

## **An Epidemiological Investigation of a Food Poisoning Outbreak in a Senior High School in Yunlin County**

### **Abstract**

An epidemiological investigation into a food poisoning outbreak on 25 September 2002 in a senior high school in Yunlin County was conducted to understand the pathogenic agents and food items associated with the incident. By analyzing 522 copies of the questionnaire collected from students, it was found that 135 students met the definition of case (70 males and 65 females), giving an attack rate of 54.7%. Attack rates by class ranged from 26.3% to 70.0%. Symptoms were abdominal pain (84.4%), diarrhea (80.0%), nausea (39.3%), dizziness (34.1%), vomiting (32.6%), weakness (31.9%), chill (28.9%), and fever (28.1%). The epidemiological curve showed that the incubation periods ranged from 5 hours to 47 hours, with a median of 18 hours. Of the ten rectal swabs, *Vibrio parahaemolyticus* type K6 was isolated in eight. By their symptoms, incubation periods and findings of laboratory testing, it was decided that the pathogenic agent of the present incident was *Vibrio parahaemolyticus*.

Single and multivariate analyses of the food items of the boxed lunch

supplied by the Weidahsiang Food Factory found that the odds ratio of food poisoning of students having the corn-carrot-bean was 1.96 times higher than the odds ratio of not having the said food item, and was statistically significant ( $p<0.05$ ). No *Vibrio parahaemolyticus*, however, was isolated in the said food item. Failure of the food factory to supply with sufficient information made it impossible to decide whether the corn-carrot-bean was the cause of the incident.

### **Introduction**

School lunch or boxed lunch is, for its convenience, a popular source of lunch at school. There often are, however, problems resulting from human negligence in the course of processing, storage and transportation of school lunch or boxed lunch supplied by food factories<sup>(1)</sup>. Data of the Bureau of Food Sanitation of the Department of Health show that in all sites of food poisoning outbreaks in 2001, school was the second most frequent site, accounting for 20.2% (36/178) of all food poisoning incidents; and the number affected, 936 in total, was the largest<sup>(2)</sup>. Schools though are under strict supervision of the education authorities, there still are occasionally food poisoning outbreaks.

At 5:00 p.m. of 25 September 2002, students of a senior high school in Yunlin County began one by one to develop gastro-intestinal symptoms. By 3-4:00 a.m. of 26 September, some 200 students and teachers had shown symptoms such as abdominal pain, diarrhea, vomiting and fever. They were admitted to the emergency department for treatment. Many of the students having symptoms had had breakfast of the same day and dinner of the day before either at home or somewhere else. There had not been students of similar symptoms recently, either. The incident was, therefore, considered to be associated with the boxed lunch on the 25<sup>th</sup>. To understand the causes, the

pathogenic agents and the food items likely to be associated with the incident, the FETP of the Center for Disease Control of the Department of Health conducted jointly with the Yunlin County Health Bureau an epidemiological investigation into the matter.

### **Materials and Method**

**Confirmation of outbreak:** Students under hospital care were visited and their medical records reviewed to confirm the diagnosis and for deciding on the definition of case. To decide on whether there had been outbreaks of diarrhea recently in the school, sick leaves of students with similar symptoms were also reviewed.

**Subjects for investigation:** The school has 1,841 students in three grades of 14 classes each. As the school had already made preliminary survey of classes with students having gastro-intestinal symptoms, it was decided to choose only classes with three and more students having gastro-intestinal symptoms for questionnaire interviewing. 484 students in 11 classes were so chosen.

**The questionnaire:** A semi-structured questionnaire containing items for personal information, source of lunch, time lunch was taken, food items eaten, time of onset of symptoms, symptoms, medical care and recovery. Copies of the questionnaire were distributed and explained to students for them to fill in on the spot.

**Definition of case:** Any student who had had the boxed lunch supplied by the Weidahsiang Food Factory on 25 September 2002 and had either two of the following symptoms, two or more diarrhea a day, or abdominal pain with nausea, dizziness, chill, vomiting, weakness and fever ( $38^{\circ}\text{C}$  and above); or two or more diarrhea a day and abdominal pain.

**Collection of specimens and laboratory testing:** Ten rectal swabs, one vomit specimen, and 15 leftovers of the Weidahsiang boxed lunch were collected.

Laboratory testing included testing for *Vibrio parahaemolyticus*, *Bacillus cereus*, *Salmonella*, *Staphylococcus aureus* and its enterotoxin, and *E. coli*.

Investigation of the environment: The Weidahihsiang Food Factory was visited to investigate into the course of food processing, sorting, storage, preparation, packing, and transportation.

