

An outbreak of *Bacillus cereus* food-poisoning in Finland associated with boiled rice

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SUMMARY

A food-poisoning outbreak caused by *Bacillus cereus* occurred in a Finnish industrial plant in January 1975. Eighteen of the 36 persons who ate a lunch including boiled rice, meat and vegetables became ill. The disease pattern was similar to previously reported short incubation time *B. cereus* food-poisonings associated with cooked rice. The median incubation time was two hours, the main symptoms being nausea, abdominal pain and vomiting. Rice and certain seasonings were the contaminated raw materials. Gas chromatographic fatty acid analysis of a bacterial cell was used as a diagnostic method as well as to identify a certain strain of *B. cereus*.

INTRODUCTION

Bacillus cereus has been recognized as a food-poisoning organism for about 25 years. Hauge, in 1950, described a type of food poisoning caused by ingestion of vanilla sauce heavily contaminated by *B. cereus*. The symptoms were rather mild: abdominal pain and diarrhoea, moderate nausea and, rarely, vomiting. The average incubation period was 10 hr. and symptoms did not usually last more than 12 hr. Most outbreaks reported have occurred in Europe (Goepfert, Spira & Kim, 1972). There have been a few published outbreaks also in the United States of America (U.S. Department of Health, Education, and Welfare, Center for Disease Control, 1973; Midura, Gerber, Wood & Leonard, 1970) and Canada (Todd *et al.* 1974). There have been two confirmed outbreaks of *B. cereus* food-poisoning also in Finland during the last three years (Dr A. Niskanen, personal communication).

Three years ago the first report of a different type of food-poisoning caused by *B. cereus* was published (Public Health Laboratory Service, 1972). Symptoms were mainly nausea and vomiting, rarely diarrhoea. Incubation period was short, 1-5 hr. The number of incidents of this type, all of which have been associated with cooked rice, usually fried rice from Chinese restaurants and take-away shops,

have already exceeded 40 in Great Britain (Gilbert & Taylor, 1975), and there has also been a similar outbreak in Canada (Lefebvre, Gregoire, Brabant & Todd, 1973).

This paper describes the investigation of an outbreak of short incubation time *B. cereus* food-poisoning that took place among the personnel of a factory in Finland. In addition to a search for methods of preventing outbreaks of this type, the use of gas chromatographic analysis of the fatty acid composition of *B. cereus* in epidemiological investigations was studied.

THE OUTBREAK

On Sunday, late January 1975, eighteen employees became ill after eating lunch in an industrial plastic plant. Thirty-six employees ate a warm meal consisting of meat, rice and vegetables. In addition milk, bread, butter, and yoghurt were served. The warm portions had been individually packed into cardboard boxes in the central kitchen on the preceding Friday and stored there in a refrigerator. The boxes were warmed up in a microwave oven in the departmental kitchen just before being eaten.

The first employees began to vomit 30 min. after the lunch that was served at about 10 a.m. All of those who became ill developed symptoms within 4 hr. after lunch. The main symptoms were nausea, vomiting and abdominal pain.

The warm meal portion was immediately suspected of being responsible for the food-poisoning cases and a sample of the meal was kept in a refrigerator till the following day and sent by the factory officers to the State Veterinary Medical Institute for bacteriological analysis. The sample was transported in a thermo-isolated package and it stayed cold until examined. Analysis revealed large numbers of *B. cereus* bacteria in the meal.

MATERIALS AND METHODS

Epidemiological methods

The population at risk was defined and questioned for disease and eating histories. Diagnosis was made according to symptoms, incubation time and duration of the illness. Food item specific attack rates were calculated. Food warming and serving histories were collected and the preparation procedure repeated. Temperature changes of the meal were followed during the preparation, storing and serving periods.

Laboratory methods

Generally accepted microbiological methods were used. Thermal shock was not given prior to examinations. Growth media, incubation temperatures and incubation times were as follows: plate count agar, 30° C., 72 hr.; calf blood agar, 37° C., 24 and 48 hr.; sulphite iron agar, 37° C., 98 hr.; violet red bile agar, 37° C.*, 24 hr. Impression smear preparations were made to examine for the presence of dead bacterial cells.

