AN OUTBREAK OF *BACILLUS CEREUS* FOOD POISONING

L. Tay
K. T. Goh
S. E. Tan

SYNOPSIS

An outbreak of food poisoning was reported in a military camp in Jurong involving 19 army personnel who became ill after breakfast on 10 November 1981. The illness was characterized by vomiting (89.5%), abdominal cramps (52.6%), diarrhoea (47.4%), headache (47.4%) and fever (10.5%). The incubation period ranged from 1¾ to 5½ hours. Fried rice sold by a food stall in a civilian canteen was implicated. *Bacillus cereus* Type 1 (flagella antigen) was isolated from the vomitus of one of the affected soldiers. The food handlers were advised on proper ways of boiling and storing cooked rice to prevent similar outbreaks from occurring in future.

INTRODUCTION

Bacillus cereus is a gram-positive, aerobic, spore-forming bacillus commonly found in the soil, on vegetables and in many raw and processed foods. It has been established as an aetiological agent of food poisoning in Europe since 1950 (1). It has been implicated in the United States only since 1968 (2, 3). In the United Kingdom, there had been reports of food poisoning following ingestion of contaminated boiled and fried rice since 1971 (4, 5).

This paper describes the first outbreak of *B. cereus* food poisoning which occurred in a military camp in Singapore.

THE OUTBREAK

On the morning of 10 November 1981, a group of army personnel became ill after their breakfast in Jurong. Epidemiological investigations carried out after the Ministry of the Environment was notified of the outbreak showed that 19 (11.3%) of the 168 persons surveyed were ill. Their clinical symptoms were vomiting (89.5%), abdominal cramps (52.6%), diarrhoea (47.4%), headache(47.4%) and fever (10.5%). Eighteen of them sought treatment at the Medical Centre, the remaining one self-medicated. All of them did not require hospitalization and were well by the next day.

An analysis of the meal-specific attack rates implicated a cooked food stall in a civilian canteen to be the source of infection (Table 1). Food specific attack rates for each food item eaten, incriminated fried rice obtained from the food stall as the vehicle of transmission (Table 2).

The median incubation period based on onset of symptoms after breakfast at 0700 hours was 2 hours 5 minutes with a range from 1¾ to 5½ hours (Fig 1).

Information obtained from the food handlers concerned revealed that the fried rice was prepared in the following manner. About half a kilogram of rice was cooked in the evening of 9 November and then left overnight in the cooker at room temperature. At 0600 hours on the next day, the cooked rice was fried in bulk with roast pork and eggs for about 20 minutes and subsequently left in a container at ambient temperature before being sold for breakfast.

**Bacteriology Section
Department of Pathology
Singapore General Hospital
Outram Road
Singapore 0316**

L. Tay, MBBS, Dip Bact.
Head

S. E. Tan
Senior Laboratory Technician

**Quarantine & Epidemiology Department
Ministry of the Environment
Princess House
Alexandra Road
Singapore 0315**

K. T. Goh, MBBS, M Sc.
Head

LABORATORY INVESTIGATIONS

The fried rice had been sold out long before the outbreak was reported and thus no food remnants were available for laboratory analysis. All the food handlers involved in the handling of food were free from cuts or sores. Only a specimen of vomitus obtained from one of the affected soldiers was available for laboratory investigations. At the laboratory, the vomitus was cultured on mannitol salt agar, MacConkey agar, phenol red-egg yolk-polymyxin agar, thiosulfate-citrate-bile salts-sucrose agar aerobically and on blood agar aerobically and anaerobically. It was also inoculated into Robertson's cooked meat broth, selenite broth and 2% alkaline peptone water. All were incubated at 35° C overnight.

LABORATORY RESULTS

On the following morning, the aerobic blood agar plate showed heavy growth of almost pure culture (95%) of gram-positive spore-bearing rods with the following characteristics:-

Diameter of each rod was more than 0.9 microns.
Spore did not bulge the sporangium.
Motility: +
Nitrate reduction to nitrite: +
Lecithinase production: +
Gelatinase: +
Voges-Proskauer: +
Starch hydrolysis: +
Fermentation of:- Glucose: Acid +
Mannitol: Acid +
Xylose: Acid -
Arabinose: Acid -
Salicin: -
Citrate (Christensen): +
Citrate (Simmons'): -

The organism was identified as *B. cereus* and sent to Dr Y. Kudoh of Tokyo Metropolitan Research Laboratory of Public Health, Japan, for serological typing. It was found to be serotype (Flagella antigen): Type 1. No other food poisoning organisms were recovered.

