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Bacillus cereus Food Poisoning Outbreak in a Kindergarten School, Bangkok, Thailand, December 2009

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Abstract

On 18 Dec 2009, the Bureau of Epidemiology was notified that 20 students from a private kindergarten school were treated for vomiting and diarrhea. An investigation was conducted to verify the diagnosis, identify source of the outbreak, and implement prevention and control measures. We conducted a descriptive and retrospective cohort study. Medical records at the hospital were reviewed. We also interviewed students, teachers and cooks at the school. A case was a student in this school who developed vomiting with at least one of the followings: fever, diarrhea or abdominal pain from 18 to 22 Dec 2009. Twenty three clinical specimens (vomitus and rectal swabs) and food samples were collected, and sent to National Institute of Health for bacterial culture. Logistic regression was used to determine the food items associated with illness. Symptoms included vomiting (100%), abdominal pain (59%), diarrhea (31%) and fever (26%). *Bacillus cereus* was isolated from three out of six vomitus specimens as well as the sweet stewed egg and pork served for school lunch on 18 Dec 2009. Thus, this outbreak was due to *Bacillus cereus* (emetic form) and the common source was likely to be the sweet stewed egg and pork (adjusted OR 2.1, 95% CI 1.0-4.4). To prevent similar outbreaks in the future, people involved in food preparation and serving should emphasize on personal hygiene and sanitary food handling practices. School administrators should exclude symptomatic cooks and food handlers from cooking.

Keywords: Bacillus cereus, food poisoning, school meals

Background

Food poisoning is a serious public health problem throughout the world.[1] In Thailand, more than 50 food poisoning outbreaks are reported every year; most are associated with schools.[2] Only 17% of these outbreaks had a specific pathogen identified. The most common pathogens identified were *Vibrio parahemolyticus*, *Salmonella spp.* and *Bacillus cereus*.[3]

Bacillus cereus is widespread in nature and frequently isolated from soil and growing plants. It is also well adapted for growth in the intestinal tract of mammals[4] and causes toxin-mediated food poisoning.[5] The bacteria is associated with two distinct types of illness: emetic syndrome caused by a heat-stable toxin and diarrhea syndrome caused by a heat-labile toxin.[6] *B. cereus* has been established as an etiologic agent of food poisoning in Europe since 1950 and in the United States since 1968.[7,8]

On 18 Dec 2009, the Bureau of Epidemiology (BOE) received a notification from a Health Center (HC) in Bangkok that 20 students from School A, a private kindergarten in Laksi District of Bangkok, were treated at a private hospital due to vomiting within an hour after eating the school lunch. On 19-22 Dec 2009, a joint BOE and HC team conducted an

outbreak investigation to determine the diagnosis, confirm the outbreak, describe characteristics of the outbreak, identify possible source(s) of infection and implement effective control and prevention measures.

Methods

Epidemiologic Investigation

We began our investigation by reviewing the national disease surveillance records on diarrhea and food poisoning in Laksi District of Bangkok, and the medical records of students treated at the hospital on 18-22 Dec 2009. We also conducted active case finding by interviewing all students, teachers and cooks who were present at the school during our visit. In addition, information from each student was validated by interview with the child's homeroom teacher. Information included sex, age, time of the lunch eaten, type and estimated amount of food eaten, onset time of signs and symptoms, and treatment. A suspected case was defined as a person who ate the lunch served in School A on 18 Dec 2009 and developed vomiting with at least one of the following symptoms: fever, diarrhea or abdominal pain from 18 to 22 Dec 2009. A confirmed case was a suspected case with laboratory confirmation of vomitus for a pathogenic agent. We analyzed descriptive data using percentage, median, range and attack rate.

A retrospective cohort study was used to determine the risk factor(s) for illness. Cohorts were students of School A who went to the school on 18 Dec 2009. A case was a student in the cohort group who had vomiting with at least one of the following symptoms: fever, diarrhea or abdominal pain. Data was analyzed using Epi Info version 3.5.1 (US CDC). Univariate and multivariate analyses were conducted, including logistic regression to control the confounding factors. Variables included in the analyses were sex, school grade and all food items served for school lunch on 18 Dec 2009. We compared food-specific attack rates and odds ratios with 95% confidence intervals.