* Media from Orion Pharmaceutical Co., Helsinki.

A micro-slide gel double diffusion test described by Casman, Bennett, Dorsey & Stone (1969) was used for the detection of staphylococcal enterotoxins after an extraction procedure developed by Reiser, Conaway & Bergdoll (1974). Samples of 100 grams of meat and rice portions of the dish were individually analysed for enterotoxins A, B, C₁ and E.

Fatty acid compositions of the isolated *B. cereus* strains were analysed by a gas chromatographic method described by Niskanen, Kiutamo, Mälkki & Nikkilä (1975).

RESULTS

The outbreak

Eighteen cases of *B. cereus* food-poisoning were identified in 36 persons sharing the lunch, thus giving an attack rate of 50%. There was no significant difference in age and sex distributions between the groups. Calculated percentage attack rates for persons who ate a specific food item, were the following: meat 51, rice 53, mixed vegetables 56, bread and butter 50, and yoghurt 43. For persons who did not eat a specific food item, the figures were: 0, 0, 0, 33, 50, and 75, respectively. Incubation times for the onset of food-poisoning were from $\frac{1}{2}$ to 4 hr. with a median of 2 hr. Nausea, abdominal pain and vomiting were the prominent symptoms. About two thirds of the affected persons had these symptoms. Only four had diarrhoea. Duration of the illness was less than 24 hr.

Microbiological examinations

The food sample analysed was heavily contaminated with *B. cereus*: the counts on blood agar were 1.7×10^8 per gram of rice and 1.0×10^6 per gram of meat. The organism was not detected in the mixed vegetables. *Staphylococcus aureus*, *Clostridia* and *Salmonella* were not found in the food remnants. Stool specimens were collected from seven persons 1–5 days after the illness, but *B. cereus* could not be found in faeces. No vomit was available for examination.

The organism was identified as *B. cereus* by its morphological, biochemical and chemical characteristics. The bacteria were Gram-positive rods more than $0.9 \mu\text{m}$ in diameter having central cylindrical spores that did not bulge the sporangium. The biochemical reactions of the strains isolated are given in Table 1. The gas chromatograms from the analyses of the fatty acid compositions of the strains followed the pattern of *B. cereus* (Niskanen *et al.* 1975).

Staphylococcal enterotoxins were not detected in the meat and rice samples. Only large Gram-positive rods were seen in the impression smear preparations.

The food preparation history

The warm portion consisted of calf meat, gravy, rice and mixed vegetables. The meat had been purchased deep-frozen. It was fresh and inspected. Long grain American rice and industrially precooked, deep-frozen vegetable assortment (pea-corn-paprika) were used. The gravy was made of beef extract, wheat flour and water. The spices used were garlic, salt, white pepper and aroma salt. All the milk products served at the lunch were made of inspected milk that was indi-

Table 1. *Biochemical reactions of the Bacillus cereus strains isolated from the food-poisoning outbreak*

(Sources of the strains: cooked rice (352), raw rice (354), garlic salt (355), white pepper (356).)

	Strain			
	352	354	355	356
Haemolysis of calf blood	+	+	+	+
Production of lecithinase	+	+	+	+
Indole formation	-	-	-	-
Reduction of nitrate	+	+	+	+
Deamination of phenylalanine	-	-	-	-
Decarboxylation of lysine	-	-	-	-
Production of acetylmethylcarbinol	+	+	+	+
Acid formation from:				
Arabinose	-	-	-	-
Rhamnose	-	-	-	-
Xylose	-	-	-	-
Glucose	+	+	+	+
Fructose	+	+	+	+
Galactose	-	-	-	-
Mannose	-	+	-	-
Lactose	-	-	-	-
Saccharose	+	+	-	-
Maltose	+	+	+	+
Trehalose	+	+	+	+
Raffinose	-	-	-	-
Inulin	-	-	-	-
Glycerol	+	+	+	+
Adonitol	-	-	-	-
Mannitol	-	-	-	-
Sorbitol	-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
Salicin	-	-	-	-

