A Clostridial or Enterococcal Food Poisoning Outbreak

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IN A SEMINAR on food poisoning, Cockburn (*1*) reported that *Clostridium welchii* was the cause of 1 percent of more than 55,000 food poisoning incidents in England and Wales during 1949–58. A high of 93 incidents for this period was recorded in 1957, mainly in group outbreaks. Most of these outbreaks were associated with cooked meat or meat products which had been left at room temperature for hours before serving. In contrast to the experience in England, there is a paucity of reports of clostridial food poisoning in the United States. McClung reported four outbreaks in 1945 (*2*). Hart and associates described an outbreak which affected more than 100 passengers on a train who had eaten a turkey dinner highly contaminated with *C. perfringens* and enterococci (*3*).

The outbreak reported in this paper occurred among persons who had attended a reunion dinner on Saturday, September 26, 1959. On the following Monday, the caterer who had prepared the food notified the Berkeley City Health Department that a large number of persons had become ill after the dinner. The hostess had refrigerated the remaining food.

The menu consisted of roast beef, barbecue sauce, chili beans (red beans with ground meat), tossed green salad with Roquefort cheese dressing, French bread with garlic and butter spread, and apple pie, all furnished by the caterer. Coffee, noncarbonated beverages, milk, and beer had been obtained from other sources.

Investigation revealed that a 22-pound roast of beef had been bought on Friday and refrigerated until 7:30 a.m. Saturday when it was roasted at 450° F. for 2½ hours. After it was removed from the oven at 10 a.m., the beef was cooled until noon when it was sliced, tied, and left at room temperature until 3 p.m. It was then placed in a 450° F. oven for only 20 minutes because the hostess came to pick up the food earlier than expected. Just before the meat was sliced, two frozen eviscerated turkeys were prepared on the cutting board and the board was highly contaminated by the liquid from the plastic bags enveloping the turkeys.

Since the other foods served seemed to have been prepared properly and there was no epidemiologic or laboratory evidence to incriminate them in the outbreak, they are not discussed further.

The meal was served at 6 p.m. and completed by 7:30 p.m. Snacks were eaten later, but it could not be learned by whom or at what time. The food was held at room temperature until midnight and refrigerated until it was picked up for examination the following Monday.

The hostess supplied the addresses and telephone numbers of the 41 guests, 9 of whom resided in Berkeley. A quick survey revealed that eight of the nine persons reported acute abdominal cramps and diarrhea, with a 9- to 14-hour incubation period. Two of the eight affected had eaten only roast beef. Epidemiologic followup was attempted for the remaining guests, who were scattered throughout California. Of the 38 guests contacted, 26 were ill;

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24 reported diarrhea and 13 acute abdominal cramps. None reported vomiting or fever. Diarrhea coupled with acute cramping was reported by 11 persons; diarrhea alone was reported by 13, and cramping alone was reported by 2 persons. The incubation period ranged from 6 to 15 hours after the main meal, with a mean, median, and mode of 10 hours. An analysis of food consumption and the clinical status of the 38 persons supplying information indicated that all of those affected consumed roast beef, although the attack rate for those who had eaten beef was only 78 percent. The attack rates for those who had eaten salad and bread were also 78 percent, but only 84 percent of those affected had eaten either one or both of these foods.

Laboratory Investigation

Laboratory examinations were sufficiently complete to rule out *Salmonella* and staphylococcal etiologies prior to the development of adequate epidemiologic histories. Foods studied included those mentioned earlier as well as scrapings from the cutting block. Stool samples, submitted in buffered glycerol saline, from some of the patients were examined. No remarkable bacteriological findings were observed with food other than the roast beef.

