AN OUTBREAK OF *BACILLUS CEREUS* FOOD POISONING IN A SCHOOL HOSTEL, KLANG

L. RAMPAL
M. JEGATHESAN
Y. S. LIM

SUMMARY

A food poisoning outbreak affected 114 female Malay students staying in a religious secondary school hostel in Klang. The students developed an illness mainly characterized by abdominal pain, nausea, vomiting and giddiness. The median incubation period in this outbreak was 2.5 hours. Laboratory examination of suspected food revealed 2.3 x 10^6 **Bacillus cereus** *organisms per gram of fried noodles.* **B. cereus** *was determined as the probable cause of this outbreak and the fried noodles the most likely vehicle for the organism. An outbreak of* **B. cereus** *food poisoning is being reported in Malaysia for the first time.*

INTRODUCTION

Food poisoning syndromes result from ingestion of foods contaminated with pathogenic microorganisms, microbial toxins or chemicals. The two main types of bacterial food poisoning are the infection type and the intoxication type. The groups of bacteria well recognized as causal agents of food poisoning outbreaks are: *Salmonella, Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum, Bacillus cereus, Vibrio parahaemolyticus* and *Escherichia coli.*[1]

In Malaysia an incidence of 9.6/100,000 population was reported in 1981.[2] The actual incidence is unknown and the above figure certainly underestimates the magnitude of the problem. This may be due to the lack of awareness, reporting, good investigation and laboratory facilities. Very often a sound epidemiological investigation alone can indicate the most likely or probable cause of the outbreak. In fact in some circumstances nothing else may be done because of the inavailability of appropriate specimens. Here was an opportunity provided for a systematic study of an outbreak and the results are presented so as to highlight the mode of investigation that can be undertaken.

Staphylococcal intoxication has always been considered to be the commonest cause of food poisoning outbreaks in this country. In this instance we are able to document, for the first time in this country, an outbreak of food poisoning that was most probably caused by an organism known as *B. cereus. B. cereus* is a well recognized causal agent of food poisoning outbreaks in many other countries,[3,4,5,6,7] and first gained widespread prominence in the seventies. The relatively short incubation period in the case of *B. cereus* reflects

L. Rampal, MBBS, MPH
Senior Medical Officer of Health
Klang District, Malaysia

M. Jegathesan, MBBS, MRCP
Head of Division

Y. S. Lim, MSc
Bacteriologist
Division of Bacteriology
Institute for Medical Research
Kuala Lumpur, Malaysia

the fact that the food poisoning is caused by preformed enterotoxin. Some *B. cereus* strains produce exotoxins that have cytotoxic properties in rabbit small intestine and guinea pig skin.[8] The relevance of this cytotoxin to the pathogenesis of human disease is uncertain. *B. cereus* strains can cause two types of food poisoning syndromes, i.e. vomiting toxin type characterized primarily by nausea and vomiting with an incubating period of one to six hours (also known as short incubation syndrome) and the diarrhoea toxin type which is manifested primarily by abdominal cramps and diarrhoea with an incubation period of eight to sixteen hours (also known as long incubation syndrome).[7,9] The illness in both types is of short duration lasting usually less than 12 hours. *B. cereus* food poisoning outbreaks of short incubation type are most often associated with fried rice that has been cooked and held warm for some period. Long incubation *B.cereus* food poisoning is associated usually with raw vegetables, meat, milk product, seasoning mixes, spices, dried potatoes and vegetable sprouts.[6,10]

Background

On being informed of a food poisoning outbreak in a religious secondary school in Klang by the hospital authorities, an epidemiological investigation was carried out. The hostel is managed by the principal and his committee.

The school where the outbreak occurred is situated two miles from Klang town. It has a student population of 1031 Malay students (679 females and 352 males). The school hostel has a population of 800 (521 females and 279 males). All meals for those staying in the hostel are prepared and served in the hostel. Five cooks and 22 food handlers assist in the preparation and handling of the food.