vidually sealed into consumer packages in a dairy plant. The bread was served presliced. After the rice was boiled it was cooled in portions of 2 kg. under cold running tap water to make the temperature drop to 15° C. Then the rice was put in 23.5 kg. lots into plastic containers (40 × 60 × 10 cm.) that were kept in a cold storage room at 9° C. for 24 hr. The individual food portions from the boiled rice and the other ingredients were made the next day. Meat was first fried in a pan after which cooked gravy was added and the mixture was transferred to an oven at 175° C. for 2 hr. The meal was then portioned into small cardboard boxes (15 × 10.5 × 2.5 cm.) that were thermally closed with thin plastic covers. The boxes were piled in containers and kept in cold storage at 9° C. for 42 hr. After that the boxes were delivered to the nearby departmental kitchens where the meal was stored in refrigerators at 4–5° C. for one hour and served after warming up in a microwave oven (2450 MHz., 1.3 kW.) for 60–90 sec.

Figure 1 indicates the actual time–temperature relations during the preparation of the warm meal.

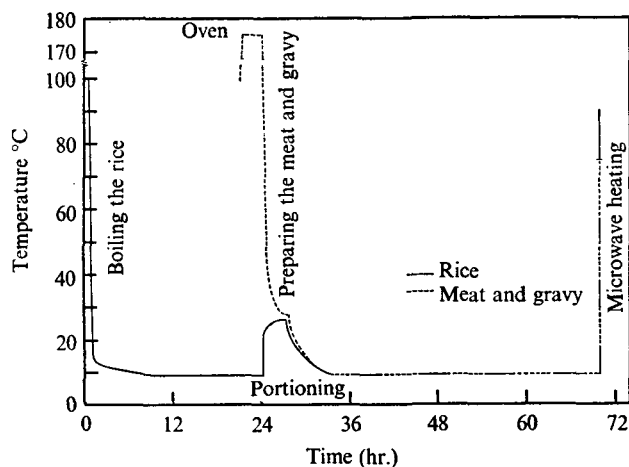


Fig. 1. Changes in temperature during the preparation of the meal, that caused the food-poisoning outbreak.

Examination of the raw-materials

The following raw-materials were examined for the presence of *B. cereus* (results in parenthesis): uncooked rice (100/g.), vegetable mixture (not found), garlic salt (100/g.), white pepper (4500/g.) aroma salt (not found). The raw meat used was no longer available for analysis. Minimum number of cells that could be detected by the dilution technique used was 100/g.

A survey was made to estimate the prevalence of rice contaminated with *B. cereus* in Finland. A package of seven different commercial rice brands was purchased from ordinary retail stores. Each package was analysed by triplicate sampling for *B. cereus*. Two of the seven packages were positive giving a prevalence rate of 29%. Both contaminated rice brands originated from the United States of America.

DISCUSSION

A case of *B. cereus* food-poisoning was defined in this study, the illness having an incubation time of up to 4 hr., with symptoms mainly nausea and vomiting, and duration less than 24 hr. The illness corresponds to the disease pattern of the previous short incubation time *B. cereus* food-poisoning outbreaks that have occurred in the United Kingdom (Public Health Laboratory Service, 1972, 1973; Mortimer & McCann, 1974; Gilbert & Taylor, 1975) and in Canada (Lefebvre *et al.* 1973).

Clinical samples were difficult to obtain: no vomit was available for examination and most of the stool specimens were obtained several days (the first 1 day, five following samples 3 days and the seventh specimen 5 days) after the symptoms had disappeared. *B. cereus* could not be detected in any of the samples. No other pathogens were present. Examination of faecal samples from patients of the former outbreaks have frequently revealed the organism in faeces (Mortimer & McCann, 1974). On the other hand *B. cereus* has been found in samples from un-

affected persons and the organism has not been isolated from faecal specimens submitted several days after the symptoms had ceased (Public Health Laboratory Service, 1973). Our failure to find the organism in faeces is probably also due to the time interval between the disease and collection of the sample.

