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Background

Food poisoning is a serious public health problem in Thailand throughout the world. In Thailand, more than 50 food poisoning outbreaks are reported each year[1], most commonly associated with schools[2]. Even though only 17 % of these Thai outbreaks had a pathogenic agent identified in recent years, *Bacillus cereus* was among the common pathogen responsible for food poisoning outbreak after *Vibrio parahemolyticus* and *Salmonella* spp.[3]

Bacillus cereus is widespread in nature and is frequently isolated from soil and growing plants but it is also well adapted for growth in the intestinal tract of mammals[4] and causes a toxin-mediated food poisoning[5]. It 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]. It has been established as an etiological agent of food poisoning in Europe since 1950 and in the United States since 1968[7,8].

On 18th Dec 2009, the Thai Bureau of Epidemiology received notification from Health Center Accreditation 53rd (HCA), Bangkok that 20 students from a private kindergarten (School A), Laksi District, Bangkok, visited a private hospital because of vomiting within an hour after eating a school lunch. On 19th-22nd Dec 2009, BOE and HCA teams conducted an outbreak investigation to verify diagnosis and outbreak, describe characteristics of the outbreak, identify possible source(s) of infection, and implement effective control and prevention measures.

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Methods

Epidemiologic investigation : We began our investigation of diarrhea and food poisoning in Laksi District, Bangkok, by reviewing National Disease Surveillance (506 report) records and medical records for students from School A who visited a hospital on 18th- 22nd Dec 09. We also performed active case finding by interviews of all students, teachers, and cooks who were present when we visited the school. In addition, each student's information was validated with the child's homeroom teacher. The data we obtained about each student included gender, age, time the lunch was eaten, estimation of the amount consumed, onset of signs and symptoms, and treatment. A suspected case was defined as illness of any person in School A who experienced vomiting with at least one of the following symptoms; fever, diarrhea, and abdominal pain during 18th -22nd Dec 09. Confirmed case was a suspected case for which there was laboratory confirmation of a pathogenic agent.

A retrospective cohort study was employed to determine the risk factor(s) for illness case. The definition of cohort was students of School A who went to school during18th- 20th Dec 09. A case-patient was a student in the cohort who had vomiting with at least one other of the following symptoms: fever, diarrhea, or abdominal pain. Data were entered and analyzed using Epi Info program version 3.5.1(US. CDC.). Logistic regression was performed for both univariate and multivariate analysis. Confounders were considered and controlled by multivariate analysis. These variables were controlled: gender, grade, and all food items served on 18th Dec. We identified the possible risk(s) in terms of odds ratios with 95% confidence intervals.

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

Environmental investigation : We surveyed kitchen, refrigerators, water supply system, and toilets. In addition, we interviewed cooks and observed food preparation, cooking, serving and cleaning process. The students' behavior, such as hand washing and eating habits, were also inspected. Five samples of drinking and used water were collected to measure residual chlorine.

Results

Epidemiologic results

From the review, in Laksi District, numbers of cases of diarrheal illness and food poisoning were higher in December 2009 than the 5-year median (Figure 1)

School A is a private kindergarten (kin) school. There are 265 students (male 133, female 132), 36 teachers, and 5 cooks. The students are divided into 4 grades: 21 students in pre-kin, 101 students of 1st kin, 70 students of 2nd kin and 73 students of 3rd kin. We collected data from 241 students (91%), 22 teachers (61%), and 5 cooks (100%).

The overall attack rate was 27% (72/268). There were 70 students (67suspected and 3 confirmed cases) and 2 suspected cases among teachers. Sixty-four ill students visited a hospital, and 30 (47%) were hospitalized. Median age of the ill person was 4 years (3- 35 years old). Ratio male: female was 1:1.

The highest attack rate (AR) among student for the 3rd kin (40%) followed by 1st kin (28%), 2nd kin (21%), and pre-kin (15%). The AR of teacher was 9%.

Clinical symptoms of illness included vomiting (100%), abdominal pain (59%), diarrhea (31%), fever (26%), and fatigue (19%)

The epidemic curve shows a common source pattern (Figure 2). The first patient had onset of symptoms at 1 pm. on 18th Dec and the last patient had onset of symptoms at 5 am. on 19th Dec. Considering clinical symptoms, lunch on 18th Dec was highly suspected to be the source of the outbreak. The shortest duration from having 18th Dec lunch to onset of the first case was 1.30 hours (3rd kin) and the longest duration was 5 hours (pre-kin).

