Outbreak of Food Poisoning Caused by *Bacillus cereus*

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ALTHOUGH Hauge (*1*) and other European investigators (*2, 3*) have established *Bacillus cereus* as an etiological agent of food poisoning outbreaks for over a decade, there have been few well-documented reports of the association of *B. cereus* with food poisoning in the United States (*4, 5*). *B. cereus* causes a form of food poisoning characterized by diarrhea, abdominal pain, and nausea with little or no vomiting. Symptoms usually occur 8–16 hours after ingestion of the contaminated food.

Hauge (*1*) found *B. cereus* to be the etiological agent in eight instances of food poisoning involving foods such as vanilla sauce, cream sauce with egg, instant meat gravy prepared from a powder, and meat preserved in gravy. Experimentally, he was able to reproduce the symptoms in himself and in four of six volunteers, each of whom had consumed 155–270 ml. of vanilla sauce containing 30–60 million *B. cereus* per ml. The mechanism responsible for inducing the disease has not been conclusively demonstrated. In common with outbreaks caused by *Clostridium perfringens*, those caused by *B. cereus* appear to require millions of living bacteria per gram of food to cause symptoms (*1, 6*).

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This paper describes and discusses a food poisoning outbreak which affected students fed through a common kitchen in a fraternity house at the University of California. *B. cereus* was found to be the etiological agent and meat loaf the vehicle.

Circumstances of the Outbreak

On May 8, 1969, an outbreak of food poisoning was reported to the city of Berkeley's Department of Public Health. Five students had reported to the university's Cowell Memorial Hospital between 1 and 5 a.m. for treatment of diarrhea, abdominal pain, and nausea with some vomiting. The patients had little or no fever. The evening meal eaten on May 7 was suspect.

An investigation by the health department revealed the following information. The fraternity had 36 members; 31 ate the dinner meal, at either the first serving at 5 p.m. or the second serving at 7:30 p.m. The meal was served in two sittings because subsequent appointments required that some persons leave early. The majority of the members ate at 7:30 p.m.

Of the 31 members who ate the meal, 15 became ill—an attack rate of approximately 48.3 percent. Of the 13 persons who ate at 5 p.m., four became ill; of the 18 who ate at 7:30 p.m., 11 became ill. The illness was characterized by diarrhea, abdominal pain, nausea with some vomiting, and little or no fever. The average incubation period in this outbreak was 10 hours. Symptoms persisted for no more than 24 hours. Six patients were interviewed directly, and informa-

tion regarding the nine others was obtained from officers of the fraternity. Illness was confined to persons who ate the evening meal at the fraternity house.

The meal consisted of meat loaf, a synthetic gravy product, mashed potatoes, string beans, lettuce salad with commercially prepared and bottled French dressing, and strawberry shortcake with whipped cream. Coffee and milk were also served.

According to the cook who had prepared the meal, the 25 pounds of ground beef used in the meat loaf was delivered by a local meat supplier at approximately 11 a.m. on May 7 and was placed in the refrigerator with a temperature of 40° F. The meat was wrapped in a paper container and was not frozen.

At 2:30 p.m. the ground beef was removed from the refrigerator and placed in a stainless steel bowl, 10 inches deep and 24 inches in diameter. Eggs, onion, garlic, catsup, and a small amount of flour were added and thoroughly mixed. The meat loaf mixture was placed in a container measuring 20 inches square and 4 inches deep and was shaped like a loaf of bread that measured at least 10 inches at its thickest section. Then the meat loaf was placed in the oven and cooked at 350° F. until 4 p.m., when it was removed, allowed to cool on a table until 4:30 p.m., covered, and placed on an oven grill set at warm temperature.

At 5 p.m. portions were sliced with a kitchen knife and served to 13 members. A synthetic gravy product which contained no meat was prepared and used separately. Flour and water were added for thickness before the gravy preparation was heated and poured into a container from which members served themselves. None of the meat loaf was mixed with this gravy product. The strawberry shortcake was served with fresh whipped cream.