Morphological, biochemical and chemical investigations were made in order to identify typical (hemolytic, large, irregular, greyish-white) colonies of *B. cereus*. Bergey's manual (Gibson & Gordon, 1974) lists the following properties of the bacterium: diameter of the cell ($> 0.9 \mu\text{m.}$), ability to reduce nitrate and inability to ferment arabinose, xylose and mannitol. Morphological and biochemical properties observed (Table 1) agree with this information as well as with the data given by Goepfert *et al.* (1972). The fatty acid compositions analysed also identify the strains from both the meal and its raw-materials as *B. cereus* (Niskanen *et al.* 1975).

Because of the similarity of the short incubation time *B. cereus* food-poisoning to the intoxication caused by *Staphylococcus aureus* the suspected food was analysed for staphylococcal enterotoxins A, B, C₁, and E. No toxin was detected. The samples were not examined for enterotoxin D, but this type is uncommon and thus the probability of the toxin having been responsible for the disease is small (Casman, 1967).

Food preference rates for affected and unaffected persons support the microbiological findings suggesting that the warm meal was responsible for the outbreak. Food item specific statistical analyses by chi square test without Yates correction (Remington & Schork, 1970) do not, however, indicate statistical differences at 10% level of significance. There were only few persons in the group that did not eat all the lunch. Everyone who did not eat meat, rice or vegetables was unaffected.

B. cereus spores can survive cooking and initiate growth in boiled rice (Gilbert, Stringer & Peace, 1974), which is an excellent growth medium for the bacterium (Raevuori & Genigeorgis, 1975). The lowest temperature limit for growth in rice is between 10 and 15° C. (Gilbert *et al.* 1974), but germination of spores is possible also at lower temperatures. During the first storage period the temperature of the rice was in the range of 10–15° C. for 5 hr. This does not permit rapid multiplication, if any. Portioning was done the following day. This procedure caused a temperature rise due to the addition of hot meat and gravy to the rice. The temperature was between 15 and 25° C. for 6 hr., allowing growth of *B. cereus*.

A microwave oven was used to warm up the portions just before serving. Gilbert *et al.* (1974) concluded, in their study of the survival and growth of *B. cereus* in boiled and fried rice, that the toxin is not destroyed in the process of frying or reheating of cooked rice. Heating time of the oven was adjusted individually by the users. The longest heating time used was 90 sec. In this treatment the temperature of the rice reaches 93° C. but that of the gravy does not exceed 75° C. These temperatures are momentary and it is evident that heat is unevenly distributed through the different parts of the portion. Food particles can also shelter the toxin from heat. The factor that causes necrotic lesions in guinea pig skin and accumulation of fluid when injected into the ligated rabbit

