Clostridium perfringens Food Poisoning: Use of Serotyping in an Outbreak Setting

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An outbreak of Clostridium perfringens food poisoning occurred among attendees of a firehouse luncheon. The predominant symptoms of diarrhea (100%) and abdominal pain (81%) among case-patients, the mean incubation period (13.4 h), and the mean duration of illness (21.2 h) were all characteristic of C. perfringens enteritis. Roast beef, although not epidemiologically implicated, was the most likely vehicle of transmission. Fecal specimens from case-patients contained a median C. perfringens spore count of greater than 10^6 and yielded isolates that were heat sensitive and predominantly nonhemolytic, produced C. perfringens enterotoxin A, and, in the majority of specimens (four of five), were identical in serotype. Food samples were negative. This outbreak demonstrates that following enumeration of C. perfringens from a suitable number of fecal specimens from case-patients, serotyping of the isolates may be helpful in implicating C. perfringens as the cause of foodborne illness. This is especially true when implicated food items test negative or are no longer available for testing.

An association of Clostridium perfringens with gastrointestinal disturbances was first noted in 1895 (11). The first large-scale outbreaks of food poisoning associated with this organism were reported in 1943 and affected approximately 250 individuals (12). By 1981, C. perfringens, the third leading cause of foodborne disease, was responsible for 1,162 reported cases of enteritis and 28 foodborne outbreaks with a known etiologic agent reported to the Centers for Disease Control (3). Although C. perfringens is now recognized as a common cause of food poisoning throughout the world, the investigation of many outbreaks is often inadequate and incomplete, owing to, among other things, ignorance of the procedures necessary to implicate this organism in foodborne illness. Although the application of serological typing of C. perfringens isolates from implicated food and/or fecal specimens from case-patients has often been disappointing in confirming the cause of an outbreak, it may be useful, as was true in our investigation.

We report an outbreak of C. perfringens food poisoning among attendees of a firehouse luncheon; although roast beef was not epidemiologically implicated, it was the most likely vehicle of transmission. The median C. perfringens spore count in fecal specimens from case-patients was greater than 10^6, and the majority of specimens (four of five) yielded spore isolates identical in serotype.

On 18 November 1984, an annual fund-raising roast beef luncheon was held by the staff of a firehouse in their fire hall in rural southeastern Maryland. As estimated 900 persons from several neighboring cities and four counties attended, although a roster of attendees was never compiled. The luncheon began at noon and was over by 6 p.m.; the food, prepared at the local elementary school and the firehouse, was served family style. Two days after the luncheon, the Maryland Deparment of Health and Mental Hygiene was

notified that gastroenteritis had developed in luncheon attendees, and an investigation was begun.

MATERIALS AND METHODS

Since many of the luncheon attendees were residents of other cities, radio and press releases were issued in the four-county area urging those who attended (whether ill or well) to call their local health department. An epidemiologic questionnaire, consisting of questions concerning demographic characteristics, food consumption histories, and symptoms of illness, was administered by telephone between 3 and 7 days after the luncheon to the 211 persons who called in. Our case definition was diarrhea (greater than or equal to three loose or watery stools per day) with an onset on 18 or 19 November in any luncheon attendee.

Fecal specimens for culturing of enteric pathogens were collected by the local health department on 19 and 20 November from 12 acutely ill luncheon attendees. Unfortunately, these specimens were not examined for C. perfringens. Despite attempts to obtain more samples, only five additional fecal specimens for C. perfringens culturing, quantitation, and characterization were obtained on 22 November (within 72 h of the onset of illness) from a different group of case-patients who were still ill, and specimens were also obtained from three well luncheon attendees. These eight fecal specimens were cultured in laboratories at the Maryland Department of Health and Mental Hygiene and at the Food and Drug Administration by standard techniques of dilution, plating, and anaerobic incubation. Confirmed spore counts were made on three different types of agar and in iron-milk medium. Isolates from the eight fecal specimens were serotyped and characterized at Food and Drug Administration and Centers for Disease Control laboratories. The relative heat resistance of C. perfringens spores in fecal specimens, the hemolytic activity of colonies produced from cells presumed to be spores, and the ability of isolates to
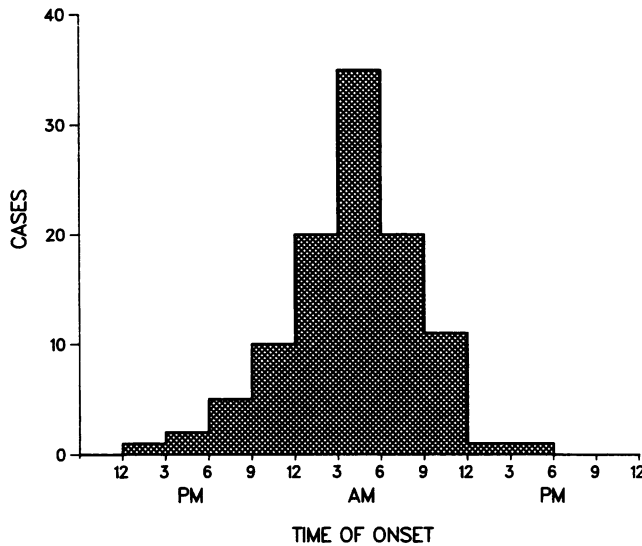
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FIG. 1. Cases of diarrheal illness, by date and time of onset, in Fruitland, Md., on 18 and 19 November 1984. Date and time of onset were unknown for six cases.