The meat was minced with scissors and a 1:10 dilution was prepared in 1 percent peptone water. The mixture was shaken with glass beads on a Kahn-type shaker for 20 minutes to produce a uniform emulsion. In an oil immersion field, a smear of the 1:10 dilution revealed a number of gram-positive rods greater than 0.9 micron in diameter. In addition, there was less than 1 gram-positive coccus per field. Because of the presence of gram-positive rods, 1 ml. of the 1:10 dilution was inoculated into 15 ml. thioglycollate broth with dextrose and incubated for approximately 18 hours. Marked gas production, an odor typical of *C. perfringens*, and the presence of a large number of asporogenous gram-positive rods greater in diameter than 0.9 micron led to a tentative conclusion that the outbreak was due to *C. perfringens*. The total count by aerobic pour plate technique was 1.7×10^8 organisms per gram on dextrose yeast plate count agar. An anaerobic count with the same agar in a phosphorus jar was 5.8×10^8 organisms per gram of food consistent with the morphology of *C. perfringens* and 4.2×10^9 organisms per gram of food which were subsequently identified as enterococci. On 1 percent egg-yolk agar with a surface streak incubated anaerobically, lecithinase positive colonies were present on the order of 2×10^8 organisms per gram.

Typical colonies were confirmed as *C. perfringens* by the following characteristics: coagulation and stormy fermentation in litmus milk; acid and gas produced promptly in glucose, maltose, glycerol, and lactose, but not in mannitol; catalase negative; nonmotile; on egg-yolk medium, colonies were surrounded by a large zone of opacity within 24 hours (anaerobically); iron not blackened; H_2S production was positive by lead acetate papers over tryptone broth but not in triple sugar iron agar; colonies showed a double zone of lysis on sheep blood agar and were considered probable type A. None of the isolates from the beef or from patients was found to be a member of Hobbs' 11 provisional serotypes of heat-resistant strains and all isolates were found by Dr. Betty C. Hobbs and by Dr. William Sadler to be heat labile types (personal communications, 1960).

The assistance of these investigators is gratefully acknowledged.

Although scrapings from the cutting block obtained 5 days after the outbreak failed to show *C. perfringens*, 5.2×10^5 enterococci per gram of scrapings were found. Identification of the enterococcus isolated from both meat and cutting block was made by the following evidence: gram-positive cocci in short to long chains; no catalase production; growth at 45° C.; growth in normal broth and broth with 6.5 percent NaCl; and alpha type hemolysis on sheep blood agar.

Discussion

It is clear that a foodborne outbreak occurred among those attending a group reunion. Since the evening meal was the first time the entire group had been together, it seems extremely unlikely that a common viral infection was the cause of the outbreak. A review of the epidemiologic and bacteriological findings does not reveal clear-cut evidence as to the etiologic

agent. Large numbers of both *C. perfringens* and enterococci were isolated from the meat. Although it is tempting to incriminate the *Clostridium*, the presence of enterococci raises serious questions.

Hobbs (*4,5*) has pointed out that *C. perfringens* may contaminate through the hands of cooks or butchers handling meat or through chopping boards. As high as 8.9 percent of carcass beef was found contaminated in England by Hobbs and Wilson (*6*). The intestinal tract of man and animals is considered to be a "normal" habitat of both clostridia and enterococci. Yamamoto and associates have shown that 28 percent of 110 turkey fecal samples yielded heat-sensitive *C. perfringens* (*7*). It is not surprising, then, that the roast in question was contaminated with both organisms, probably at the caterer's establishment as suggested by the finding of large numbers of enterococci on the cutting block.

From the history of the preparation of the meat, it is clear that inadequate cooking times were allowed and that ample opportunity for growth of contaminating organisms was afforded. Hobbs is quoted as demonstrating that on freshly boiled lamb slices without gravy, a mixture of spores and asporogenous *C. welchii* increased from approximately 260,000 to 19.5 million in 3 hours and in slices with gravy to 2.5 and 46 million organisms in 2 and 3 hours respectively, at a temperature range of 102–120° F. (*8*).

The literature is scanty regarding the "food poisoning" properties of enterococci and under what conditions such poisonings may occur. One cannot disregard the possibility of a synergistic relationship between enterococci and heat-labile *C. perfringens*. The outbreak reported here is similar to that reported by Hart and associates (*3*) in which large numbers of both types of organisms were present on the suspected turkey.