Two suspected cases among teachers were identified from active case finding. Both had the same school lunch as the students on 18th Dec. No other teacher had the school lunch. The first ill teacher was a 35-year old woman, who ate the school lunch at 12 noon of 18th Dec. She experienced abdominal pain and vomiting at 2:30 p.m. that day. Her clinical' symptoms improved without treatment. The second ill teacher was a 33-year old woman, who had lunch with the ill teacher but her symptoms of abdominal pain and vomiting did not begin until 4:30 pm. She took Norfloxacin for 1 day and her symptoms improved. Interviews indicated that 2 cooks had been ill before the outbreak occurred. One was a 60-year old woman who had abdominal pain and diarrhea on 13th Dec. She took an anti-diarrheal drug, and her symptoms improved. The second ill cook was a 40-year old woman who had vomiting and abdominal pain on 15th Dec. She took Norfloxacin for one day, and her symptoms improved. Neither of these cooks were absent from work while they were ill.

This school provides soy milk every morning and lunch to all students. Lunch times are set by grade (Figure 2). Lunch on 18th Dec. was composed of rice, eggs, and pork with brown sauce and watermelon. The amounts of food were different by grade, pre-kin to 2nd kin: 1 bowl of rice (about 3 tablespoons), 2 little pieces of egg (one egg divided into 6 little pieces), a tablespoon of chopped pork, and 4 pieces of pitted watermelon (1 cm^3 per piece). The amounts of food for 3rd kin are double quantity, and watermelons were non-pitted.

From analytic, 241 students were evaluated, including the 70 who were ill. At lunch on 18th Decmber, 14 students (20%) who later became ill asked for more rice, eggs and pork with brown sauce, and 19 students (28%) who later became ill asked for more watermelon. The food-specific attack rates were quite similar for all the listed food items (table1).

Univariate analysis showed that all the food items in the lunch were associated with illness, but after adjusting for potential confounders, e.g., gender, grade and all food items, we found that eating more than one little piece of egg led to an adjusted odds ratio of 2.14 (Table 1).

Laboratory results

Six emesis and 2-rectal swab specimens were obtained from six admitted sick students. Five hand-swab and 5 rectal-swab specimens were obtained from all 5 cooks. Two rectal swab specimens were obtained from 2 teachers who had been ill after eating the school lunch on 18th December. Three specimens of leftover food from the lunch on 18th Dec were also tested.

Three emesis specimens of students were positive for *B.cereus*, as was a specimen of one food item (brown soup). Other specimens were all negative for bacteria.

Environmental results

There were 5 cooks (A- E). D and E have fixed job descriptions: D is a cook helper and E prepares only soy milk, Job duties of A, B, and C change every day (Table 2). Cook B got sick on 15th Dec and D got sick on 13th Dec. The preparation processes of lunch on 18th Dec were started in the evening of 17th Dec (Table 3)

Kitchen area : From the survey, there were 2 kitchens, one for making only soymilk and other for cooking children's food (Figure 3). Cooked and raw foods were kept in the same refrigerator where it was opened very often. Dishes and utensils were clean by hands and were put on the table for dry. However, from our observation, there were many leaves fall down and much dust (Figure 4).

Food serving : Generally, all students will have lunch at the cafeteria. However, the cafeteria had been prepared for a Christmas's party on 18th Dec, thus only pre-kin students had lunch there. All other students had lunch in their classrooms. Cooks carried food by the same trolley from cafeteria to every classroom, by passing playgrounds. The foods were cover by cleaned cheesecloth. (Figure 5).

Eating behavior : Students can ask for more food and they use their own glass. They don't wash their hands before eating.

Water supply and toilet : The school uses tap water. Drinking water is distilled water. There is a toilet in every classroom, and soap is provided. Residual chlorine level in 2 samples of use water (kitchen, toilet) and 3 samples of drinking water (kitchen, cafeteria and classroom) were less than 0.2 ppm.