produce *C. perfringens* enterotoxin A were determined at the Food and Drug Administration laboratory. Methods for these procedures are described elsewhere (6). In addition, three isolates of *C. perfringens* from each of the five case-patients were serotyped at the Centers for Disease Control laboratory (7).

Several food items obtained from samples of three leftover roast beef meals were analyzed at the Maryland Department of Health and Mental Hygiene by standard bacteriologic methods. Methods of food preparation at both the elementary school and the firehouse kitchen were scrutinized. Food-specific attack rates were calculated. Statistical testing was done with the Fisher exact test and the chi-square test.

RESULTS

Of the 211 persons who were interviewed, 112 met the case definition; the crude attack rate was 53.1%. The male/female case-patient ratio was 0.75:1. The mean age of case-patients was 45.7 years, with a range of 5 to 84 years; age-specific attack rates were highest for those over 70 years of age (70.4%) and lowest for those 60 to 69 years of age (37.5%), with no apparent trend. The epidemic curve is shown in Fig. 1. The mean incubation period was 13.4 h, with a range of 4 to 20 h. The mean duration of illness was 21.2 h, with a range of 1 to 96 h. No one was hospitalized. The predominant symptoms in case-patients were diarrhea (100%), abdominal pain (81%), nausea (32%), loss of appetite (32%), and weakness (32%).

Analysis of food consumption histories which allowed for "yes," "no," or "not sure" responses revealed that 11 of the 13 food items (6 vegetables, 3 beverages, rolls, and butter) served were each consumed by less than 70% of the case-patients, whereas the two remaining food items (roast beef and dumplings in broth) were each consumed by more than 95% of the case-patients. Of the 207 persons who ate roast beef, 111 (54%) became ill, compared with 1 of 3 persons (33%) who did not ($P > 0.30$; Fisher two-tailed exact test); of the 193 persons who ate dumplings in broth, 106 (55%) became ill, compared with 5 of 16 persons (31%) who did not (chi-square = 2.44; $P > 0.10$). Since this was a roast

beef and dumpling luncheon, very few persons, whether they became ill or not, did not eat roast beef or dumplings and, thus, neither could be statistically significantly associated with illness.

The dumplings (plain dough) were boiled in fresh broth (not made from meat juices) for 10 to 15 min in the firehouse kitchen just prior to being served warm. Of the 50 roasts, most weighing between 12 and 18 lb (ca. 5.4 to 8.2 kg) (some up to 25 lb [ca. 11.3 kg]), half were cooked on 16 November and half were cooked on the following day at the local elementary school. The roasts were placed in 4-in. (ca. 10-cm)-deep pans, two to a pan, covered with foil, and cooked in a convection oven at 350°F (ca. 176.7°C) for 4 h. After the roasts were cooked, the meat juices were drained and discarded. The roasts were placed in single layers on shallow cookie pans (1 to 2 in. [ca. 2.5 to 5 cm] deep), six to eight per pan, covered with foil, stacked on a food rack, and allowed to cool in a kitchen office with a built-in cooling unit (temperature, 45 to 46°F [ca. 7.2 to 7.8°C]) for 3 to 4 h. They were stacked on shelves against one wall in a dry storage room with a similar cooling unit (temperature, 45 to 48°F [ca. 7.2 to 8.9°C]) for further cooling and awaited transport to the firehouse by refrigerator truck on the morning of the luncheon. Depending on the day they were prepared, the roasts were sliced and served cold at the firehouse 18 to 48 h after they had been cooked. The roasts were prepared by two school cafeteria employees, neither of whom had a recent history of acute illness. Both wore gloves during food preparation.