The successful isolation and quantitation of organisms in food left after the snack period of the reunion may have been due to a set of unusually favorable circumstances. The hostess refrigerated the food until it was picked up for laboratory investigation, there was no delay in processing the specimens, and the specimens were not frozen at any time prior to examination. L. deS. Smith has suggested that undue harshness in blending or freezing of specimens may significantly and rapidly decrease numbers of *C. perfringens* (personal communication and unpublished paper, 1960). It is common practice to freeze food samples allegedly involved in a food poisoning outbreak until sufficient epidemiologic information is developed to incriminate a particular food or foods. If information is obtained from patients which indicates a possible clostridial food poisoning, this procedure may need reevaluating to require holding some of each sample at refrigerator temperature as well as at freezing temperature.

In the outbreaks reported by Hobbs (*4*) and by Cockburn (*1*) the organisms were heat resistant. However, heat resistance could be lost in as little as one passage on an artificial medium. The organisms isolated from the meat and from the patients were heat sensitive and did not belong to Hobbs' provisional serologic groups. The question still remains whether great numbers of heat-sensitive strains of *C. perfringens* can per se be the primary cause of clostridial type of food poisoning. It may be that a heat-sensitive strain in conjunction with other organisms such as enterococci can be the incitant of food poisoning.

The findings in this outbreak once again point up the necessity of either keeping food hot enough or cold enough to prevent the multiplication of micro-organisms of all types. Removal of heat-resistant organisms such as clostridia from a roast cannot be accomplished short of ruining the food from a gastronomic point of view. Therefore, the serving of food maintained continuously hot or cold is one of the prerequisites to prevention of such outbreaks. The outbreak highlights the need for attention to cutting-block sanitation to prevent excessive transfer of organisms between foods of various types. Advances in knowledge regarding the etiologies of food poisoning require close cooperation of epidemiologists, investigators, sanitarians, and laboratorians to the end that specimens are collected and processed promptly by adequate procedures.

Summary

Investigation of a food poisoning outbreak among 26 dinner guests revealed that the roast

beef served had been prepared by a caterer who did not allow adequate time for roasting the meat and who also prepared frozen eviscerated turkeys on the cutting block just before using the block to slice the meat.

Bacteriological analysis of leftover food revealed no conclusive evidence as to the etiologic agent. However, large numbers of heat-labile *Clostridium perfringens* and enterococci were isolated from samples of the meat and enterococci were also found in scrapings from the cutting block.

The findings pointed up the necessity of keeping food sufficiently hot or cold to prevent the multiplication of micro-organisms of all types, as well as the need for apprising food-handlers as to the importance of cutting-block sanitation.

AUTHORS' NOTE: We wish to acknowledge the discussion of the significance of enterococci associated with other pathogens in food poisoning outbreaks in "*Salmonella infantis* Isolated From Ham in Food Poisoning Incident," by Dr. Robert Angelotti and associates, which appeared in *Public Health Reports*, September 1961, after this paper was prepared for publication.

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Seminars on Hepatitis

Seminars on viral hepatitis will be held in 12 States and Puerto Rico during 1962 to help State and local health officials control spread of the disease.

The Public Health Service's Communicable Disease Center and the States' public health associations are jointly sponsoring the seminars, which are being held in conjunction with annual meetings of the State associations.

Many of the nation's leading specialists in hepatitis will be among the participants. They will review the available information about identification, prevention, and control of hepatitis, including ways to assure prompt reporting of cases.

With more than 1,000 cases occurring every week, hepatitis now ranks third among the communicable diseases reported to the Public Health Service, exceeded only by measles and streptococcal infections. The disease seems to occur in 5- to 7-year cycles, but even the number of cases during the low years of the cycles has been gradually increasing.

In 1961, the number of reported cases reached an all-time high of 73,000, a 46 percent increase over the previous high, which occurred in 1954. In 1962 about 18,000 cases were reported during the first 13 weeks compared with about 23,000 cases during the same period last year.

Extensive research is underway on the development of a hepatitis vaccine, but at present the principal protective measures are community sanitation and personal hygiene. Gamma globulin is sometimes given to family contacts of hepatitis patients as a preventive measure.