TABLE 1: ANALYSIS OF MEAL-SPECIFIC ATTACK RATE OF A FOOD POISONING OUTBREAK AT JURONG, NOVEMBER 1981

MEAL	Took Specified Meal			Did not take specified meal			% ILL DIFF.	P VALUE
	ILL	WELL	% ILL	ILL	WELL	% ILL		
Military cookhouse 9.11.81								
Lunch	1	54	11.5	12	95	11.2	0.3	NS
Dinner	7	42	14.3	12	107	10.0	4.3	NS
Night snack	0	20	0	19	129	12.8	-12.8	NS
10.11.81 Breakfast	1	30	3.2	18	119	13.1	-9.9	NS
Civilian canteen 10.11.81								
Stall 1	18	11	62.1	1	138	0.7	61.4	1.4×10^{-13}*
Stall 2	0	44	0	19	105	15.3	-15.3	NS

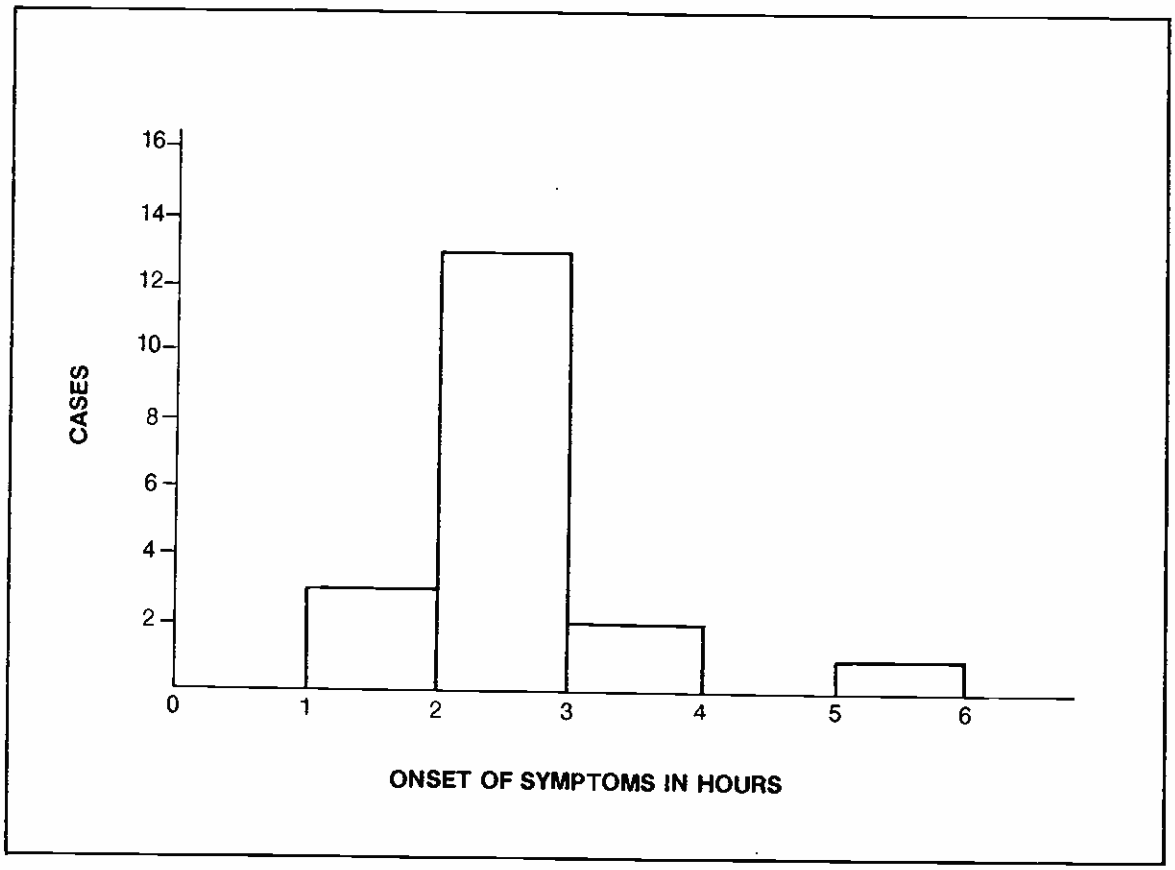
NS = Not statistically significant
* High statistically significant
Stall 1 = Cooked food
Stall 2 = Drinks & snacks

TABLE 2: ANALYSIS OF FOOD-SPECIFIC ATTACK RATE OF A FOOD POISONING OUTBREAK AT JURONG. NOVEMBER 1981

FOOD ITEM	Ate specified Food			Did not eat specified Food item			& ILL DIFF.	P VALUE
	ILL	WELL	% ILL	ILL	WELL	% ILL		
Fried rice	17	0	100.0	2	149	1.3	98.7	2.1×10^{-21}*
Fried vermicilli	1	9	10.0	18	140	11.4	-1.4	NS

NS = Not statistically sigificant
* Highly statistically significant

FIG 1. TIME DISTRIBUTION OF 19 CASES OF FOOD POISONING AT JURONG, NOV 1981.



DISCUSSION

B. cereus is the aetiological agent of two types of food poisoning: one primarily manifested by diarrhoea and abdominal pain 8-16 hours after ingestion of contaminated food diarrhoeal-type or long-incubation period syndrome), the other by nausea and vomiting with an incubation period of only 1-5 hours (vomiting-type or short-incubation period syndrome).

The diarrhoeal-type syndrome has been reported in the US and particularly in Europe, implicating foods like meat, poultry, vegetables, dessert dishes and sauces (1, 6, 7, 8). The vomiting-type syndrome has been reported in the United Kingdom, Canada and USA frequently associated with the consumption of cooked (usually fried) rice (4, 5, 9, 10, 11).

