Research Article

Waterborne Food Poisoning Outbreak of *Bacillus Cereus* in Primary School Sabah East Malaysia

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Abstract

On 15 Feb 2012, food poisoning outbreak occurred in a primary school that never reported any incident before. A team was sent to identify the risk factor and to institute control measures. A retrospective cohort study design was applied. All persons who attended the school canteen meal on 15 Feb 2012 were interviewed, using a standard questionnaire. Environmental investigations included observations of kitchen, water supply, sanitation, food-handling procedures and the collection of environmental samples for microbiological analysis. 33 of 188 people had upper gastro-intestinal symptoms. All were students whereas school staff members ate food from outside the canteen. Mean incubation period was 30 minutes. Of 15 foods, *nasi kuning* was associated with the outbreak, RR 3.8 (95% CI 2.19-6.56). Kitchen cleanliness was poor. Temporary shortage of water supply caused them to use untreated water from the village well. Food handler practiced unhygienic food preparation. *Bacillus cereus* was isolated from water tank, preparation table surface and hand towel. *Nasi kuning* was most likely the source of *B. cereus sp.* outbreak probably from cross-contamination during food handling as a result of poor hygienic practices and using untreated water tank. The outbreak stopped with closure of school canteen. Food premises inspection must ensure all water is potable.

Keywords: *Bacillus cereus sp*., Food poisoning outbreak, Waterborne, Primary school.

Introduction

Food poisoning by *Bacillus cereus* mostly presented with acute nausea vomiting, abdominal pain and loose stool. It continues for less than a day and is rarely fatal. In epidemic, diagnosis is made by cultures to estimate organisms present. If organisms are isolated from two or more ill persons, it may confirm the diagnosis. Availability of toxin test is valuable.[1] *B. cereus* is a bacterium of Gram-positive, aerobic spore forming rod and mostly β-hemolytic strain. It produces exotoxins and manifests as emetic or diarrheal enterotoxin. Emetic type is highly stable, resisting heat, pH and proteolysis.[2] In animal studies, 9-12 µg/kg is found to be an emetic dose.[3] Emetictoxin production is linked to temperature 12-15°C[4] and starts sporulation.[5] Liver failure cases and *B. cereus* poisoning had been associated with mitochondrial toxin.[6] In laboratory diagnostic, it can be grown aerobically at 37°C on blood agar culture media, and it manifests as ground glass appearance.[7] The real incidence of *B. cereus* illness is unknown due to clinical similarity of *Clostridium perfringens* (diarrheal) or *Staphylococcus aureus* (emetic), and probably of under-diagnosis because of minimal symptoms. In USA, only 1.5% *B. cereus* illness was reported from 1990 to 2002, whereas in Norway, 33% of foodborne cases were reported from 1988 to 1993.[8] Hungarians liked to eat spicy meat attributed to top three common causes of *B. cereus* illness from 1960 to 1968. Improper food storage caused *B. cereus* spores to survive.[9] Food poisoning in Kota Kinabalu schools had frequent episodes and a cause for concern.

Episode of acute food poisoning outbreak in Kota Kinabalu school canteen happens every two months in different schools. Most of the canteen operators are aware about the minimum health needs and routine enforcements by the authorities. Student's absenteeism and parent concern are implicated.

'L' primary school in Kota Kinabalu comprised of 320 students and 30 school staffs members, who had never reported any food poisoning episode. The school was equipped with treated water, good sanitation and proper waste disposal. The ministry of education gives food supplement packs everyday during recess time as part of the subsidized program to 118 eligible students. The school canteen prepares the food which is only given to eligible students from school assessment during early part of the year. On 15 Feb 2012, 4.50 pm district public health office received a notification that six students from 'L' primary school had sudden onset of abdominal pain, vomiting and diarrhea. All were treated as outpatients. The event was preceded by eating food from their school canteen at about 10.00 a.m. the same day. Rapid assessment team from health office was activated on the same day to verify the outbreak, followed by outbreak investigation. The objectives of the investigation are to determine the extent of the outbreak and to describe the distribution of cases according to the place, person and time of the illness. It also tried to find out the most likely source of the outbreak, organism that caused outbreak and suggest preventive measures for future outbreaks.