Laboratory Investigation

Clinical specimens were collected, including vomitus and rectal swabs from students, rectal swabs from teachers, and hand and rectal swabs from cooks. All specimens were sent to the Thailand National Institute of Health (NIH) for bacterial culture. Food remnants from the lunch served on 18 Dec 2009 were also obtained and sent to NIH for testing.

Environmental Investigation

We surveyed the school kitchen, refrigerators, water supply system and toilets. In addition, we interviewed cooks and observed food preparation such as cooking, serving and cleaning. Hand washing and eating habits of the students were also evaluated. Five samples of drinking water and pipe water were collected to measure the residual chlorine.

Results

Epidemiologic Results

The surveillance data from Laksi District showed that number of diarrhea illness and number of food poisoning were higher in December 2009 than that of the five-year median (Figure 1 and 2).

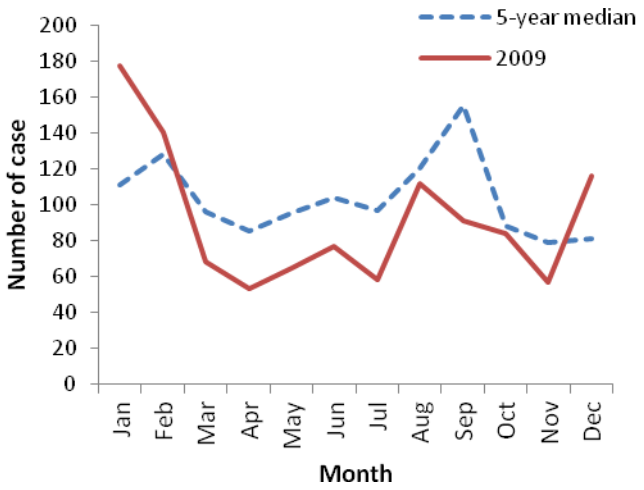

Figure 1. Number of diarrhea cases by month of onset compared with five-year median, Laksi District, Bangkok, Thailand, 2009

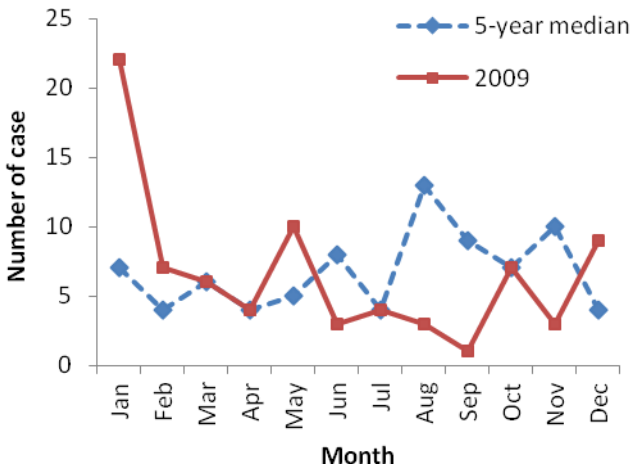

Figure 2. Number of food poisoning cases by month of onset compared with five-year median, Laksi District, Bangkok, Thailand, 2009

