JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1988, p. 471–474
0095-1137/88/030471-04$02.00/0

Vol. 26, No. 3

Characterization of an Outbreak of *Clostridium perfringens* Food Poisoning by Quantitative Fecal Culture and Fecal Enterotoxin Measurement

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Received 23 September 1987/Accepted 1 December 1987

Published criteria for implicating *Clostridium perfringens* as the cause of food-poisoning outbreaks include finding a median fecal *C. perfringens* spore count of $>10^6$/g among specimens from ill persons. We investigated a food-poisoning outbreak with the epidemiologic characteristics of *C. perfringens*-related disease in a nursing home in which the median fecal spore count for ill patients (2.5×10^7/g) was similar to that for well patients (4.0×10^6/g), making the etiology of the outbreak uncertain. All ill and well patients tested had eaten turkey, the implicated food item. *C. perfringens* enterotoxin was detected by reverse passive latex agglutination in fecal specimens from six of six ill and none of four well patients who had eaten turkey ($P = 0.005$), suggesting that this organism had caused the outbreak. This investigation suggests that detection of fecal *C. perfringens* enterotoxin is a specific way to identify this organism as the causative agent in food-poisoning outbreaks.

Clostridium perfringens is a common cause of food-poisoning outbreaks in the United States (2). The illness is characterized by diarrhea and abdominal cramps without fever (1, 2, 21). Symptoms typically begin 7 to 15 h after consumption of food contaminated with high concentrations of the organism and last ≤24 h (21).

Epidemiologic criteria have been established to confirm whether *C. perfringens* is the etiologic agent in outbreaks, since it is common for *C. perfringens* spores to be present in small numbers in human fecal specimens and raw food samples (11). These criteria include a median fecal *C. perfringens* spore count of $>10^6$/g in ill persons (1, 11, 21); $>10^5$ organisms per g in epidemiologically implicated food items (1, 2, 11, 21); and identification of *C. perfringens* of the same serotype in most ill persons, but not in well persons (2). Recently, however, there have been several reports that *C. perfringens* spore counts of $>10^6$/g may be found in fecal samples taken from institutionalized well patients at times when no outbreak had occurred (24, 26).

Detection in fecal samples of *C. perfringens* enterotoxin, the substance thought to mediate the pathogenesis of diarrhea due to this organism (6, 7, 19), has been suggested to be the definitive method of implicating this organism as the cause of illness (13, 24). In this report, we describe the investigation of an outbreak of *C. perfringens* food poisoning in a nursing home in which the detection of enterotoxin in fecal specimens from ill but not well patients helped to confirm the etiology of the illness.

Outbreak. On 16 January 1986, the Epidemiology Division of the Vermont Department of Health was informed by the infection control nurse at a nursing home that many patients had been ill with diarrhea that day and the previous night. The illnesses were generally mild; no patient required intravenous therapy. The home is a skilled-nursing facility housing 142 long-term, chronically ill patients. Most patients are bedridden, communicate poorly, and are fed and bathed by the staff. About 130 staff members attend the patients. Meals for patients are prepared in a central kitchen.

MATERIALS AND METHODS

Epidemiologic investigation. Since many patients were unable to answer questions reliably, the nursing-home staff compiled a list classifying the patients by illness status during the period 15 to 17 January 1986. The dietary status of each patient was determined from dietary-office records. The nursing staff was instructed to obtain fecal and serum specimens from ill and well patients on each of the five wards and in each of the three dietary groups (regular, ground, and unrestricted). Medical charts were reviewed for patients from whom clinical specimens were obtained to verify illness and food consumption status. Food served to patients was assumed to have been eaten unless a notation to the contrary was present in the chart. In addition, on 16 and 17 January a questionnaire concerning food consumption and symptoms during the period 13 to 17 January was administered to nursing-home staff members. Fecal specimens were not collected from staff members.

The nursing-home kitchen, food storage areas, and food transport carts were inspected by a health department sanitarian. The source and preparation of all food items served during the week were reviewed. Food samples were collected and transported on ice for bacteriologic analysis.

Laboratory investigation. Whole fecal specimens were collected in sterile containers without preservative. Portions were transferred immediately into buffered glycerol-saline and Cary-Blair medium for later culture for bacterial pathogens. A portion of each specimen without preservative was stored at −70°C for later *C. perfringens* spore count and enterotoxin analysis.

Fecal specimens were analyzed for *Salmonella*, *Shigella*, and *Campylobacter* species and *Bacillus cereus* by standard laboratory methods (15). Qualitative cultures and quantita-

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tive fecal spore counts for *C. perfringens* were performed by the method of Hauschild (11).