Methodology

Based on preliminary information, we defined a case as any person among students and teachers from Primary School 'L' who developed any of the following symptoms: acute diarrhea (three or more loose stools within 24 hours), abdominal pain or vomiting within 48 hours after eating meal served, and had any meal three days before 15 Feb 2012 from the school canteen. District health officer and team searched the case by telling 'L' primary school administrator to provide a name list of students who had attended school canteen. Students were identified and called through classroom announcement and instructed to gather in the school hall. Ten interviewers among health inspectors from district health office were briefed about the questionnaire; they interviewed students by face-to-face method. The contents of customized questionnaires collected include information on class grade, gender, clinical symptoms, time of onset of symptoms and consumption of food items in the past three days. Apart from interview, medical records of cases were reviewed at the nearest health clinic. Incidences by class level and gender were calculated and epidemic curve was also created. Based on descriptive epidemiology findings, a hypothesis was generated that one or more food items served at the canteen were contaminated with the causative agent. To test this hypothesis, a retrospective cohort study was conducted. The study population defined as person who ate at the canteen on 13-15 Feb 2012 and did not have any symptoms prior to eating in the canteen. The expose group means the interviewee ate the food based on the menu list. Information on demographic characteristics and potential risk food items were compared between exposed and unexposed group. Overall attack rate was calculated based on case definition. The analysis used was univariate for food items calculation of relative risk (RR), 95% confidence interval (CI), using Chi-square tests and p-value <0.05 was considered as statistically significant. The survey was conducted from 16 to 20 Feb 2012. The data was analyzed by using SPSS. The vomitus sample was collected from person with suspected food poisoning and sent to laboratory at Public Health Lab Kota Kinabalu for isolation and detection of common possible entero-pathogens. Bacteriological investigation included direct and Gram staining and culture in basic and selective media. Health inspector and food technologist conducted sampling suspected served food as a proxy, environmental sampling of utensils, tables, hand towels, and water tap and tank. Samples from food handler including nasal and finger swabs were sent to public health laboratory. Evaluation of the kitchen cleanliness was conducted with standardized evaluation format. The food technologist conducted the HACCP assessment.

Results

33 cases of 188 students met the clinical case definition who had attended school canteen meal on 13-15 Feb 2012 with overall attack rate of 17.5%. The most common symptoms were vomiting, abdominal pain, headache and some diarrhea symptoms. Almost all cases were from level 2 (standard 4-6). They had later recess time compared to level 1 and probably had different food batches. There was no difference between the proportion of male and female. The first case-patient developed symptoms at 10.30 a.m., 15 Feb 2012 and outbreak peaked at 10.45 a.m. The shape of the epidemic curve suggested a point source outbreak. The mean incubation period from the time of food being served at the school canteen to the

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onset of illness was 0.5 hours (range 0.5-4.0 hours). No new cases were observed after 48 hours from exposure. All cases were treated as outpatients. In-depth interviews with case patients claimed that they had started the meal at 10 a.m. during recess time, and most of them had consumed *nasi kuning* 15/34 (44.1%) from the canteen. The hypothesis was any person who had meal in the school canteen on 15 Feb 2012 and had symptoms of gastrointestinal tract was exposed to food components of recess meal which contained causative agent (see Figs. 1-3).