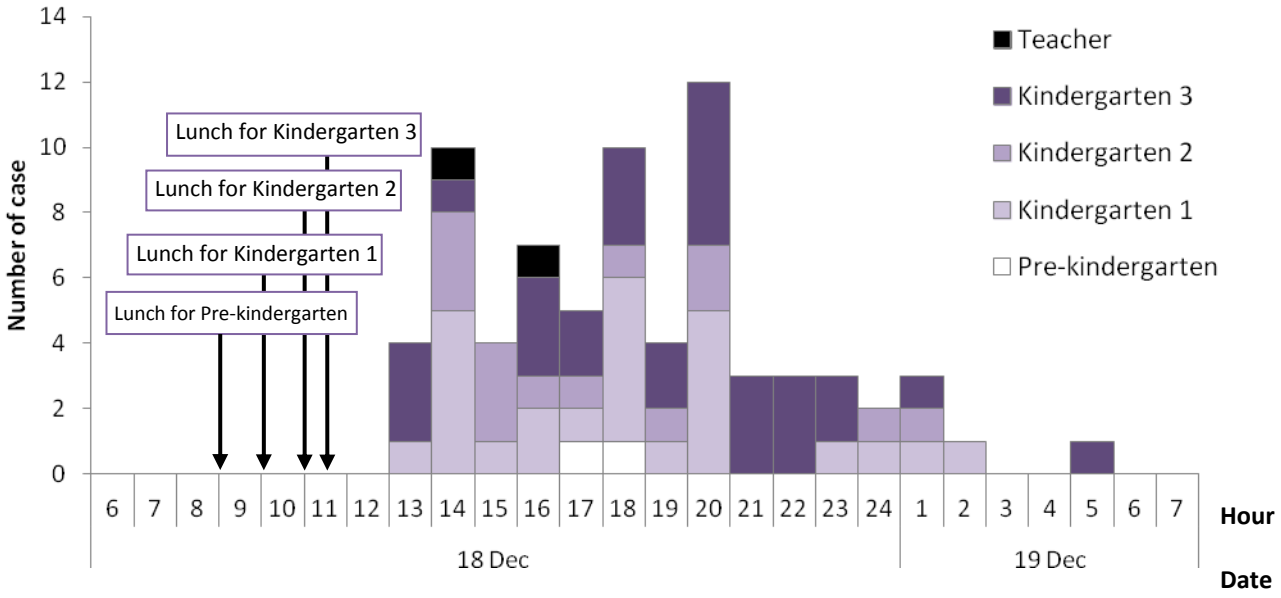

Figure 3. Number of food poisoning cases by occupation, grade and date and time of onset in School A, Bangkok, Thailand, 18-19 Dec 2009 (n=72)

The School A is a private kindergarten school with 265 students (133 males and 132 females), 36 teachers and five cooks. The overall attack rate was 27% (72/268). There were 70 cases (67 suspected and three confirmed) among students and two suspected cases among teachers. Sixty-four ill students received treatment at a hospital and 30 (47%) of them were hospitalized. Median age of the ill persons was four years, ranged from three to 35 years. The male to female ratio was 1:1. The highest attack rate was observed among students in Kindergarten 3 (Table 1).

Table 1. Number and attack rate of food poisoning cases by occupation and grade in School A, Bangkok, Thailand, 18-19 Dec 2009

Occupation	Number of case	Attack rate (%)
Student		
Pre-kindergarten	21	15
Kindergarten 1	101	28
Kindergarten 2	70	21
Kindergarten 3	73	40
Teacher	36	9

Clinical symptoms of illness included vomiting (100%), abdominal pain (59%), diarrhea (31%), fever (26%) and fatigue (19%). The epidemic curve is consistent with a common source outbreak (Figure 3). The first case had onset of symptoms at 1 pm on 18 Dec 2009 while the last case at 5 am on 19 Dec 2009. The school lunch served on 18 Dec 2009 was highly suspected to be the source of the outbreak. The median incubation period was seven hours (inter-quartile range 5.0 to 9.5 hours).

Two suspected cases among teachers were identified from active case finding. Both teachers had the same school lunch as the students on 18 Dec 2009. No other teacher ate the school lunch or became ill. The first ill teacher was a 35-year-old woman, who ate the school lunch at noon on 18 Dec 2009. She experienced abdominal pain and vomiting at 2:30 pm on that day. Her clinical symptoms improved without treatment. The second ill teacher was a 33-year-old woman, who had lunch with the first ill teacher, yet her abdominal pain and vomiting began at 4:30 pm on that day. She took Norfloxacin for one day and her symptoms improved.

Interviews indicated that two cooks had been ill before the outbreak occurred. One was a 60-year-old woman who had abdominal pain and diarrhea on 13 Dec 2009. She took an anti-diarrheal drug and her symptoms improved. The second cook was a 40-year-old woman who had vomiting and abdominal pain on 15 Dec 2009. Her symptoms improved after taking Norfloxacin for one day. Neither of these cooks were absent from work while they were ill.

In addition, the school provided soy milk to all students every morning and lunch. Lunch times were set by grade levels (Figure 3). Lunch on 18 Dec 2009 included rice, sweet stewed egg and pork, and watermelon. The amount of food served was different by grade levels. Students in pre-kindergarten, Kindergarten 1 and Kindergarten 2 received a bowl of rice (about three tablespoons), two pieces of sweet stewed egg (one egg divided into six pieces), a tablespoon of chopped pork and four pieces of pitted watermelon (1 cm^3 per piece). The amount of food for

students in Kindergarten 3 was double than that of students in lower grade levels. Watermelon served for Kindergarten 3 students were not pitted.