MATERIALS AND METHODS

Epidemiologic Methods

Of the 800 Malay students who stay in the hostel, 665 students (467 females and 198 males) were interviewed. The 114 cases (all females) of food poisoning were medically examined and interviewed. Information was obtained regarding the meals taken.

The principal of the school, wardens, supervisor, all the cooks and food handlers were also interviewed to obtain information about the outbreak. The interviews were carried out systematically with the objective of identifying the patients, their clinical manifestations and factors related to the outbreak Individual rectal and nasal swabs were taken from the cook and food handlers and sent to the laboratory.The incriminated food left unconsumed was collected in sterile containers and sent to the laboratory for bacteriological examination. Also ascertained was the ingredients, method of preparation and distribution of the implicated food.

Case definition: a case was defined as the occurrence of nausea, vomiting, abdominal pain in a previously healthy person,or isolation of *B. cereus* from a stool specimen.

Bacteriological Methods

The implicated food, fried noodles, was submitted by the Public Health Inspector to the Bacteriology Division, Institute for Medical Research, for bacteriological examination. A portion of the food was taken and emulsified with quarter-strength Ringer solution. It was then streaked directly onto blood agar (BA), MacConkey agar (Mac), desoxycholate citrate agar (DCA) and thiosulphate citrate bile sucrose agar (TCBS), and incubated at 37°C for 24 hours. The food was also inoculated into selenite F (SF), alkaline peptone water (APW) and Robertson's cooked meat medium with 10% salt (RCM) enrichment broths. After 24 hours incubation at 37°C, a loopful each from SF, APW and RCM was streaked onto DCA, TCBS and BA, respectively and incubated at 37°C for 24 hours.

27 rectal swabs from the food handlers were received for processing. After overnight incubation at 37°C, swabs from SF and RCM were plated onto DCA and BA respectively and incubated at 37°C for 24 hours. Nasal swabs from 29 food handlers were also submitted to the laboratory. They were dipped into tryptic soy broth and immediately plated onto BA and incubated at 37°C for 24 hours.

Suspect colonies grown on BA were picked and coagulase test was done to confirm the presence of coagulase-positive *Staphylococcus aureus.* From Mac, DCA and TCBS plates, Colonies resembling *Escherichia coli, Salmonella, Shigella, Vibrio cholerae* and *Vibrio parahaemolyticus* were tested serologically and biochemically. Using quarter-strength Ringer solution as a diluent, 10-fold serial dilutions were made from the food. *S. aureus* and *B. cereus* counts were made on Baird-Parker agar (BP) and mannitol-egg yolk-polymyxin agar (MYP), using the surface spread plate method. The plates were incubated at 37°C for 48 hours, after which colony counts were performed. The method of Lancette and Harmon[11] was followed to identify and confirm *B. cereus.* The enterotoxin production for *S. aureus* was performed using the optimal sensitivity plate method as described by Robbins *et al.* [12]

RESULTS

Attack Rate

Of a total of 467 girls (ages 13 – 18) examined, 114 (24.4%) were ill. None of the 198 boys interviewed were ill.

Clinical Manifestations

Table I shows the number and percentages of signs and symptoms in the outbreak. The majority of the signs and symptoms were related to the upper gastrointestinal tract (abdominal cramps), nausea and vomiting . These symptoms were compatible with the clinical syndrome of *B. cereus* vomiting toxin type of food poisoning.

TABLE 1
NUMBER AND PERCENTAGE OF SIGNS AND SYMPTOMS IN THE OUTBREAK IN 114 CASES

Symptoms	Number with symptoms	% with symptoms
Abdominal pain	97	85.1
Nausea	89	78.1
Vomiting	82	71.9
Giddiness	80	70.2
Fever	6	5.3
Diarrhoea	3	2.6

Incubation Period

Table II shows that the incubation period was short. The range was half an hour to eight hours. The incubation period after eating the breakfast at the hostel was one to three-and-a-half hours in the majority of the cases. The median incubation period was two-and-a-half hours.

Epidemic Curve

Fig. 1 shows the distribution of 114 cases by time of onset of disease. The epidemic curve shows a rapid onset and a sharp rise in the cases after an hour. It was point source (common source) epidemic. Cases occurred rapidly after first onset, reaching a peak point within three hours and then declined rapidly.