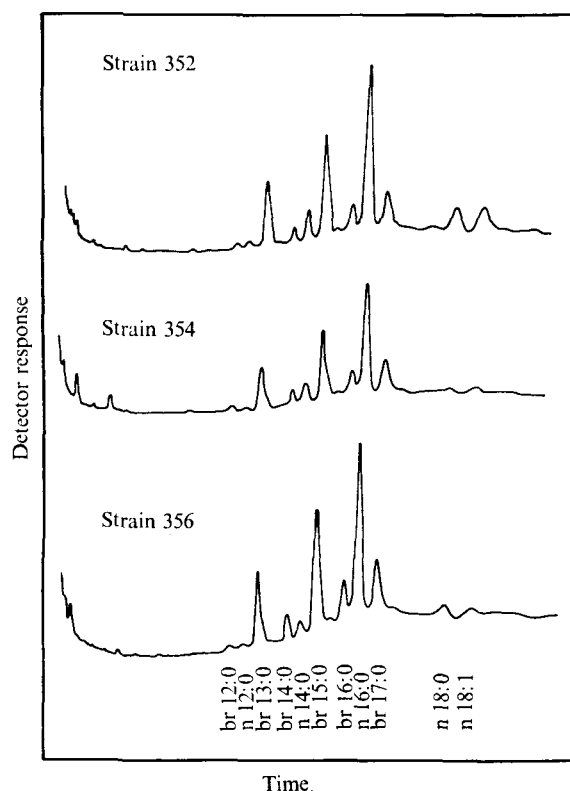


Fig. 2. Gas chromatograms of the *Bacillus cereus* strains isolated from the food-poisoning outbreak. Sources of the strains: cooked rice (352), raw rice (354), white pepper (356).

ileal loop has been suggested as being also responsible for the food-poisoning action of *B. cereus* (Spira & Goepfert, 1972; Glatz & Goepfert, 1973). The toxin is stated to be rather heat labile, being inactivated at 56° C. in 5 min. (Glatz & Goepfert, 1973).

Spices and rice are common sources of aerobic sporeformers and potentially dangerous in respect of *B. cereus* food-poisoning outbreaks (Goepfert *et al.* 1972; Gilbert *et al.* 1974). In an American study Kim & Goepfert (1971) found 40% of spice samples analysed to be contaminated with *B. cereus*. Nygren (1962) reported an isolation rate of 72% in Sweden. Rice was not included in the studies mentioned above. Gilbert & Taylor (1975) found *B. cereus* in 88% of samples of uncooked rice grains. Our survey of the prevalence of *B. cereus* in commercially available rices in Finland indicated a prevalence rate of 29%. The number indicates only the proportion of rice brands having a contamination level of ≥ 100 per gram and thus cannot be directly compared with the values given above. Rice, garlic salt and white pepper were the contaminated raw materials in this outbreak.

Serological methods for *B. cereus* have not yet developed to the extent that they could be used as a tool in epidemiological investigations. Taylor & Gilbert (1975) recently developed a serotyping scheme that is based on 18 sera prepared against flagellar antigens of the bacterial cell. In this study gas chromatographic

analysis of the fatty acid composition of the isolated bacilli was tentatively used for epidemiological studies. Gas chromatograms for three strains isolated from this outbreak are shown in Fig. 2. All the strains can be identified as *B. cereus*. The gas chromatographic method thus proved to serve as a valuable aid in the identification of the isolated strains. Quantitative differences in fatty acid compositions are so small that they do not allow decisive determination of the origin of *B. cereus* in the infected food. However, it can be concluded that the strain 354 that was isolated from raw rice resembles the food-poisoning strain, 352, more than the one isolated from white pepper, 356. The most important quantitative differences are in the 14-carbon fatty acids of the strains (Fig. 2). In this case the role of *B. cereus* spores in the seasonings is less important owing to the long heating time of the gravy. Heat treatment of the gravy was remarkably more effective than that of the rice, thus giving a smaller probability for the survival of spores in spices, despite their higher initial concentration.

To prevent further outbreaks, rice and spices of good microbiological quality should be chosen. Temperatures in excess of 10° C. should be avoided during the storage period of boiled rice. The whole portion of meal should be prepared at the same time in order to avoid the biphasic temperature curve which enables *B. cereus* to grow in rice.