Discussion

In the past two years, three outbreaks of *B.cereus* have been reported to the Thai Bureau of Epidemiology including 2 outbreaks in 2009 and one outbreak in 2008. All of these outbreaks occurred in schools[9,10]. The common pitfalls found in the three outbreaks were food prepared long before serving, inadequate reheating, and not excluding cooks who have gastroenteritis symptoms from handing foods.

In the outbreak reported here, the findings consistence with *B.cereus* (emetic form) infection[11], as most clinical symptoms of patients were vomiting and abdominal pain. The median incubation period was short, and laboratory results found *B.cereus* in patients and in food. Eggs with brown soup were most likely cause of this outbreak because brown soup was found *B. cereus* and egg was identified as a risk factor by statistical association. The contamination could occur at any several points in the preparation and serving because the cook cooked, cut eggs with bare hands, serving process as the cooks carried food passed the ground, this can contaminated with *B.cereus* because of its spore are commonly found in soil[11,12], utensils drying because dishes and utensils were put on the table outside kitchen that's has much dust.

The 3rd kin students had the highest attack rate, the possibility is the longest duration from cooking to serving (about 2 hours), cause growth and spore forming of *B. cereus* and food providing of this grade's students were double amount of other grades.

Limitations

We anticipated several limitations in this outbreak investigation. The outbreak occurred among small children, so the information we got from them might not be accurate because they might not remember the food items and might not understand the questions asked. Some of them could not describe all of their symptoms and signs.

Exposure misclassification might reflect recall bias on the part of teachers who could recall what the ill children had eaten for lunch more accurately than they could recall what the well children had eaten. There might have been confounders we did not identify.

Since 1971, more than 40 incidents of *B.cereus* food poisoning associated with the consumption of cooked rice have been reported[13] but in our investigation no rice was left for testing that has been served at lunch on December 18th.

Recommendations

During the investigation, we gave health education to students, teachers, and cooks about food poisoning, primary care of gastrointestinal symptoms. The surveillance was launched 1 days after the outbreak occurred for seeking patients with GI symptom(s) among students, teachers and cooks.

Several potential risks of contamination were observed. Food hygiene and sanitation should be emphasized in school, including cooks requiring that wash their hands before handling food, wear gloves while cooking, use separate equipments for handling of raw food and cooked food such as cutting board, store raw food and cooked food separately, and assure that refrigeration is adequate to store food safety. In addition, foods should not remain at ambient.

temperature after being cooked and foods should be thoroughly cooked. A sick cook should not use an antibiotic or anti-diarrheal drug without visiting a physician. Students should always wash their hands before eating and after using the toilet. School administrators should exclude symptomatic cooks from cooking and should require them to have a medical check-up each year. Water supplier should be confirmed as having adequate chlorine levels at all times.

Conclusion

A common-source food poisoning outbreak occurred in a kindergarten school in Bangkok, Thailand, in December 2009. *B.cereus* was the causative organism. Lunch of 18th Dec was the most suspected. Major recommendation should emphasize food sanitation, especially food storage, preparation including cooking and serving. No additional cases of food poisoning for persons associated with this school after 19th Dec 09.

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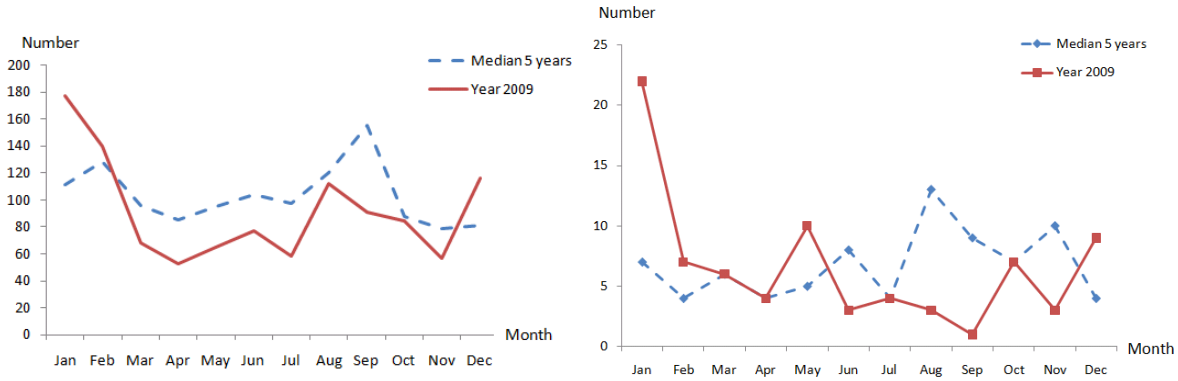