The remaining meat loaf was returned to the oven maintained at 250° F. and served to the other 18 members at 7:30 p.m. A small portion that was left over from the second serving was placed in the refrigerator.

Investigation

The remainder of the suspected meat loaf, which was stored at 40° F. following the second serving, was available for culture. Microscopic examination of a 1:10 dilution of the meat loaf revealed the presence of large gram-positive rods. Serial dilutions were made, and the number of viable organisms was determined by plate counts. The plates were incubated aerobically and anaerobically at 35° C. for 48 hours. The total aerobic plate count of the meat loaf was 76×10^6 per gram, of which 70×10^6 per gram were identified as *B. cereus*.

This aerobic sporeformer had the following characteristics which identify it as *B. cereus* (*7*).

Gram-positive rods more than 0.9 microns in diameter
Spores that did not bulge the sporangium
Motile
Acid from glucose and salicin
Production of acetylmethylcarbinol
Liquefaction of gelatin
Peptonization of litmus milk
Reduction of nitrates to nitrites
Hemolysis of sheep blood
Production of lecithinase

The remaining organisms were identified as gram-positive cocci: streptococci, not enterococci, and coagulase-negative micrococci.

The synthetic gravy and the flour were also examined. The total plate count for the gravy was less than 10^2 organisms per gram and for the flour was 10^3 per gram. No mashed potatoes, string beans, or whipped cream used in the dessert was available for examination.

A stool culture collected from the cook was negative for the usual enteric pathogens as well as for *B. cereus*. Since stool examinations for *B. cereus* are not recommended (*1*), specimens were not collected from the students. Because of the widely variant attack rates and the laboratory data indicating large numbers of bacteria in the meat loaf, a limited study was conducted to determine the temperature of the meat loaf during its initial cooking.

Twenty-five pounds of ground beef were ordered from the same meat distributor with the request that it be the same quality purchased by the fraternities and sororities. Using the same utensils and oven with the oven temperature indicator reading 350° F., a meat loaf was prepared according to the directions given by the cook.

Three iron constanton thermacouples were used with a Honeywell potentiometer for determining temperatures. One thermacouple was in-

serted into the center of the meat loaf and one 4 inches from either end. The meat loaf was placed in the preheated gas oven and cooked with the gas oven temperature indicator reading 350° F. (176° C.); the actual oven temperature was 291° F. (144° C.) as determined by the use of a fourth thermacouple.

At the end of 1 hour, the thermacouples registered the following temperatures. The oven temperature was 345° F. (174° C.), the center of the meat loaf was less than 77° F. (25° C.), the front portion was 88° F. (31° C.), and the back portion was 79° F. (26° C.). At the end of 1½ hours, the maximum time period the meat loaf could have been cooked at 350° F., the oven temperature was 370° F. (188° C.), the center of the meat loaf was still less than 77° F. (25° C.), the front was 108° F. (42° C.), and the back was 102° F. (39° C.). It took 2 hours and 20 minutes for the center of the meat loaf to reach 77° F. (25° C.).

Discussion

The epidemiologic aspects of the various *B. cereus* outbreaks are quite characteristic. The incubation period is usually from 8 to 16 hours, and the illness is characterized by diarrhea, abdominal pain, nausea with little or no vomiting, and no fever. Recovery within 24 hours is usual.

Previously reported outbreaks have been associated with puddings, sauces, or meat combinations which were prepared in large quantities some hours before eating and which were stored at temperatures that would have allowed g owth of the organism. The incriminated food usually showed *B. cereus* in excess of 10^6 organisms per gram of food.

B. *cereus* outbreaks are in many ways very similar to those caused by *C. perfringens*. Therefore, every laboratory should have available a protocol to determine the presence and the relative numbers of aerobic and anaerobic organisms. Microbiological examinations of both food and stool specimens are important for diagnosis of *C. perfringens*. *C. perfringens* dominates the fecal flora, whereas *B. cereus* is not excreted in large numbers (*1*).

In this outbreak, the symptomology, the incubation period, the duration of symptoms, and the number of *B. cereus* organisms in the meat loaf indicated that food poisoning had occurred and that *B. cereus* was the etiological agent. Having confirmed that food poisoning had occurred, an attempt was made to ascertain what foodhandling practices contributed to the outbreak.