REFERENCES

- CASMAN, E. P. (1967). Staphylococcal food poisoning. *Health Laboratory Science* **4**, 199.
- CASMAN, E. P., BENNETT, R. W., DORSEY, A. E. & STONE, J. E. (1969). The microslide gel double diffusion test for the detection and assay of staphylococcal enterotoxins. *Health Laboratory Science* **6**, 185.
- GIBSON, T. & GORDON, R. E. (1974). *Bacillus*. In *Bergey's Manual of Determinative Bacteriology* (ed. R. E. Buchanan and N. E. Gibbons), 8th ed., p. 529. Baltimore: The Williams & Wilkins Co.
- GILBERT, R. J., STRINGER, M. F. & PEACE, T. C. (1974). The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. *Journal of Hygiene* **73**, 433.
- GILBERT, R. J. & TAYLOR, A. J. (1975). Das Auftreten von *Bacillus cereus*-Lebensmittelvergiftungen in Grossbritannien. *Archiv für Lebensmittelhygiene* **26**, 38.
- GLATZ, B. A. & GOEFFERT, J. M. (1973). Extracellular factor synthesized by *Bacillus cereus* which evokes dermal reaction in guinea pigs. *Infection and Immunity* **8**, 25.
- GOEFFERT, J. M., SPIRA, W. M. & KIM, H. U. (1972). *Bacillus cereus*: food poisoning organism. A review. *Journal of Milk and Food Technology* **35**, 213.
- HAUGE, S. (1950). Matforgiftningen fremkalt av *Bacillus cereus*. *Nordisk Hygienisk Tidsskrift* **31**, 189.
- KIM, H. U. & GOEFFERT, J. M. (1971). Occurrence of *Bacillus cereus* in selected dry food products. *Journal of Milk and Food Technology* **34**, 12.
- LEFEBVRE, A., GREGOIRE, C. A., BRABANT, W. & TODD, E. (1973). Suspected *Bacillus cereus* food poisoning. *Epidemiological Bulletin, Canada* **17**, 108.
- MIDURA, T., GERBER, M., WOOD, R. & LEONARD, A. R. (1970). Outbreak of food poisoning caused by *Bacillus cereus*. *Public Health Reports, Washington* **85**, 45.
- MORTIMER, P. R. & MCCANN, G. (1974). Food poisoning episodes associated with *Bacillus cereus* in fried rice. *Lancet* **i**, 1043.
- NISKANEN, A., KIUTAMO, T., MÄLKKI, Y. & NIKKILÄ, E. (1975). Nachweis von *Bacillus cereus* in Lebensmitteln: Gaschromatographische Analyse der Zusammensetzung der bakteriellen Fettsäuren. *Archiv für Lebensmittelhygiene* **26**, 15.
- NYGREN, B. (1962). Phospholipase C-producing bacteria and food poisoning. *Acta pathologica et microbiologica scandinavica, Supplementum* **160**, 1.

- PUBLIC HEALTH LABORATORY SERVICE (1972). Food poisoning associated with *Bacillus cereus*. *British Medical Journal* **i**, 189.
- PUBLIC HEALTH LABORATORY SERVICE (1973). *Bacillus cereus* food poisoning. *British Medical Journal* **iii**, 647.
- RAEVUORI, M. & GENIGEORGIS, C. (1975). Effect of pH and sodium chloride on growth of *Bacillus cereus* in laboratory media and certain foods. *Applied Microbiology* **29**, 68.
- REISER, R., CONAWAY, D. & BERGDOLL, M. S. (1974). Detection of staphylococcal enterotoxins in foods. *Applied Microbiology* **27**, 83.
- REMINGTON, R. D. & SCHORK, M. A. (1970). *Statistics with Applications to the Biological and Health Sciences*, p. 242. Englewood Cliffs: Prentice-Hall Inc.
- SPIRA, W. M. & GOEFFERT, J. M. (1972). *Bacillus cereus*-induced accumulation in rabbit ileal loops. *Applied Microbiology* **24**, 341.
- TAYLOR, A. J. & GILBERT, R. J. (1975). *Bacillus cereus* food poisoning: a provisional serotyping scheme. *Journal of Medical Microbiology* **8**, 543.
- TODD, E., PARK, C., CLECNER, B., FABRICIUS, A., EDWARDS, D. & EWAN, P. (1974). Two outbreaks of *Bacillus cereus* food poisoning in Canada. *Canadian Journal of Public Health* **65**, 109.
- U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE. CENTER FOR DISEASE CONTROL (1973). *Bacillus cereus* food poisoning – United Kingdom. *Morbidity and Mortality Weekly Report* **22**, 348.