A total of 241 students were interviewed, including 70 ill students. Fourteen students (20%) who asked for more rice, sweet stewed egg and pork, and 19 students (28%) who asked for more watermelon during lunch on 18 Dec 2009 became ill later. The attack rates by level of exposure were shown in Table 2.

Table 2. Attack rates by amount of food served during lunch in School A, Bangkok, Thailand, 18 Dec 2009

Food item	Quantity of food	Attack rate (%)	Chi-square	P-value
Soy milk (glass)	0 1 2	10 24 43	10.7	0.005
Rice (bowl)	1 2 3	23 35 78	6.7	0.10
Sweet stewed egg (piece)	1 2 3 4 8	29 21 40 37 88	11.3	0.001
Pork (teaspoon)	0 1 2 4	100 22 100 75	14.4	0.001
Watermelon (1 cm^3 piece)	1 2 3 4 5	0 20 17 22 20	9.1	0.003
Bread (piece)	0 1 2 3	50 23 41 100	10.0	0.007

Table 3. Analysis of food served during lunch in School A, Bangkok, Thailand, 18 Dec 2009

Food item	Crude OR* (95% CI)	Adjusted OR** (95% CI)
Soy milk (glass)	2.2 (1.3-3.7)	1.7 (0.4-6.8)
Rice (bowl)	2.0 (1.3-3.0)	0.7 (0.2-2.5)
Sweet stewed egg (piece)	1.6 (1.2-1.9)	2.1 (1.0-4.4)
Pork (teaspoon)	2.1 (1.4-3.2)	0.8 (0.2-4.0)
Watermelon (1 cm^3 piece)	1.2 (1.1-1.4)	1.1 (0.8-1.3)
Bread (piece)	2.0 (1.2-3.5)	0.5 (0.1-2.1)

** Logistic regression*

*** Adjusted for sex, grade and all food items*

Univariate analysis showed that all the food items served during the lunch were associated with illness. However, after adjusting for sex, grade and all food items, eating more than one piece of egg had an adjusted odds ratio of 2.1 (Table 3).

Laboratory Results

Six vomitus and two rectal swab specimens from six ill students admitted to the hospital were obtained on 19 Dec 2009. Five hand swab and five rectal swab specimesn were obtained from all five cooks. Two rectal swab specimens were obtained from two ill teachers. Three specimens of leftover food (soup of sweet stewed egg and pork, watermelon and bread) from the lunch on 18 Dec 2009 were also tested.

Three vomitus specimens from ill students and the soup of sweet stewed egg and pork were tested positive for *B. cereus*. All other specimens were negative for bacteria.

Environmental Results

There were five cooks (A-E) in the School A. The Cooks D and E had their fixed job descriptions; D was a cook's helper and E prepared only soy milk. However, the duties of Cooks A, B and C were changed every day. The Cook D was ill on 13 Dec 2009 and B on 15 Dec 2009. The preparation process for the lunch served on 18 Dec 2009 began in the evening of 17 Dec 2009 (Table 4).

Table 4. Food handling processes for lunch served on 18 Dec 2009 in School A, Bangkok, Thailand

Date and time	Food handling processes	Food handler*
17 Dec 2009		
16:00-17:30	- Purchased food at market.	B
	- Prepared sweet stewed eggs, put into soup and allowed standing at room temperature for 2 hours before refrigerated it.	C, D
18 Dec 2009		
06:00-09:30	- Reheated sweet stewed eggs and cut eggs into pieces.	B, C, D
	- Boiled the chopped pork.	D
	- Prepared watermelon.	C, D
	- Combined all ingredients into children's bowls for lunch.	A, D
09:30-10:30	- Placed lunch bowls on serving trolley and covered with cloths.	A, D
10:30-11:00	- Served lunch to pre-kindergarten.	A, D
11:00-11:30	- Served lunch to Kindergarten 1 and 2.	A, D
11:30 to noon	- Served lunch to Kindergarten 3.	A, D

** Food handler B had diarrhea on 15 Dec 2009.*
Food handler D had vomiting and diarrhea on 13 Dec 2009.

The ingredients used for the sweet stewed egg and pork were water, sugar, garlic, star anise seed, coriander roots, cinnamon and dark soy sauce. The water was boiled with all other ingredients. The boiled eggs were divided into little pieces, with one egg into six pieces. The pork was chopped and boiled in another pot. The soup, the chopped pork and egg pieces were mixed together and served.