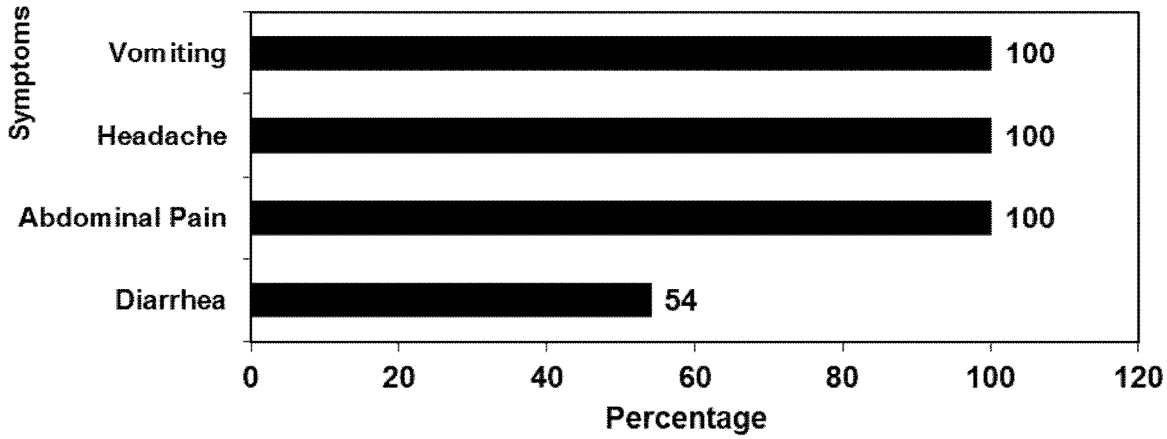

Figure 1.Percentage of Cases with Symptoms (n=33)

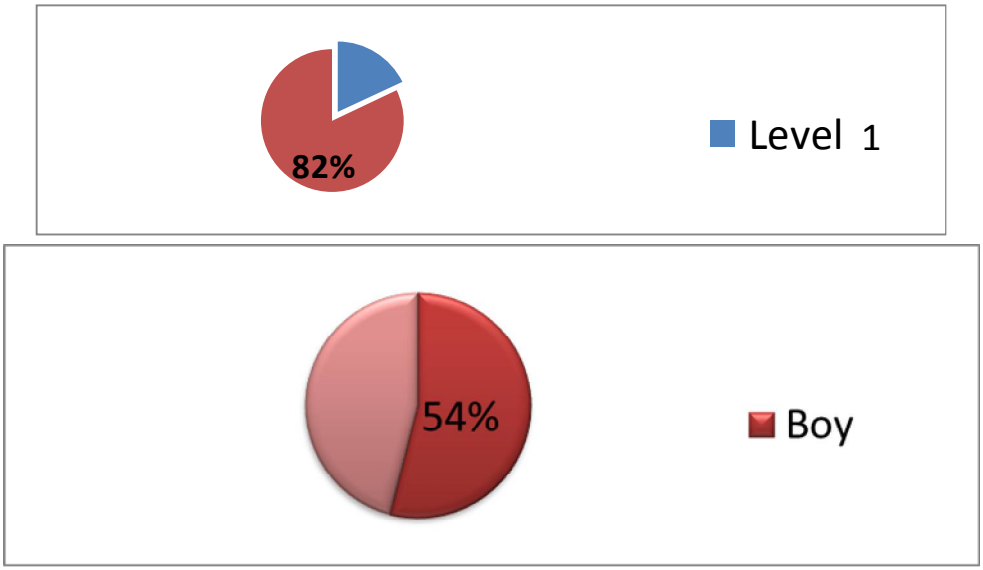

Figure 2.Percentage of Student Level (Standard) and Gender among Cases (n=33)

