in vegetable, leaf mustard, rice and corns were not found significantly related to the outbreak. The odds ratio of getting food poisoning for those who had had shrimps with carrot was 5.16 times higher than those who hadn't had this item (95% confidence interval at 1.54-580.30). The odds ratio of those who had had pork in black pepper was 1.95 times higher than those without (95% confidence interval at

1.19-12.85). The odds ratio of those who had had fried fish with pineapple was 1.76 times higher than those without (95% confidence interval at 1.27-8.93). Through stepwise multiple logistic regression by controlling mutual interference of variables, it was found that shrimps with carrot, fried fish with pineapple and pork in black pepper were each statistically related to the outbreak.

Table 3. Attack Rates by Food Items

Food item	Eaten			Not eaten			Relative Odds Ratio (3)/(6)	95% Confidence Interval
	Sick (1)	Not sick (2)	Attack rate (3)=(1)/[(1)+(2)]	Sick (4)	Not sick (5)	Attack rate (6)=(4)/[(4)+(5)]		
Shrimp	91	50	64.5%	1	7	12.5%	5.16**	1 54-580 30
Steamed egg	84	47	64.1%	7	9	43.8%	1.47	0 71-7.73
Sliced meat	67	40	62.6%	19	15	55.9%	1.12	0 56-3.09
Fried fish	81	39	67 5%	10	16	38.5%	1.76*	1 27-8.93
Pork	84	45	65.1%	6	12	33 3%	1.95*	1.19-12 85
Leaf mustard	74	49	60.1%	17	9	65 4%	0.85	0.29-2.08
Rice	80	53	60.1%	12	5	44.4%	0.85	0.16-2.06
Corns	72	45	61 5%	20	13	60 6%	1.02	0.43-2 45

*statistically significant, X 2 - test , $p<0.05$
**Fisher's exact test (two-tailed), $p<0.05$

2. Findings from laboratory testings

Human specimens and leftovers

Vibrio parahaenzolyticus K6 was isolated from all 11 rectal swabs. Laboratory testings by the Taipei City Health Bureau also isolated from the steamed egg, pork in black pepper and leaf mustard Vibrio parahaemolyticus of serotypes K64, K22 and K34 (Table 4). From pork in black pepper, Staphylococcus aureus of enterotoxin-genic types A and B was also isolated. Bacillus cereus was found in all food items but rice and fried fish. No Salmonella, pathogenic E. coli and Shigella were isolated from these food specimens.

Table 4. Findings of Laboratory Findings

Food Item	*E coli*	*V parahaemolyticus* (Cl-U/gm)	*S. aureus* (Cl-U/gm)	*Salmonella*	*B cereus* (CFU/gm)	Pathogenic *E coli*	*Shigella*
Rice	negative	negative	not found	negative	not found	negative	negative
Shrimp, Corns	4.6×10^{2}	negative	not found	negative	6.8×10^{8}	negative	negative
Steamed egg	larger than 1.1×10^{3}	positive (K64)	not found	negative	1.2×10^{8}	negative	negative
Pork	negative	positive (K22)	(1.6×10^{5}) enterotoxin types A,B	negative	9.4×10^{7}	negative	negative
Sliced meat	larger than 1.1×10^{3}	negative	not found	negative	1.6×10^{8}	negative	negative
Fried fish	negative	negative	not found	negative	not found	negative	negative
Mustard	negative	positive (K34)	not found	negative	7.6×10^{7}	negative	negative

Environment specimens

The two swabs from the food handlers' hands, two from the kitchen table, two from knives and one from the chop-board collected by the Health Center were sent to the Taipei City Health Bureau for laboratory testings. The results are shown in Table 5. With the exception of Vibrio parahaemolyticus K48 found on the hand of a food handler, no pathogens were isolated from other specimens.

Table 5. Findings of Laboratory Testings

Specimen	*E. coli*	*V parahaemolyticus* (Cl-U/gm)	*S aureus* (Cl-U/gm)	*Salmonella*	*B cereus* (Cl-U/gm)	Pathogenic *E coli*	*Shigella*
Lu XX	positive	positive (K48)	negative	negative	negative	negative	negative
Shen XX	negative	negative	negative	negative	negative	negative	negative
Chop-board	positive	negative	negative	negative	negative	negative	negative
Knife	negative	negative	negative	negative	negative	negative	negative
Knife	negative	negative	negative	negative	negative	negative	negative
Table	positive	negative	negative	negative	negative	negative	negative
Table	positive	negative	negative	negative	negative	negative	negative

Discussion

From the information collected through epidemiological investigation and the statistical analysis, it was found that the incubation period of the present outbreak was 14 to 18 hours, and the major symptoms were abdominal pain and diarrhea. These facts correspond with the specific features of Vibrio parahaemolyticus-induced food poisoning. Vibrio parahaemolyticus was isolated from rectal swabs of patients, food leftovers and the hand of a food handler. It was therefore decided that

Vibrio parahaemolyticus was the most likely pathogenic agent of the present outbreak. Though Bacillus cereus and Staphylococcus aureus were also isolated from the food specimens supplied by the First Squad, the boxed lunch from which specimens were collected were left under room temperature for a while and the food had already become putrid. Bacillus cereus is a normal colony commonly found in the environment with pathogenicity at an amount larger than 10^5 /gm. The Bacillus cereus isolated must have multiplied under room temperature. Therefore, the likelihood of Bacillus cereus as the pathogenic agent was removed. The Staphylococcus aureus isolated could have also been the result of the food being left under room temperature for too long. However, Staphylococcus aureus is toxin-genic, food could have been contaminated by it in the process of preparation.