Exposure Rate

Table III shows that there were only two food items served for breakfast at the hostel. 113 out

TABLE II
FREQUENCY DISTRIBUTION OF INCUBATION PERIOD

Incubation period (hours)	Number of cases	Cumulative frequency
0.5 –	3	3
1.0 –	13	16
1.5 –	17	33
2.0 –	26	59
2.5 –	29	88
3.0 –	9	97
3.5 –	7	104
4.0 –	3	107
4.5 –	2	109
5.0 –	0	109
5.5 –	2	111
6.0 –	1	112
6.5 –	0	112
7.0 –	1	113
7.5 –	0	113
8.0 –	1	114

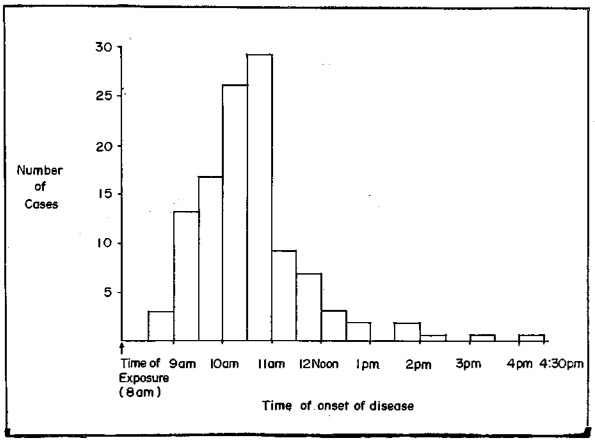

Fig. 1 Distribution of 114 cases by time of onset of disease (Epidemic Curve).

of 114 cases (all females) consumed both tea and noodles. No male was ill. There was no real difference in the exposure rates for tea (among females) for those ill and not ill. There was a real difference (among females) for noodles among those ill and not ill. For males, out of 198 males interviewed although 185 were exposed to both tea and noodles, none became ill.

Food History Attack Rate

Only two food items were served for breakfast on that day. Table IV shows that for noodles there is a high percentage ill persons in the 'Ate' column and low percentage of ill persons in the 'Did Not Eat' column. Noodles also has a greater difference in percentage between those who ate and those who did no eat when compared to tea. Noodles was therefore suspected as being the responsible vehicle.

Test for Statistical Significance

The difference in the attack rates for fried noodles among exposed and not exposed is highly significant (chi square value = 9.5, $p < 0.005$).

Bacteriological Results

From the implicated food, coagulase-positive *S. aureus* and *B. cereus* were isolated from the BA plates and *Salmonella agona* was recovered from the DCA plated from the SF enrichment broth. The other pathogens, namely, *E. coli*, *Shigella*, *V.* cholerae and *V. parahaemolyticus* were not isolated. Counts for coagulase-positive *S. aureus* and *B. cereus* were found to be 8×10^4/g and 2.3×10^6/g respectively. The strain of coagulase-positive *S. aurues* isolated from the fried noodles was a non-enterotoxin producer. All the rectal swabs examined yielded no *Salmonella*, *Shigella* or coagulase-positive *S. aureus*. From three of the 27 nasal swabs examined, coagulase-positive *S. aureus* was isolated. Of the three strains of coagulase-

TABLE III
EXPOSURE RATES BY ARTICLE OF FOOD CONSUMED BY SEX

Sex	Type of food	Ill (114 females) (no male)		Not ill (353 females) (198 males)		Real difference (a – b)
		Number exposed	Exposure rate (%) (a)	Number exposed	Exposure rate (%) (b)	
Female	Tea	113	99.1	350	99.2	– 0.1
	Noodles	113	99.1	308	87.3	11.8
Male	Tea	0	0	186	93.9	–93.9
	Noodles	0	0	186	93.9	–93.9

TABLE IV
FOOD HISTORY ATTACK RATE

Sex	Food items	Number of persons who ate specified food				Number of persons who did not eat specified food				
		Ill	Not ill	Total	Percentage ill (a)	Ill	Not ill	Total	Percentage ill (b)	Real difference (a – b)
Female	Tea	113	350	463	24.4	1	3	4	25	– 0.6
	Noodles	113	308	421	26.8	1	45	46	2.2	24.6
Male	Tea	0	186	186	0	0	12	12	0	0
	Noodles	0	186	186	0	0	12	12	0	0

positive *S. aureus* isolated, only one nasal isolate was found to be an enterotoxin D producer.