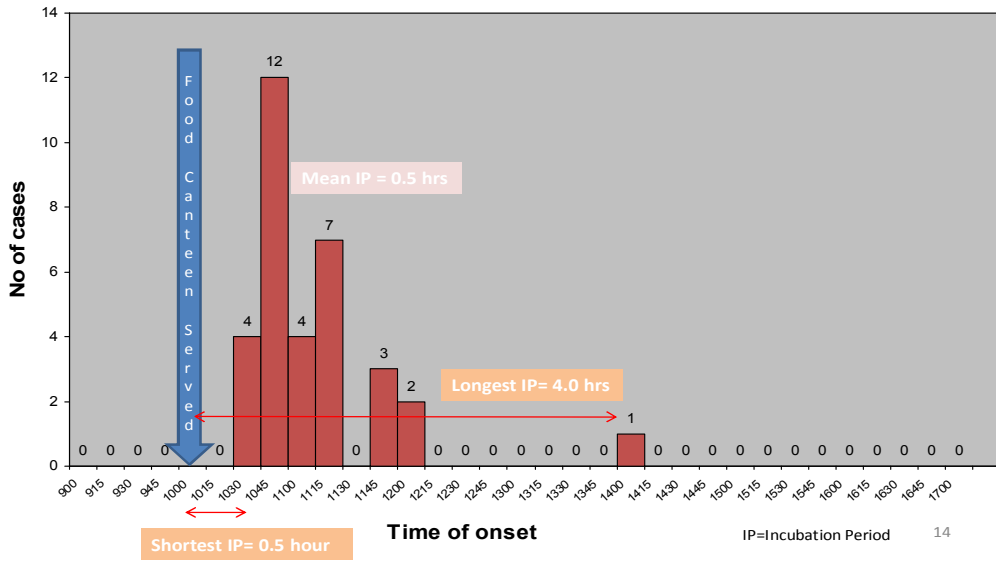

Figure 3.Epidemic Curve of the Food Poisoning Outbreak at Primary School Canteen, 15 Feb 2012 (n=33)

Based on the food eaten from the menu list of food supplement program, a total of 15 food items were recorded from food recall between 13 and 15 Feb 2012. 188 students had eaten at least one of the 15 types of food and were recruited for the cohort study. Anyone who ate *nasi kuning* 3.8 times (RR: 3.8, 95% CI 2.19-6.56) was more likely to develop symptoms than those who did not; this relative risk was statistically significant (see Table 1).

Table 1.Food-Specific Attack Rates and Relative Risks of Acute Food Poisoning in 'L' Primary School Canteen, 15 Feb 2012

No.	Food	Eat			Did not Eat			RR	95% C.I
		Ill	Not Ill	Attack Rate (%)	Ill	Not Ill	Attack Rate (%)		
1	Spring rolls	1	3	25.00	29	155	15.76	1.59	0.28-8.94
2	Turmeric Rice *(nasi kuning)*	15	16	48.39	20	137	12.74	3.80	2.19-6.56
3	Chicken Dates	2	3	40.00	29	154	15.85	2.52	0.82-7.76
4	Fried Rice	1	3	25.00	29	155	15.76	1.59	0.85-2.94
5	Nasi goreng	3	7	30.00	25	153	14.04	2.14	0.77-5.88
6	Doughnut	2	3	40.00	31	152	16.94	2.36	0.77-7.24
7	Fried Vermicelli	6	14	30.00	27	141	16.07	1.87	0.87-3.96
8	White Rice	3	5	37.50	31	149	17.22	2.18	0.84-5.63
9	Fried Wings	2	4	33.33	30	152	16.48	2.02	0.62-6.56
10	Sausages	2	5	28.57	29	152	16.02	1.78	0.52-6.02
11	Fried Nuggets	2	5	28.57	31	150	17.13	1.67	0.49-5.61
12	Fried Noodles	5	18	21.74	27	138	16.36	1.33	0.56-3.10
13	Cold Drinks	1	8	11.11	26	153	14.53	0.76	0.11-5.02
14	Mango Syrup	1	8	11.11	30	149	16.76	0.66	0.10-4.32
15	Popcorn	1	11	8.33	28	148	15.91	0.52	0.07-3.52

N=188

Of 33 cases, only two students had vomitus samples from suspected food poisoning which were sent to public health laboratory. The results showed one positive culture for *B. cereus*. The other sample showed no growth. This may be because of inadequate vomitus sample. Rapid test for toxin was used to detect *B. Cereus* toxin. No hematology or biochemistry test was conducted (see Table 2).