Vibrio parahaemolyticus, gram-negative, is one of the major pathogenic agents of gastroenteric infections. By antigens, the bacilli come in 13 0-antigen groups and 65 K sub-groups. The pathogenic ones can produce Kanagawa phenomenon to induce human red blood cells to produce beta-hemolysis hemolysin.[2,3,4] The bacilli grow at a temperature from 10 to 40℃, optimally under 35-37℃. Growth is inhibited at pH values lower than 5.0 and higher than 11.0. They are often found offshore in fish, shellfish and crustaceans. In coastal countries such as the United Kingdom, the United States, New Zealand, south-east Asian countries, Taiwan and Japan, Vibrio parahaernolyticus is a common pathogenic agent of food poisoning. The bacilli fission and grow rapidly, double in 12 to 18 minutes[5-10] . Some reports maintain that Vibrio parahaemolyricus can induce food poisoning by the contamination of food through hands, rags, kitchen utensils such as knives and chop-boards [11]. The incubation period is from 6 to 24 hours, averaging 15-17 hours. The average morbid period is 2 days. 95% of the patients will develop such symptoms as diarrhea and abdominal pain. Diarrhea is often sudden and watery. Other symptoms include fever, headache, nausea, vomiting and dehydration. A few patients may have bloody stool or mucus in stool. Deaths are rare[12,13]

On 3 June 1997, some participants, staff and security officers of the National Anti-Drug Conference, after having had the boxed lunch supplied by a restaurant, had developed some gastroenteric symptoms, the attack rate being as high as 55.7%. A total of 650 lunch boxes was supplied on that day. The job required the 10 cooks and workers sufficient time to prepare. The cooking started at 5:00. By the report of the cooks, the food for the day was prepared in that order: steamed egg, fried fish with pineapple, pork in black pepper, shrimps with carrot, sliced meat with vegetable, corns and leaf mustard.

Statistical analyses found that shrimps with carrot, fried fish with pineapple and pork in black pepper were each related to the food poisoning. The pork in black pepper for instance, was processed the night before, kept in the freezer with sea food, and then heated and cooked. If the temperature was not high enough, by

being kept for too long, Vibrio parahaemolyticus could have grown. Sea food was also served. In this case, food containers could have been contaminated; the cooked food kept aside for cooling could have been contaminated by the sea food sauce, by hands, rags, kitchen utensils such as knives and chop-boards. Cook's hands are the best medium of food contamination and Vibrio parahaemolyticus was isolated from the swab of a cook's hand. Therefore, the pork in black pepper was the likely food item that induced the outbreak. Bacilli had been isolated from shrimps with carrot and fried fish in pineapple. The two food items were found statistically significant, the chances of their inducing the food poisoning could not be eliminated. The steamed egg and leaf mustard, though statistically not significant, the fact that Vibrio parahaemolyticus was isolated from their specimens suggested that they could have either been contaminated by other food in the lunch boxes or cross-contaminated in the process of preparation.

The lunch was supplied either in Japanese style or in paper boxes. The food items and the time of preparation for the two types of lunch were the same. The lunch in paper boxes was prepared at 8:00 in the morning and kept under room temperature. Food for the lunch in Japanese style was heated till 11:00 before packing. The attack rates for the two types of lunch served were significantly different. It therefore seemed that though the food for the lunch had already been contaminated, different methods of preservation produced different results. The speculation that the incident was related to the consumption of leftovers was found not valid. On 4 June, most patients visited by the Chung-Chen Health Center at clinics and hospitals were duty officers of the First Squad. On 3 June, some duty officers had had the food in paper boxes supplied by the restaurant for lunch and dinner. They didn't know that the food they had had for dinner was in fact the same food in Japanese. Style served for lunch and re-packed in paper boxes later. Workers of the Chung-Chen Health Center, not knowing the details, misunderstood that the leftovers were related to the incident.

Recommendations

Upon findings of the present investigation, the following recommendations are made:

1 .Sea food, either for eating raw or cooked, should be kept separately from other food to avoid cross-contamination.

2.Cooked food, before packing, should be kept either in heater or away from uncooked food to prevent pathogenic agents from growing.

3.In preparing for boxed lunch in large quantity, restaurants should strictly follow the principle of ¡§eat soon after cooking¡¨ to reduce the time of food being exposed to room temperature for pathogenic agents to multiply.

Acknowledgement

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