Method of preparation and distribution of the fried noodles

The ingredients used were noodles, cooking oil, blended dried chillies with shrimp paste, blended garlic, bean sprouts, ikan bilis, and blended big onions. It was prepared as follow: cooking oil was heated in a steel cauldron (Fig. 2). Blended chillies, big onions and garlic were added and fried until slightly brown. The bean sprouts were then added. Lastly noodles were added and fried. Fig. 2 shows that the food was prepared in two steel cauldrons by five cooks. All the five cooks assisted in the preparation of the noodles in both the cauldrons. The cooked noodles were then transferred to the three aluminium basins. Noodles from cauldron 1 was transferred by three female food handlers into the first basin and then to 279 plates meant only for the boys. The balance of the fried noodles from cauldron 1 was transferred to the second basin by three different female food handlers. The second basin was filled up (by these latter food handlers) with noodles taken from cauldron 2. Noodles from the second basin was transferred into 261 plates for girls only. The same food handlers who filled up the second basin, transferred the remaining noodles in cauldron 2 into a third basin and from this basin into 260 plates for girls only.

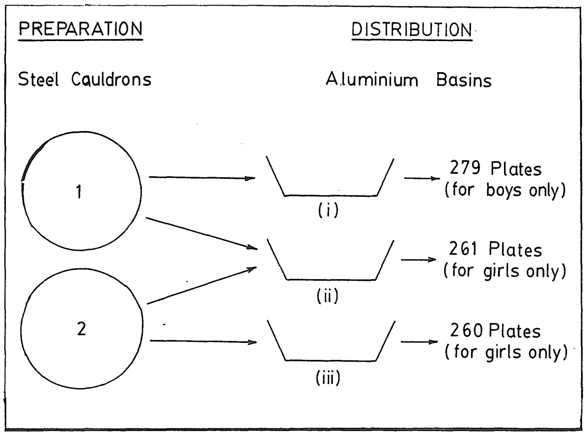

Fig. 2 Method of preparation and distribution of noodles.

The results indicate that the most likely cause of the food poisoning outbreak was *B. cereus*, and the fried noodles the most likely vehicle.

DISCUSSION

Past studies[4,5] have demonstrated that *B. cereus* food poisoning may be manifested by symptoms and incubation period resembling staphylococcal intoxication. In these outbreaks, persons who consumed boiled and fried rice contaminated by *B. cereus* usually experienced vomiting one to five hours later. Although we have also isolated *S. agona* in the implicated food, we have ruled this microorganism out as a likely cause of the food poisoning outbreak because the epidemiological picture was not consistent with common source *Salmonella* food poisoning outbreak. In the case of *S. aureus* although the clinical manifestations and incubation period were somewhat consistent to that of *S. aureus* intoxication, we have also ruled this organism out because the isolate from the implicated food was a non-enterotoxin producer and also the staphylococcal count was rather low.

B. cereus appeared to be the most likely cause of this outbreak for the following reasons: the clinical manifestations and the incubation period were consistent with that expected of the vomiting toxin type of *B. cereus.* Furthermore, the *B. cereus* count in the implicated food was 2.3×10^6/g which is generally considered as high enough to initiate an outbreak. The fact that all the 114 cases were females indicates that the three most likely events which occurred as shown in Fig. 2 were: the fried noodles in cauldron 2 was contaminated before cooking or while cooking; the fried noodles was contaminated while being transferred into the second or third aluminium basins, or being transferred into the 521 plates meant for the females. The third, but, most unlikely event that could have occurred was that a portion of the fried noodles in cauldron 1 was contaminated and the whole of this contaminated portion was transferred into the second aluminium basin, filling half of it and that is why only 114 girls fell ill.