Figure 1 Number of diarrhea (left picture) and food poisoning cases (right picture) by month of onset, Laksi District, Bangkok, Thailand, 2009 compared with 5-year median

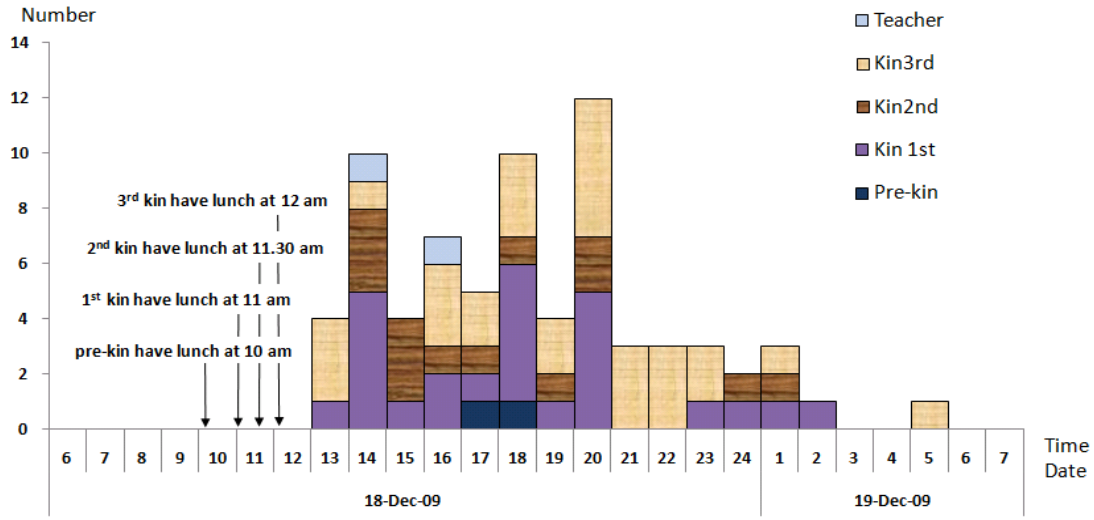

Figure 2 Number of food poisoning cases by occupation, grade, date and time of onset, School A, Bangkok, Thailand, 18th-19th December 2009 (n= 72)

Table 1 Attack rates and results of analysis by specific food, School A, Bangkok, Thailand, 18th Dec 2009

Food item	Attack rate (%)	Crude OR* (95% CI)	Adjusted OR** (95% CI)
Soya milk (glass)	20	2.20 (1.30- 3.74)	1.70 (0.42- 6.80)
Rice (bowl)	20	1.99 (1.33- 2.99)	0.65 (0.17- 2.48)
Egg (little piece in brown soup)	22	1.55 (1.24- 1.94)	2.14 (1.04- 4.41)
Pork (teaspoon)	20	2.10 (1.38- 3.20)	0.78 (0.16- 3.91)
Watermelon (1 cm3 piece)	20	1.23 (1.08- 1.39)	1.05 (0.82- 1.32)
Bread (piece)	19	2.02 (1.17- 3.47)	0.50 (0.11- 2.14)

* From logistic regression

** Adjusted for gender, grade, all food items

Table 2 Cooks' duty schedule of school A, Bangkok, Thailand, during 17th - 18th Dec. 2009

Date	Go to market	Main cook	Serving food	Cook helper	Soy milk maker
17th Dec	B	A	C	D	E
18th Dec	C	B	A	D	E

Table 3 Food preparation, cooking and serving processes of lunch menu of 18th Dec 09, school A, Bangkok, Thailand