Data processing and analysis: Epi-info was used for the key-in, validation and data processing. Attack rates and distribution of symptoms and incubation periods were calculated. Association of food items with the incident was analyzed. Estimated odds ratios (EOR)<sup>(3)</sup> and their 95% confidence intervals were used to express the statistical significance of the differences. Mantel-Haenszel odds ratios<sup>(4)</sup> were used for the cross analysis of various food items.

## Results

All copies of the questionnaire distributed, 522 in total, were returned, at a return rate of 100%. Of them, 274 were males (52.5%), and 248 females (47.5%). 135 of them met the definition of case, 70 males (51.9%), and 65 females (48.1%), giving an attack rate of 54.7% (Table 1). Table 2 gives the attack rates by class, ranging from 26.3% to 70.0%. Symptoms were abdominal pain (84.4%, 114/135), diarrhea (80.0%, 108), nausea (39.3%, 53), dizziness (34.1%, 46), vomiting (32.6%, 44), weakness (31.9%, 43), chill (28.9%, 39), fever (28.1%, 38), and others (4.4%, 6). Sporadic cases occurred at 5:00 p.m. of 25 September; the number of cases increased after 2:00 a.m. of 26<sup>th</sup>, and reached a climax between 4 and 6:00 a.m. The epidemiological curve showed that the incubation periods ranged from five hours to 47 hours, with a median of 18 hours and a mode of 18 hours.

Four HACCP (hazard analysis and critical control point) food factories namely the Weidahihsiang, Sungzhilin, Zhumin and Meijing, supplied the boxed

lunch of the day. Boarding students were given lunch by the school. A few students brought their own lunch to school. Questionnaire interview showed that 247 students had the boxed lunch of the Weidahsiang, and of them, 135 had developed symptoms, giving an attack rate of 54.7%. 18 students had the Sungzhilin boxed lunch, and one became ill, giving an attack rate of 5.6%. 17 students had the Zhumin boxed lunch and none became ill, giving an attack rate of 0.0%. 37 students had the Meijing boxed lunch and one became ill, giving an attack rate of 2.7%. 200 students had the school lunch and two became ill, giving an attack rate of 1.0%. Three students had their own lunch and none became ill, giving an attack rate of 0.0% (Table 1). More students having the Weidahsiang boxed lunch became ill, and the difference was statistically significant ( $p$  values  $<0.05$ ), indicating that the boxed lunch supplied by the Weidahsiang was most probably associated with the food poisoning outbreak.

Analyses of the food items eaten from the Weidahsiang boxed lunch on 25 September, as shown in Table 3, showed that the odds ratio of food poisoning of students who had the pork in green pepper was 1.72 times higher than the odds ratio of those who did not have the said food item, the 95% confidence interval being 0.98-3.03. The odds ratio of the students who had the corn-carrot-bean was 2.39 times higher, the 95% confidence interval being 1.38-4.17. The odds ratio of the students who had the bamboo shoots was 1.43 times higher, the 95% confidence interval being 0.83-2.47. The odds ratio of students who had the cucumber was 2.14 times higher, the 95% confidence interval being 1.23-3.75. The odds ratio of the students who had the eggs cooked with dried radish was 1.15 times higher, the 95% confidence interval being 0.65-2.03. The odds ratio of the students who had the chicken legs was 1.54 times higher, the 95% confidence interval being 0.87-2.72. The odds ratio of the student who had the rice was 1.06 times higher, the 95%

confidence interval being 0.58-1.93. The odds ratio of the students who had the tempura was 0.92 times higher, the 95% confidence interval being 0.33-2.59. The odds ratio of the students who had the hot dog was 1.25 times higher, the 95% confidence interval being 0.61-2.56. The odds ratio of the students who had the boiled dumplings was 1.25 times higher, the 95% confidence interval being 0.16-11.05. Of all, the 95% confidence intervals of the corn-carrot-bean and the cucumber did not cover 1.0, and were statistically significant. Cross analysis of these two food items by adjusting the food item cucumber found that the Mantel-Haenszel odds ratio was 1.96, indicating that the odds ratio of having the corn-carrot-bean was 1.96 times higher than that of not having it; the 95% confidence interval was 1.08-3.56, and was statistically significant. When the food item corn-carrot-bean was adjusted, the Mantel-Haenszel odds ratio was 1.62, indicating that the odds ratio of having the food item cucumber was 1.62 times higher than that of not having it; the 95% confidence interval was 0.87-3.03, which was, however, not statistically significant. That the likely food item causing the present food poisoning outbreak was the corn-carrot-bean was indicated.