Quantitative accumulation of *B. cereus* in a food depends on the number of organisms initially present, the suitability of the food as a growth medium, and the length of time during which growth is possible. Kjellander and Nygren (*8*) reported an investigation of the occurrence of *B. cereus* in various types of foods. Of 514 food samples, 26 percent of the meat products, 77 percent of the milk products, and 51 percent of the vegetables, fruits, and nuts contained *B. cereus*, often in considerable numbers.

Information regarding factors influencing the growth of *B. cereus* in food is scanty. Since sporeforming bacteria such as *B. cereus* can survive moderate heat, spores surviving in cooked food may germinate and multiply rapidly when the food is held at favorable temperatures.

The overall attack rate among the students who ate the meal was 48.3 percent. The attack rate among the students who ate at 5 p.m. was 30.7 percent, and for those who ate at 7:30 p.m. it was 61.1 percent. The difference in these attack rates suggests the possibility that the organisms multiplied in the meat loaf.

Jensen (*9*) reported that when foods are held between 50° F. (10° C.) and 120° F. (49° C.) for 4 to 8 hours, they are in the danger zone for bacteria growth associated with food poisoning. Also, Castellani and co-workers (*10*) studied the temperature required to kill certain food poisoning organisms in stuffed turkey. (*B. cereus* was not included in this study.) These investigators found that the organisms in stuffing multiplied during initial stages of the roasting process. An internal temperature of 165° F. was required to kill the organisms in the stuffing. A post-oven rise of 5° to 10° F. in temperature occurred in the center of the stuffing if the turkey was allowed to stand at room temperature for 20 minutes. The period of multiplication is longer in larger turkeys because the rate of heat penetration into the stuffing is slower. Cooking should not be interrupted until the lethal temperature (165° F.) is reached.

In view of Jensen's and Castellani's reports, the data obtained in our experiment suggest that the major part of the cooking and holding time of the meat loaf may have been a period for microbial growth. In the prevention of bacterial food poisoning outbreaks, emphasis must be placed on proper temperature control, that is, keeping food hot enough or cold enough to retard germination of spores or growth of organisms.

Further, the importance of selecting cooking utensils which will permit thorough penetration of heat cannot be overlooked. Self-insulation of food due to its inherent density and the shape or size of its container probably has contributed to as many food poisonings as the other more obvious methods of mishandling, such as incorrect temperatures.

The search for organisms like *B. cereus* deserves emphasis because in nearly half the food poisoning outbreaks reported annually in the United States no etiological agent is identified. In 46 percent of the foodborne disease outbreaks reported in 1968, the etiology either was not specified or was unconfirmed (*5*).

Microbiologists in all laboratories charged with determining the causative agents and pinpointing the source in outbreaks of foodborne diseases should become familiar with procedures for identifying *B. cereus* and become aware of the significance of the numbers of the organisms present. With such knowledge a better understanding of the etiology of many outbreaks as well as the role of *B. cereus* and of other bacteria can be achieved.

Summary and Conclusions

A food poisoning outbreak affected 15 members of a fraternity at the University of California, Berkeley. The students developed an illness characterized by diarrhea, abdominal pain, nausea with little vomiting, and little or no fever. The average incubation period in this outbreak was 10 hours. Laboratory examination of the suspect food revealed 70 x 10^6 *Bacillus cereus* organisms per gram of meat loaf, but the more common agents usually incriminated in bacterial food poisoning were not observed. *B. cereus* was determined as the probable cause of this outbreak and the meat loaf the most likely vehicle for the organisms.

Few documented reports of *B. cereus* food poisoning have been reported in the United States. Laboratory investigations of foodborne outbreaks of gastroenteritis should include determinations of the total numbers of bacteria, kinds of bacteria, and the relative numbers of each kind involved. This practice would lead to increased recognition of outbreaks due to *B. cereus* and give a better indication of the organism's importance among the causes of foodborne diseases in the United States.

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Tearsheet Requests

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