A week before the outbreak, the school had suffered shortage of water supply. The canteen operator had brought water from outside school for washing and cooking. In-depth interview of canteen operator

showed that the source of water was from the nearby village well. On-site visit found the water was not chlorinated and was poorly preserved. The water samples cultured positive for *B. Cereus* (see Table 3). The potable water was mainly for washing and cleansing, whereas for drinking purposes bottled water was used. *Nasi kuning* preparation was studied by the food technologist (see Fig. 4). The rice pack was bought from the nearby supermarket and stored in the canteen owner's house. A Few packs of rice were sent to the canteen every morning. The rice was usually rinsed at the canteen backyard by using water tank as early as 5.00 a.m. The water tank supplied water from village well during the events. The rice was inadequately cooked for 20 minutes, then transferred and stored inside the enclosed cool box for 3 hours. At 9.00 a.m., the rice was prepared and divided into few small plastic packets. The preparation table was positive for *B. cereus*. *Nasi kuning* packet had been served on the table and preparing for students to take it during recess time at 10.00 a.m. The canteen has four operators. Only one had attended food-handling course. Nobody had annual health check-up and was vaccinated for anti-typhoid. One temporary cook was hired for one week before the outbreak. Nobody had upper respiratory tract infection or acute gastroenteritis symptoms during the outbreak. During inspection on notification, the leftover food and drinks were disposed of and all equipment and food utensils had been cleaned. Few leftover food such as chicken dates, fried chicken wings, and few items of dried food were sampled and sent to the laboratory. No food sample was positive for *B. cereus*. Kitchen hygiene and sanitation assessment was rated as 60% and considered as below acceptable level. Food handler practiced unhygienic food preparation by not wearing standard cooking outfit. The hand towel used by food handler was positive for *B. cereus*. The limitation of this investigation was canteen operators had cleared up all the food and had cleaned the kitchen before rapid assessment team arrived.

Table 2.Laboratory Results

Sample Category	Sample Type	Result
Food	Chicken dates	Negative for *B. cereus*
	Fried wings	Negative for *B. cereus*
	Fried peanut	Negative for *B. cereus*
	Fried anchovy	Negative for *B. cereus*
	Chocolate Bun	Negative for *B. cereus*
Drink	Cold drink	Negative for *B. cereus*
	Mango syrup	Negative for *B. cereus*
	Sarsi syrup	Negative for *B. cereus*

Table 3.Environmental Sampling Results

Sample Category	Sample Type	Result
Kitchen environment samples	Food preparation table	Positive for *B. cereus*
	Gas stove handler	Negative for *B. cereus*
	Chopping board	Negative for *B. cereus*
	Serving food table	Positive for *B. cereus*
	Backyard table	Positive for *B. cereus*
	Pipe outlet	Negative for *B. cereus*
	Blender	Negative for *B. cereus*
	Washing hand sink	Negative for *B. cereus*
	Hand towel	Positive for *B. cereus*
Water supply	Water tank	Positive for *B. cereus*
Food handler	Finger nail	Negative for *B. cereus*
	Nasal swab	Negative for *B. cereus*