A field visit to the Weidahsiang Food Factory on 27 September revealed that the factory was divided into four sections for food processing, the pre-processing section, the cooking section, the waiting section, and the packing section. The processing site was clean; and all utensils were washed and kept on portable racks, which were 30 cm above the ground. Cooking tools were clean and in good order. All food items though were kept in the same freezer, raw and cooked foods were kept separately. No vectors such as flies, rats and cockroaches were found. The area outside the pre-processing section (for processing raw food materials) was disorderly with thick grasses.

Vectors were likely to breed there. Health conditions of the kitchen staff were fair. No wounds were found on their hands.

Laboratory testing of the ten rectal swabs had isolated *Vibrio parahaemolyticus* type K6 in eight, and *Staphylococcus aureus* in one (no enterotoxin of *S. aureus* was isolated). No pathogenic agents of food poisoning were isolated from the vomit specimen. No pathogenic agents of food poisoning were isolated from the 15 food leftovers.

### **Discussion and Conclusion**

In September 2002 since school began, only one or two students in a day visited the school health room for any one of the symptoms such as abdominal pain, diarrhea, nausea, dizziness, vomiting or fever. In the present investigation, however, 139 students in 11 classes developed within three days after the incident one or more of the said symptoms, indicating that this was a food poisoning outbreak.

*Vibrio parahaemolyticus* has an incubation period of two to 48 hours, averaging 15 to 17 hours. 95% of cases will develop symptoms such as diarrhea, abdominal pain, fever, headache, nausea, vomiting, and some even bloody stool. Fatal cases are relatively rare<sup>(3,6,7)</sup>. Of the nine cases admitted to the Joseph Hospital of Huwei, four were blood tested. The WBC counts of three of them were higher than 10,000/ $\mu$ l, suggesting that the pathogenic agents of the present incident were bacterial. The questionnaire interview revealed that the attack rate of those who had eaten the Weidashiang boxed lunch was as high as 54.7%. Distribution of the incubation periods of the 135 cases (Figure 2) also showed a single peak, and ranging from five hours to 47 hours, with a median of 18 hours, suggesting that the outbreak was an infection from exposure to a common source of longer incubation period. Further, the

clinical symptoms of abdominal pain, diarrhea and nausea, and the isolation of *Vibrio parahaemolyticus* from rectal swabs suggested that the pathogenic agent of the present incident was *Vibrio parahaemolyticus*<sup>(3)</sup>.

Analyses of the food items suggested that the corn-carrot-bean was the cause of the outbreak. Though *Vibrio parahaemolyticus* was isolated in eight (80%) rectal swabs, no pathogenic agents of food poisoning (including *Vibrio parahaemolyticus*) were isolated from the food leftovers. *Vibrio parahaemolyticus* is generally found in fishery products<sup>(1)</sup>. They multiply by two folds in 12 to 18 minutes under optimal conditions (30-37°C). They can cause infection when the number of colonies reaches 10<sup>5</sup> per gram<sup>(8)</sup>. Poisoning by *Vibrio parahaemolyticus* is always due to inadequate freezing or cooking of fishery products, and eating them raw. Some reports suggest that *Vibrio parahaemolyticus* causes infection by indirect contamination of food through kitchen knives, chopping board, rags, utensils and fingers<sup>(2,8,9,10)</sup>. The boxed lunch supplied by the Weidahsiang on 25 September contained no fishery products. Why was the corn-carrot-bean the cause of the incident? The food supplied by the Weidahsiang, upon investigation, was either manufactured or processed by the factory and was not purchased from other sources, suppliers of vegetables, meat, cooked food and frozen food should have nothing to do with the incident. The only exception was, the corns, carrots, beans and meatballs came from a certain supplier. The supplier also sold fish balls and other fishery products. It was likely that the meatballs had already been contaminated by *Vibrio parahaemolyticus* before they reached the Weidahsiang. The meatballs were kept in the same freezer with the corn-carrot-beans, and whether they had been contaminated by *Vibrio parahaemolyticus* then required further investigation. If the corn-carrot-beans were contaminated, they would likely cause infection because chances were



that they would have not been properly cooked by quick frying for some 100 students at one time. The Weidahsiang failed to provide information on the total number of boxed lunch supplied on that day, to what customers they were supplied, and the food items of the boxed lunch. Whether there were fishery products in the boxed lunch supplied to others was unknown. Further investigations were required to understand when, in the course of preparation, processing or storage after production, the corn-carrot-beans were contaminated.