Date and time for preparing and cooking processes of lunch of 18th Dec 09, School A, Bangkok	Processes of preparing, cooking, serving food and symbol of cooks
17th Dec 09 4.00 pm. - 5.30 pm.	- B bought one hundred eggs, pork chop and watermelon from the market, - C and D boiled and peel the eggs then put them in brown sauce, boiled and left for 2 hours before refrigerated.
18th Dec 09 6.00 am. -9.30 am.	– B reheat eggs in brown soup then C, D was scooped and cut an egg in to six pieces by thread that she heated in boiling water before cut and she cut with bare hands, then put cut eggs in empty pot for serving. – B heated pork chop in boil water and put in empty pot. – D cooked rice in a big pot. – C and D cleaned, cut watermelons to small pieces with bare hands (use the same cutting board with meat) and put in empty pot. – While serving, A and D put rice, eggs, pork chop and brown soup together in children's bowl with different serving spoon.
9.30 am. - 10.30 am.	- A, D put food pots in the trolley and covered the pot by cleaned cloths, moved to cafeteria, divided food to small dishes and covered by cloths.
10.30 am. - 11.00 am.	- A, D serving to prekindergarten
11.00 am. - 11.30 am.	- Moved trolley to 1st , 2nd kindergarten's classrooms and serving food
11.30 am. - 12.00 am.	- Moved trolley to 3rd kindergarten's classrooms and serving food

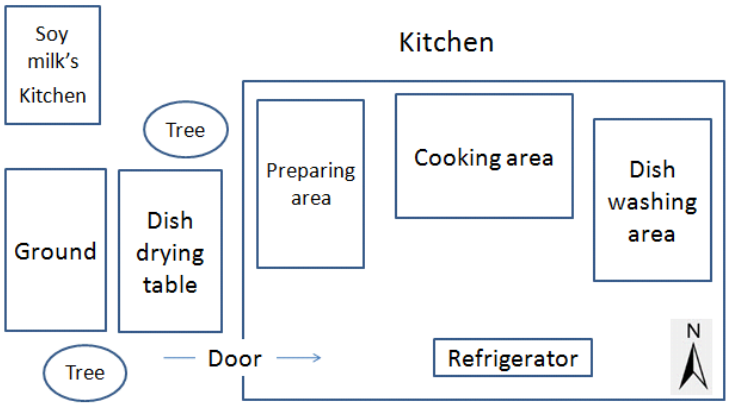

Figure 3: Kitchen area of School A, Bangkok, Thailand, 18th December 2009

Figure 4 Table is locating outer the kitchen for dry dishes and other utensils, School A, Bangkok, Thailand, 18th December 2009

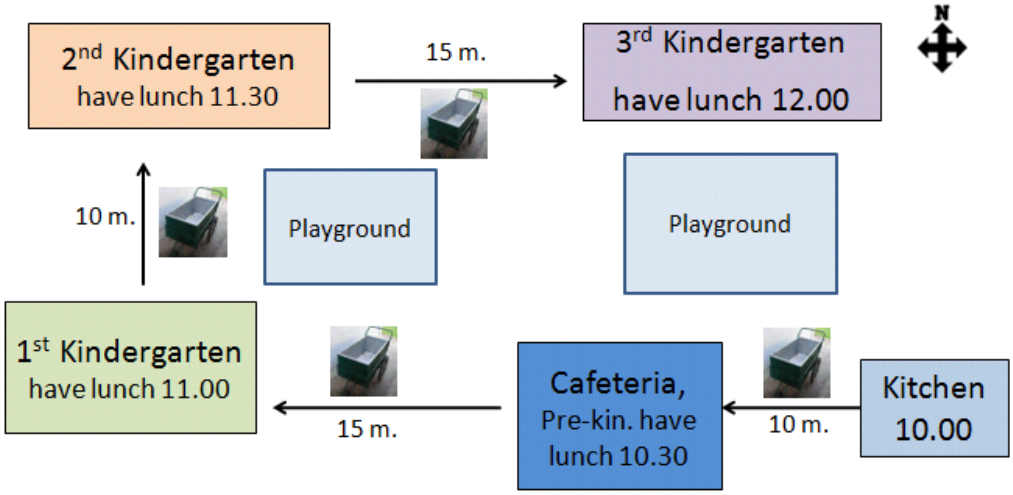

Figure 5 Flow of food carrying on 18th Dec of school A, Bangkok,Thailand, 18th December 2009