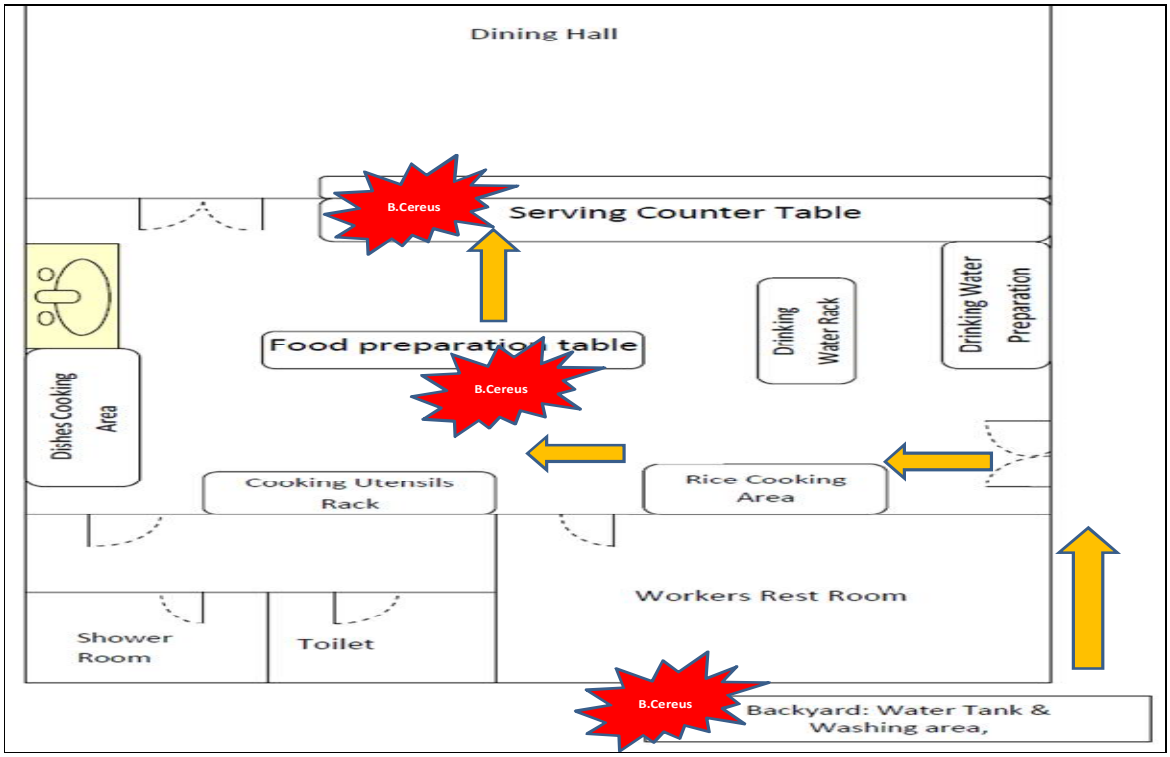

Figure 4.Aerial View of Turmeric Rice Preparation Flowchart

Discussion

The outbreak was verified with 17.5% of students who came down with similar symptoms suggestive of an outbreak of food poisoning from common point source. Various upper gastrointestinal tract symptoms were suggestive of entero-pathogenic organism involvement with incubation period between 0.5 and 4 hours. There is no specific group described as being of special risk for *B. cereus* food-borne disease. Study has postulated that a person with low peptic acidity might be prone to *B. cereus* diarrheal disease [10]. In this outbreak, anyone who ate *nasi kuning* was 3.8 times more likely to develop symptoms than those who did not. *Bacillus Cereus sp*. was positive from food handler hand towel, food preparation, serving tables and water tank. Cross contamination from water tank and inadequate cooking at canteen might be possible causes of outbreak. Shortage of water supply caused them to use untreated water. Temporary food handler practiced unhygienic food preparation and longer holding time. Inadequate cooking time and delayed cooling probably causing population and packing will increase *B. cereus* food poisoning.