HACCP is a universally accepted excellent method of controlling food safety<sup>(10-14)</sup>. HA (hazard analysis) conducts a series of scientific and systematic assessment and analyses on food during their courses of manufacturing from the collection of source materials, through processing, packing, flow of products, to final products reaching the hands of consumers to understand the possibilities of all hazards. CCP (critical control point) develops effective measures and conditions to eliminate or reduce to the acceptable minimum of any food hazards at a certain point, step or procedure of high risks during the course of manufacturing. The Bureau of Food Sanitation of the Department of Health, to assure safety of food and beverages, is promoting the HACCP system. Execution of the HACCP is based on the Good Hygienic Practice (GHP). The GHP regulates, in accordance with Paragraph 1 of Article 20 of the Law on the Management of Food Sanitation, the work sites, facilities and quality assurance of food industries in the manufacturing, processing, seasoning, packing, transportation, storage, and sales of food or food additives. The Weidahsiang though is an HACCP food factory, for its failure to adequately comply with the GHP regulations, it had brought about a food poisoning outbreak. Supervision on food industries should not be relaxed even they are HACCP factories. Efforts should be made

to supervise food industries to develop self-control system of food sanitation to early detect problems in the course of food manufacturing, and not to wait until the final products are inspected or sample-tested for contamination, nor until food poisoning outbreaks occur.

### **Acknowledgement**

The authors wish to express their appreciation to the Yunlin County Health Bureau and the National Huwei Senior High School for their cooperation in the investigation.

**Prepared by: Wang HH<sup>1,2</sup>, Jiang DD<sup>3</sup>, Yu WP<sup>1,4</sup>**

1. FETP, Division of Surveillance and , CDC, DOH
  2. Nursing Department, National Taiwan University Hospital
  3. Division of Surveillance, CDC, DOH
  4. Meat Inspection Division, Bureau of Animal and Plant Quarantine, Council of Agriculture, the Executive Yuan
- For further communication, please direct correspondence to Jiang DD.

### **References**

1. Lin CH. *Vibrio parahaemolyticus*. J of Nosocomial Infection, 1997; 7(5): 299-302.
2. Department of Health. Incidents of Food Poisoning in the Taiwan Area, 2001.
3. Chin J. Control of Communicable Diseases Manual, 17<sup>th</sup> ed. Washington, DC, APHA 2000; 110-111, 202-212.
4. Rosner B. Fundamentals of Biostatistics, 2<sup>nd</sup> ed. Boston, Massachusetts, PWS Publishers 1986; 347-352.
5. Kelsey JL, Thompson WD, Evans AS. Methods in Observation Epidemiology. Oxford University Press, 1986; 148-178.
6. Adkins HJ, Escamilla J, Echeverria P, et al. Symptoms associated with diarrhoeal illness at San Lazaro Hospital, Manila in 1983 and 1984.

- Southeast Asian Trop Med Public Health 1989; 20(2): 207-214.
7. Joseph SW, Colwell RR, Kaper JB. *Vibrio parahaemolyticus* and related halophilic Vibrios. *Crit Rev Microbiol* 1982; 19(1): 77-124.
  8. Sanyal SC, Sen PC. Human volunteer study on the pathogenicity of *Vibrio parahaemolyticus*. Tokyo, Japan Saikon Publishing Co., 1974; 227-230.
  9. Pan TM. *Vibrio parahaemolyticus* and food poisoning. *Health Report*, 1995; vol 5 No. 1; 30-37.
  10. Lee TF, Chen KT, et al. Report on a food poisoning outbreak in a school. *Epidemiology Bulletin*, 1995, vol 11 No. 2, 23-32
  11. Tseng CH, Hsu CL, et al. Principles and Practice of HACCP. Huanan University of Science and Technology Press, 2000; 5-15, 44-47.
  12. Bureau of Food Sanitation, DOH. Food and Beverage Sanitation. [http://food.doh.gov.tw/life/default\\_com.html](http://food.doh.gov.tw/life/default_com.html)
  13. Appendix 8, Seafood HACCP Regulations, Fish and Fisheries Products Hazards and Controls Guidance, 3<sup>rd</sup> ed., 2001, US Food and Drug Adm, Center for Food Safety and Applied Nutrition. <http://www.cfsan.fda.gov/~comc/haccp4.html>.
  14. Pathogen Reduction/HACCP and HACCP Implementation, Food Safety and Inspection Service, US Dept of Agriculture. <http://www.fsis.usda.gov/oa/haccp/imphaccp.htm>.