Our survey showed that there were two kitchens; one for making only soy milk and the other for cooking. Cooked and raw foods were kept in the same refrigerator which was opened frequently. Dishes and utensils were cleaned by hands, and were put on a table outside the kitchen to dry. We observed that many leaves and dust fell onto that area (Figure 4).

Figure 4. Table outside the kitchen to dry dishes and other utensils in School A, Bangkok, Thailand, 18 Dec 2009

Usually, all students eat lunch in the school cafeteria. However, on 18 Dec 2009, the cafeteria was used to prepare for a Christmas party. Thus, only pre-kindergarten students had lunch there on that day. All other students ate lunch in their classrooms. Cooks used the same trolley to carry food from the cafeteria to every classroom, passing through the playground. Students did not wash their hands before eating. Students could ask for more food if they want.

The school used tap water for washing dishes and hand washing. Drinking water was filtered. There was a toilet in every classroom and soap was provided. The residual chlorine level of two tap water samples (kitchen and toilet) and three drinking water samples (kitchen, cafeteria and classroom) were less than 0.2 ppm, which was lower than the standard level.

Discussion

In the past two years, three outbreaks of *B. cereus* have been reported to the Thailand Bureau of Epidemiology, including two outbreaks in 2009 and one in 2008. All of these outbreaks occurred in schools.[9] Common problems associated with all three foodborne outbreaks were long standing time before serving, inadequate reheating and not excluding cooks with gastroenteritis symptoms from handling food. Suspected foods of those outbreaks were fried rice, noodle and fish balls.

In this outbreak, our findings were consistent with *B. cereus* (emetic form) infection.[6,10] The median incubation period was short, and *B. cereus* was isolated from patients and the soup of sweet stewed egg and pork. In addition, the egg was identified as a risk factor by statistical association. Thus, the most likely source of infection in this outbreak was the sweet stewed egg and pork. It had been a long interval from preparation of food until serving. The food items could be contaminated at any point of several preparation processes because the cooks used bare hands and same equipment for handling of raw and cooked food, and left food at ambient temperature after being thoroughly cooked.

The fact that students in Kindergarten 3 had the highest attack rate might reflect the delay in serving their lunch (about two hours between reheating or cooking and serving) as well as larger portion of food than those given to the lower grades. The longer time interval from food preparation to serving could have provided more opportunity for the bacteria to multiply.

Limitations

We identified several limitations in this outbreak investigation. Since the outbreak occurred among young children, the information we collected from them might not be accurate because they might not remember the food items or understand the questions. Some of them could not describe all of their symptoms. Furthermore, it was less likely that the teachers could observe and remember what each student had eaten.

Exposure misclassification might reflect recall bias that teachers could remember the food items eaten by the ill children more than that of the other children. There might have been other confounders that we did not identify.

Since 1971, more than 40 incidents of *B. cereus* food poisoning associated with consumption of cooked rice have been reported,[11] yet in our investigation, none of the rice that was served at lunch on 18 Dec 2009 was available for testing.

Conclusion

A common source food poisoning outbreak occurred in a kindergarten school in Bangkok, Thailand during

December 2009. *B. cereus* was the causative organism. The sweet stewed egg and pork served for school lunch on 18 Dec 2009 was the likely source of infection.

Actions Taken

During our investigation, we provided health education to students, teachers and cooks about food poisoning and general care for persons with gastrointestinal symptoms. A special surveillance was launched for one day after the outbreak to detect more patients with gastrointestinal symptoms among students, teachers and cooks. No additional case of food poisoning was reported in this school since 19 Dec 2009.

Recommendations

Food hygiene and sanitation should be emphasized among all cooks and other food handlers in schools to wash their hands before handling of food, wear gloves while handling of food, use separate equipment for handling raw and cooked food such as cutting board, store raw and cooked food separately, and assure adequate refrigeration for safe food storage. In addition, they should not allow food to stand at ambient temperature after being thoroughly cooked. Students should always wash their hands before eating and after using toilet. School administrators should exclude symptomatic cooks and food handlers from cooking and provide medical check-up at least once a year. All water supplies and drinking water should be monitored for adequate chlorine level at all times.

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