The fecal specimens obtained from 12 case-patients on 19 and 20 November were negative for *Salmonella* and *Shigella* species. Fecal specimens obtained on 22 November from the eight other luncheon attendees (five case-patients and three well persons) were also negative for these enteric pathogens as well as for *Staphylococcus aureus* and *Bacillus cereus*. Fecal specimens from two of the three well persons, including one of the two persons who had prepared the roast beef and one of only three persons identified at the luncheon who did not eat roast beef, were positive for heat-sensitive *C. perfringens* (9), as were specimens from the five case-patients (Table 1). The median *C. perfringens* spore count in the case-patient specimens that were quantifiable was greater than 10^6/g with all media used (Table 1). Isolates from fecal specimens from the five case-patients and one well person were predominantly nonhemolytic and produced *C. perfringens* enterotoxin A, and four of the five case-patient specimens yielded isolates of the same serotype (Hobbs type 13) (Table 1). The nontypeable isolates might all have been similar to each other, i.e., they, too, might have been derived from a single parent organism, but without a serological identity this cannot be established. Past investigations have incriminated more than one strain (7). Samples of all food items from three leftover dinners, including three small samples of roast beef, had negligible coliform counts and were negative for routine enteric pathogens, *S. aureus*, *B. cereus*, and *C. perfringens*.

DISCUSSION

The basic laboratory criteria established by Hauschild (8) for confirming *C. perfringens* as the cause of food poisoning are (i) a median spore count of greater than or equal to 10^6/g in fecal specimens from patients and (ii) isolates of the same identifiable serotype from the majority of fecal specimens from patients. These criteria were fully met by the findings of this investigation. Furthermore, the predominant symptoms

TABLE 1. Characteristics of *C. perfringens* isolates[a]

Specimen source	Spore count[b] on or in[c]:				Hemolysis[d]	Heat resistance of spores at 100°C (min)	CPEA (>100 ng/ml)[e]	Serotype[f]
	SPS agar	TSB agar	TSC agar	Iron milk medium				
Case-patient								
A	QNS[g]	172	140	110[h]	NH	15	+	13 or NT (1:2)
B	0.03	0.02	0.015	0.01	NH/H (2:1)	15	+	13 or NT (2:1)
C	7.8	1.4	1.2	1.1	NH	15	+	NT (0:2)
D	3.2	7.0	8.0	2.4	NH	15	+	13 (3:0)
E	6.5	3.0	4.0	4.6	NH/H (100:1)	15	+	13 (3:0)
Well person								
A	ND[i]	1	ND	1	H	15	−	ND
B[j]	ND	3	ND	1	NH	15	+	ND
C[j]	ND	0	ND	1	ND	ND		ND

[a] All isolates were confirmed as *C. perfungens* by the Association of Official Analytical Chemists method. They were nonmotile and nitrate, lactose, and gelatin positive.

[b] Reported as number of organisms × 10^6 per gram of stool for case-patients and as number of organisms × 10^3 per gram of stool for well persons.

[c] SPS, Sulfite-polymyxin-sulfadiazine; TSB, Trypticase (BBL Microbiology Systems)-soy-sheep blood; TSC, tryptose-sulfite-cycloserine without egg yolk.

[d] NH, Nonhemolytic (incomplete hemolysis on TSB agar); H, hemolytic; NH/H, both nonhemolytic and hemolytic (ratio at population level present on TSB agar).

[e] +, Present; −, absent.

[f] 13, Typeable isolates agglutinated with Hobbs reagent 13; NT, isolates nontypeable with 91 reagents. Numbers in parentheses represent ratios of typeable to nontypeable isolates.

[g] QNS, Not enough sample for quantitation.

[h] Most probable number (three tubes per dilution). Strains produced stormy fermentation in iron-milk medium within 18 h at 46°C.

[i] ND, Not determined.

[j] Cooked roasts.

of diarrhea and abdominal pain, the mean incubation period of 13.4 h, and the mean duration of illness of 21.2 h are all characteristic of *C. perfringens* type A enteritis (1). The occurrence of this outbreak in the fall is also consistent with seasonal patterns characterizing foodborne *C. perfringens* outbreaks, in contrast to the more common occurrence of other common foodborne enteric diseases in the summer (3).