**Table 1. Attack Rates by Food Factories**

Factory	Weidahsiang	Sungzhilin	Zhumin	Meiching	School	Self	Total
No. with symptoms	135	1	0	1	2	0	139
No. ate	247	18	17	37	200	3	522
Attack rate (%)	54.7	5.6	0.0	2.7	1.0	0.0	26.6

**Table 2. Attack Rates by Class**

Grade/Class	1/11	1/12	1/13	2/2	2/3	2/4	2/6	2/9	3/1	3/6	3/12	Total
No.	30	29	30	19	12	20	18	16	26	19	28	247
No. of cases	19	17	17	5	8	14	7	7	12	10	19	135
Attack rate (%)	63.3	58.6	56.7	26.3	66.7	70.0	38.9	43.8	46.2	52.6	67.9	54.7

**Table 3. Analysis of Single Food Items**

Food Item	Taken/Not Taken	Ill	Not Ill	Odds Ratio	95%CI																																																																																							
Pork with black pepper	Taken	95	65	1.72	0.98~3.03																																																																																							
	Not Taken	40	47			Corn-carrot-bean*	Taken	90	51	2.39	1.38~4.17	Not Taken	45	61	Bamboo shoot	Taken	84	60	1.43	0.83~2.47	Not Taken	51	52	Cucumber*	Taken	68	36	2.14	1.23~3.75	Not Taken	67	76	Eggs with dried radish	Taken	91	72	1.15	0.65~2.03	Not Taken	44	40	Chicken leg	Taken	95	68	1.54	0.87~2.72	Not Taken	40	44	Meatball	Taken	82	55	1.60	0.93~2.76	Not Taken	53	57	Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05
Corn-carrot-bean*	Taken	90	51	2.39	1.38~4.17																																																																																							
	Not Taken	45	61			Bamboo shoot	Taken	84	60	1.43	0.83~2.47	Not Taken	51	52	Cucumber*	Taken	68	36	2.14	1.23~3.75	Not Taken	67	76	Eggs with dried radish	Taken	91	72	1.15	0.65~2.03	Not Taken	44	40	Chicken leg	Taken	95	68	1.54	0.87~2.72	Not Taken	40	44	Meatball	Taken	82	55	1.60	0.93~2.76	Not Taken	53	57	Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110						
Bamboo shoot	Taken	84	60	1.43	0.83~2.47																																																																																							
	Not Taken	51	52			Cucumber*	Taken	68	36	2.14	1.23~3.75	Not Taken	67	76	Eggs with dried radish	Taken	91	72	1.15	0.65~2.03	Not Taken	44	40	Chicken leg	Taken	95	68	1.54	0.87~2.72	Not Taken	40	44	Meatball	Taken	82	55	1.60	0.93~2.76	Not Taken	53	57	Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110															
Cucumber*	Taken	68	36	2.14	1.23~3.75																																																																																							
	Not Taken	67	76			Eggs with dried radish	Taken	91	72	1.15	0.65~2.03	Not Taken	44	40	Chicken leg	Taken	95	68	1.54	0.87~2.72	Not Taken	40	44	Meatball	Taken	82	55	1.60	0.93~2.76	Not Taken	53	57	Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																								
Eggs with dried radish	Taken	91	72	1.15	0.65~2.03																																																																																							
	Not Taken	44	40			Chicken leg	Taken	95	68	1.54	0.87~2.72	Not Taken	40	44	Meatball	Taken	82	55	1.60	0.93~2.76	Not Taken	53	57	Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																																	
Chicken leg	Taken	95	68	1.54	0.87~2.72																																																																																							
	Not Taken	40	44			Meatball	Taken	82	55	1.60	0.93~2.76	Not Taken	53	57	Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																																										
Meatball	Taken	82	55	1.60	0.93~2.76																																																																																							
	Not Taken	53	57			Rice	Taken	98	80	1.06	0.58~1.93	Not Taken	37	32	Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																																																			
Rice	Taken	98	80	1.06	0.58~1.93																																																																																							
	Not Taken	37	32			Tempura	Taken	10	9	0.92	0.33~2.59	Not Taken	125	103	Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																																																												
Tempura	Taken	10	9	0.92	0.33~2.59																																																																																							
	Not Taken	125	103			Hot dog	Taken	26	18	1.25	0.61~2.56	Not Taken	109	94	Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																																																																					
Hot dog	Taken	26	18	1.25	0.61~2.56																																																																																							
	Not Taken	109	94			Dumpling	Taken	3	2	1.25	0.16~11.05	Not Taken	132	110																																																																														
Dumpling	Taken	3	2	1.25	0.16~11.05																																																																																							
	Not Taken	132	110																																																																																									

\*Statistically significant; 95% confidence intervals did not cover 1.0