The value in carrying out such investigations is that these epidemiological investigations will show the most probable vehicle of transmission, the causative organism and also the factors which contribute to the chain of events. This information can be used for the establishment of sensible control and preventive measures. As long as people have to consume food, particularly in this modern era, where food is usually mass produced, there will always be the danger of common source outbreaks implicating large numbers of people.

It is therefore even more important that sound food hygiene practices are inculcated amongst those responsible for the preparation, distribution and handling of food. Epidemiological knowledge about food poisoning in a particular country will serve as a useful basis for any proposed education programme aimed at achieving the above objectives. Health staff responsible for investigating food poisoning outbreaks must be aware of the similarity between *B. cereus* food poisoning syndrome (with short incubation period) and the well established foodborne illness caused by *S. aureus* and also the similarity between *B. cereus* food poisoning syndrome (long incubation period) and the foodborne illness caused by *C. perfringens.* Lack of awareness of these may cause *B. cereus* to be overlooked as a potential causative agent in some outbreaks.

Microbiologists should become familiar with procedures for identifying *B. cereus* and be aware of the significance of the numbers of organisms present. Physicians should be aware of the epidemiology of *B. cereus* food poisoning. With this knowledge a better understanding of the etiology of food poisoning outbreaks as well as the role of *B. cereus* and other bacteria can be achieved.

ACKNOWLEDGEMENTS

The authors wish to thank Dato Dr Hj Talib bin Latif, Director General, Ministry of Health, Malaysia for permission to publish the paper; Dr G. F. de Witt, Director, Institute for Medical Research, Kuala Lumpur and Dr Chong Chee Tsun, State Director, Medical and Health Services, Selangor for their encouragement, the staff of District Health Office, Klang and Division of Bacteriology I.M.R., for their technical assistance. Special thanks are also due to Prof Jane Baltazar I.P.H. University of the Philippines, for her critical review and Puan Fauziah Jaafar for typing out the manuscript..

REFERENCES

[1] Hobbs B C, Gilbert RJ. *Food poisoning and hygiene: Bacterial and other microbial causes of food poisoning.* London: Edward Arnold Publishers Ltd, 1978; 25-35.

[2] Unit Penerangan Pengurusan Kesihatan, Bahagian Kesihatan Kementarian Kesihatan. *Incidence of the notifiable communicable diseases in Malaysia by state,* 1981.

[3] Hauges. Food poisoning caused by aerobic spore forming bacilli. *J Appl Bacteriol* 1955; 18: 591-595.

[4]News and notes. Food poisoning associated with *Bacillus cereus. Br Med J* 1972; 1: 189.

[5]News and notes. Bacillus food poisoning. *Br Med J* 1973; 3: 647.

[6]Portnoy BL, Geepfert JM, Harmon SM. An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. *Am J Epidemiol* 1976; 103(6): 589-594.

[7]Midura T, Gerber M, Wood R, *et al.* Outbreak of food poisoning caused by *Bacillus cereus. Public Health Reports* 1970; 85: 45-48.

[8]Turabull PCB. Studies on the production of enterotoxins by *Bacillus cereus. J Clin Path* 1976; 29: 941.

[9]Mortimer PR, Mc Cann G. Food poisoning episodes associated with *Bacillus cereus* in fried rice. *Lancet* 1974; 1: 1043.

[10]Kim HU, Geepfert J M. Enumeration and identification of *Bacillus cereus* in foods. One 24 hour presumptive test medium. *Appl Microbiol* 1971; 22: 581.

[11]Lancetre G A, Harmon S M. Enumeration and confirmation of *Bacillus cereus* in foods: collaborative study. *J Assos of Anal Chem* 1980; 63(3): 581-586.

[12]Robbins R, Gould S, Bergdoll M S. Detecting the enterotoxigenicity of *Staphylococcus aureus* strains. *Appl Microbiol* 1974; 28: 946-950.