Selection bias may have been operating in this investigation, since those who were ill or not ill were self-selected for interviews. This process may have resulted in biased estimates of demographic, illness, and/or food consumption information. The fact that the reported symptoms, incubation period, duration of illness, and laboratory findings were all consistent with *C. perfringens* enteritis makes selection bias in terms of these parameters less likely, although still possible. Furthermore, in terms of food consumption, it is difficult to postulate that those who were ill or not ill and who ate certain foods would be more likely to call in to be interviewed than those who did not eat those foods. Although roast beef could not be epidemiologically implicated, owing to the few numbers of people who did not eat it, its frequent association with foodborne *C. perfringens* outbreaks in general (15) and the manner in which it was stored and cooled in this outbreak are supportive of it being the most likely vehicle of transmission.

The ubiquitous distribution of *C. perfringens* in nature is well documented. Not only does the fecal flora of 95% of normal adults include *C. perfringens* (2), but this organism has also been isolated from raw fruits and vegetables (18), from soil (16), from dust and fomites in the kitchen (13), and from processed as well as carcass meats (veal, beef, lamb, and pork) (4, 9). Although dumpling consumption was highly correlated with roast beef consumption in this investigation, dumplings were not a likely vehicle of transmission, since they were boiled just prior to being served. In the preparation of the roast beef, the cooking process may have killed vegetative forms of *C. perfringens*, but some spores may have survived, germinated, multiplied, and produced toxin if

suitable temperatures were maintained, as was probably the case during the long cooling process in the office and storage room. Neither of the storage areas, made of cinder block construction and poorly insulated doorways, was designed for refrigeration of perishable items. The internal temperatures of the foil-covered racked roasts were likely much higher than the ambient temperatures, thus allowing for the rapid growth of organisms. Increases of 100,000-fold in the number of *C. perfringens* organisms have been noted in barbecued chicken kept at 45°C for 8 h (14). Contamination might also occur after cooking as a result of contact with sporebearing kitchen utensils, hands, and dust (8). If the food is not adequately reheated to destroy the multiplied vegetative cells, its consumption may produce illness. The failure to recover *C. perfringens* from the three roast beef samples may be a reflection of the small amount sampled and/or contamination of only some of the roasts which were consumed.

Nonhemolytic strains of *C. perfringens*, as noted in all of our case-patients, have been implicated frequently in foodborne outbreaks, whereas the heat resistance of strains varies greatly (5, 6, 9). The relatively large amounts of toxin (greater than 100 ng/ml) produced by isolates in all of our case-patient specimens have been noted previously in other outbreaks in strains which had a definite association with food poisoning (6) (Table 1). Nonhemolytic isolates of *C. perfringens* which also produced *C. perfringens* enterotoxin A were noted in one well person, one of the two cooks who helped prepare the roasts. Although not ill, she did eat roast beef and may have acquired the organism from that source but in less than the threshold number for causing illness; the lower number detected in her feces tends to support this explanation. Enterotoxin-positive isolates are rare in normal feces, but they do occur. The only "true" control had a fecal specimen with a low spore count, and the isolates were hemolytic and did not produce *C. perfringens* enterotoxin A.

The usefulness of serotyping in outbreaks in Great Britain has been noted by Stringer et al. (17). Thorough investiga-

tions of outbreaks within a more restricted geographical area and diligent preparation of new reagents whenever nontypeable strains have been encountered are no doubt responsible for the high success rate in that country. Serotype information, without organism or spore quantitation in food or fecal specimens, respectively, has been used in other published reports implicating *C. perfringens* as the etiologic agent (10, 19). In our investigation, four of the five case-patient fecal specimens yielded isolates of the same identifiable serotype. A second, nontypeable isolate may also have been involved in this outbreak. It could not be determined whether the five nontypeable isolates were related to each other. The facts that a case-patient (C) had a very high *C. perfringens* spore count, while the two tested isolates failed to agglutinate, and that two case-patients (A and B) also yielded nontypeable isolates support this speculation.

The Centers for Disease Control criteria, as contrasted with Hauschild's (8), for implicating *C. perfringens* as a cause of foodborne illness rely only on organism and spore counts in food and feces, respectively (15). These criteria occasionally may be inadequate, as this outbreak demonstrates, because of a lack of satisfactory food samples. The enumeration of *C. perfringens* from a suitable number of fecal specimens (at least five) from case-patients and subsequent serotyping of the isolates can be helpful in implicating *C. perfringens* as the cause of foodborne illness. This is especially true when implicated food items test negative or are no longer available for testing.

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