Studies carried out with experimental animals showed that the vomiting-type syndrome may be caused by a heat-stable toxin produced by some *B. cereus* strains and capable of causing vomiting when fed to monkeys (12). *B. cereus* strains which cause the diarrhoeal-type syndrome had been found by Turnbull to elaborate a heat-labile enterotoxin that activates intestinal adenylate cyclase and results in intestinal fluid secretion (13).

The short incubation period ranging from 1¾ to 5½ hours, the clinical features with vomiting as the predominant symptom and the implication of fried rice as the vehicle of transmission in this outbreak were features quite characteristic of a typical vomiting-type sydrome of *B. cereus* food poisoning.

In this outbreak, the spores of the organism, presumably in the rice, probably survived the cooking process. During storage at room temperature, the spores probably germinated, multiplied and then produced the heat-stable toxin, which was probably not inactivated when the cooked rice was fried in bulk with other ingredients the following morning. Tests carried out by Gilbert et al (14) found that the spores of *B. cereus* survived cooking and were capable of germination and outgrowth. The optimum temperature for growth in boiled rice was between 30° C and 37° C and growth also occurred during storage at 15° C and 43° C. In most outbreaks, large numbers of *B. cereus*, ca. 10^6 — 10^9 were isolated from food remnants.

Gilbert and Parry (15) carried out serological typing of cultures of *B. cereus* from 84 outbreaks of food poisoning in seven countries. They found that in 35 of the 61 outbreaks associated with the vomiting-type syndrome, foods, clinical specimens or both yielded H-serotype 1 only. In addition, Type 1 strains together with other serotypes were isolated in seven other outbreaks. Types 3, 4, 5, 8 or a mixture of serotypes were present in 14 other outbreaks. On the other hand, two of the nine diarrhoeal-type outbreaks yielded serotype 1, whilst Types 2; 6, 8, 9, 10 and a mixture of Type 12 and an untypable strain were responsible for one outbreak each. The *B. cereus* strain responsible for this outbreak was also found to belong to Type 1, the predominant serotype associated with the vomiting-type syndrome.

As a preventive measure, the food handlers were advised to store all cooked rice after boiling at a temperature of not less than 63° C or to cool it quickly and then keep it in the refrigerator within two hours of cooking. Rice should also be boiled in smaller quantities on several occasions during the day, thereby reducing the storage time before frying.

ACKNOWLEDGEMENT

The authors wish to thank Dr Yasuo Kudoh, Chief, Section of Enteric Diseases, Department of Microbiology, Metropolitan Research Laboratory of Public Health in Tokyo, Japan, for doing serotyping of the *B. cereus* isolated.

REFERENCES

1. Hauge S. Food poisoning by aerobic spore-forming bacilli. J Appl Bact 1955; 18: 591-5.
2. U.S. Department of Health, Education and Welfare, National Communicable Disease Center, Atlanta, Georgia, Foodborne outbreaks. Annual Summary. 1968, page 32.
3. Midura T, Gerber M, Wood R, Leonard A R. Outbreak of food poisoning caused by *Bacillus cereus*. Public Health Rep 1970; 85: 45-9.
4. Public Health Laboratory Service. Food poisoning associated with *Bacillus cereus*. British Medical J 1972; 1: 189.
5. Public Health Laboratory Service. Bacillus cereus food poisoning. British Medical J 1973; iii: 647.
6. Geopfert J M, Spira W M, Kim H U.: Bacillus cereus: food, poisoning organism. A review J of Milk and Food Technology. 1972; 35: 213-27.
7. Schmitt N, Bowmer E J, Willoughby B A: Food poisoning attributed to *Bacillus cereus*. Canadian J Public Health 1976; 67: 418-22.
8. Giannella R A, Brasile L A: Hospital foodborne outbreak of diarrhea caused by *Bacillus cereus*: Clinical, epidemiological and microbiologic studies. J Infect Dis 1979; 139: 366-70.
9. Mortimer P R, McCann G: Food poisoning episodes associated with *Bacillus cereus* in fried rice. Lancet 1974; 1: 1403-5.
10. Lefebune A, Greojoire C A, Brabant W. Todd E: Suspected *Bacillus cereus* food poisoning. Epidemiological Bulletin, Canada 1973; 17: 108-10.
11. US Department of Health, Education and Welfare, Center for Disease Control. Foodborne and Waterborne disease outbreaks. Annual Summary, 1974, 1976, pg 26.
12. Melling J, Caspel B J, Turnbull P C B et al: Identification of a novel enterotoxigenic activity associated with *Bacillus cereus*. J Clin Path 1976; 29: 938-40.
13. Turnbull P C B: Studies on the production of enterotoxins by *Bacillus cereus*. J Clin Path 1976, 29: 941-8.
14. Gilbert R J, Stringer M F, Peace T C: The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. J Hyg, Camb. 1974; 73: 433-44.
15. Gilbert R J, Parry J W: Serotypes of *Bacillus cereus* from outbreaks of food poisoning and from routine foods. J Hyg, Camb. 1977; 78: 69-74.