Study has found that *B. cereus* spores are able to survive in hot environment and eliminating other species of vegetative bacteria.[11] The spore is not easily removed by regular cleaning of surfaces.[12] Due to spore resiliency, any food with pH >4.8 cannot be excluded as a *B. cereus* vehicle.[13.] Poor compliance to standard food preparation procedures such as inadequate cooling or storage at ambient temperature at <60°C, might cause *B. cereus* growth.[14] All of these faults were observed in the assessment of the school canteen kitchen. *Bacillus cereus* poisoning of emetic type is commonly associated with consumption of fried and cooked rice.[13] pasta, pastry and noodles[15], whereas consumption of roteinaceous foods, sauces and vegetables, meat products, soups, puddings and milk products[16] are associated with diarrhoeal type. The differences reflected *B. cereus* poisoning types with variety of food vehicles.[13] In 1955, Hauge decribed one outbreak episode attack rate was 82%, others 50-75%.[17] Most persons who consumed contaminated food became ill and low risk of secondary cases. *Bacillus cereus* poisoning of diarrheal type mostly happened in institutions such as schools.[18] There were five outbreaks in Britain implicating *fried rice* as the vehicle.[19] It was associated with food preparation in *"takeout"* restaurants, consumed pasta salad, mayonnaise, turkey loaf, hot chocolate from vending machines, boiled beef, sausage, chicken soup, macaroni cheese, vanilla sauce, and puddings. In agricultural product such as rice, 25-50% *B. cereus* is normal flora and abundance in soil.[20] Most of the

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contamination occurs before cooking. If the food is prepared at 30-50°C and slow cooling, *B. cereus* will germinate and multiply.[21] Studies have found that 93% of sampled cooked rice had viable *B. cereus*, about less than 105/g.[22] Human feces do not play a role in transmission of *B. cereus,*[23] even though up to 40% of asymptomatic person had *B. cereus* in the stool.[24] The short incubation period with high attack rate provides support for *B. cereus* pathogenic mechanism.[18] The emetic type ranged from 1 to 6 hours and predominant upper gastrointestinal tract symptoms. Diarrhea only affect one-third of them. Culture of suspected food product is the way to relate outbreak. It is considered significant if the suspected food product contains more than 105/g *B. cereus* toxin.[25] Isolation of *B. cereus* in a healthy person is not appropriate for confirmation unless negative stool cultures.[24] Phage typing is a reliable source to implicate source.[26,27] In summary, *B. cereus* a spore forming bacteria causes food poisoning illness and an emetic type. It is always present in uncooked rice and heat-resistant spores may survive during cooking. In this outbreak, the most likely mechanism of poisoning is by cross-contamination; the spores of *B. cereus* are available at different surfaces, causing hard to control. Contamination of *nasi kuning* probably occurred on the preparation table and further cross-contamination during serving food. The risk factors of outbreak are food handler who was on temporary basis, most of them had health status unknown and food handlers did not take care of their personal hygiene. Apart from food handler, another risk factor was the food preparation during rinsing rice at the backyard by using contaminated water tank, keeping at room temperature before food being served and possibly rice was undercooked. Lastly, the water supply was untreated water from village well was the main risk factor for this outbreak.

We instructed school canteen to be closed on 15 Feb 2012 for two weeks under PCID Act 1988.[28] We gave them notice for major cleaning. Public health officer gave health talks to all food operators on hygiene. They need to do health check-up and get vaccinated within a week. District health educator had organized health talk and pamphlet distribution to most school children. The students learned about spoiled food identification and method of poisoning notification to school administrator. Acute Gastroenteritis surveillance has showed a declining trend toward the mid-year 2012. Village well was closed on 18 Feb 2012. The community was aware about the situation and agreed to be closed and buried it. We did follow up a few months later and found it was permanently demolished.

We recommend other school canten operators to submit their health certificate copies to district health office. Routine practice of cooling rice or any food at room temperature should be changed; we advised them to keep food at proper temperature above 60°C. While serving food, we strongly advised them to use the warmer. We urged the school adminisration to ensure all water sources for food preparation are safe. We also provided health office hotline to them if they suspect any food contaminats or ill school children. Parent Teacher Association was briefed to do canteen cleanliness assessment and to be monitored by the health teacher.

In conclusion, there was an outbreak of food poisoning at 'L' Primary School. The most likely vehicle of transmission was contaminated *nasi kuning* (turmeric rice) probably from cross contamination tables and hand towel. The most likely organism involved was *B. cereus sp.* from untreated water supply. It was probably sporulation developed from insufficient cooking time and poor hygiene practiced by temporary food operators.

Conflict of Interest: None

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