The *C. perfringens* enterotoxin level was measured for all patients for whom sufficient fecal specimens were available. Each specimen was homogenized (1 g of feces in 9 ml of phosphate-buffered saline with 0.05% Tween 20), the mixture was centrifuged (15,000 × *g* for 30 min at 4°C), and the supernatant fluid was passed through a membrane filter (pore size, 0.22 μm; EGWP; Millipore Corp.). Enterotoxin was detected both by reverse passive latex agglutination (RPLA), using a commercial kit (Oxoid USA) as described by Harmon and Kautter (9), and by four-layer sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (16, 18). In the RPLA testing, agglutination determined by visual inspection was considered a positive result. In the ELISA, an optical density at 405 nm of ≥0.2 after correction for background fecal control values was considered positive. The concentration of enterotoxin per gram of feces was determined from the optical density value. The specificity for *C. perfringens* enterotoxin has been shown to be high for both the ELISA (18) and RPLA test (17).

Serum samples were tested for anti-enterotoxin antibody by using the ELISA method of Niilo and Cho (20), except that each ELISA well was coated with 0.5 μg of enterotoxin or human hemoglobin as a negative control. An optical density at 405 nm of ≥0.4 after correction for background serum control values was considered positive; the titer reported is the reciprocal of the last dilution yielding a positive ELISA.

Enterotoxin production by clinical isolates of *C. perfringens* from all patients from whom viable isolates were available was measured by ELISA with cultures that had been heat shocked and then subcultured in Duncan-Strong medium (5) as previously described (18). *C. perfringens* FD-1 (a known enterotoxin-negative strain) and *C. perfringens* NCTC-8239 (a known enterotoxin-positive strain) were included as controls (18).

Serotyping of *C. perfringens* isolates from all nursing-home patients who submitted fecal specimens was performed at the Botulism Laboratory, Centers for Disease Control, by the whole-cell agglutination method previously described (4, 10).

RESULTS

Epidemiologic investigation. A case of outbreak-related diarrhea was defined as any nursing-home patient reported by the nursing staff, or any member of the nursing-home staff self-reported by questionnaire, to have experienced loose or watery stools in the 48 h following noon on 15 January 1986. From nursing reports, 70 (49%) of 142 patients met this case definition. Vomiting and fever were not commonly reported. Symptoms resolved in most patients within 24 h. Attack rates for patients ranged from 28% to 56% on the five nursing-home wards. The attack rate was 57% for patients on regular diets, 47% for those on ground diets, and 32% for those on unrestricted diets who ate in the cafeteria and had a choice of food items.

Questionnaires were completed by 113 (87%) of 130 staff members. Sixteen (14%) staff members met the case definition. They reported related symptoms including abdominal cramps (81%), nausea and vomiting (38%), and fever (13%). The median duration of illness in staff members was 15.5 h. The onset times of diarrheal illness in staff and in ill patients whose medical charts were reviewed because they submitted fecal specimens are shown in Fig. 1.

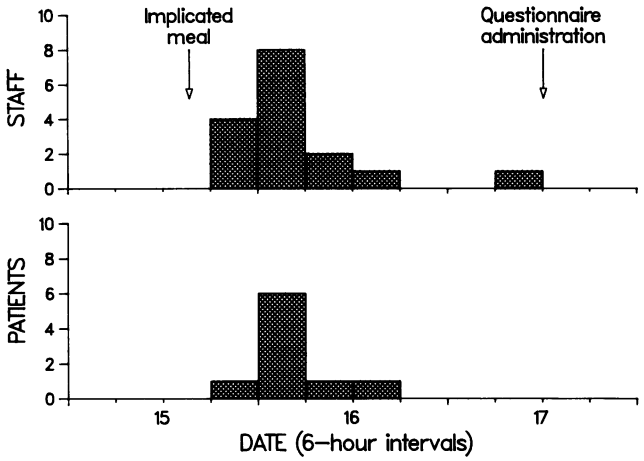

FIG. 1. Diarrheal illness among staff members and patients submitting stool specimens, by time of onset.

Of the staff members who ate nursing-home cafeteria-prepared food for lunch on Wednesday, 15 January 1986, 10 (59%) of 17 who ate turkey were ill compared with 0 (0%) of 8 who did not eat turkey (relative risk = infinity, $P = 0.008$; two-tailed Fisher exact test).

All patients, including members of all three dietary groups, had been served turkey for lunch on Wednesday. All ill and well patients submitting fecal cultures were likely to have eaten turkey on the basis of a review of dietary and medical records. The median incubation period from the implicated Wednesday noon meal to the onset of symptoms was 12 to 18 h in ill staff and ill patients submitting fecal specimens (Fig. 1).

Several violations of food handling practices were identified. The implicated turkey was prepared from frozen whole turkeys that had been thawed overnight in a refrigerator, cooked the following day (reported internal temperature of approximately 190°F [88°C] on a single measurement of one turkey), cooled at room temperature, and refrigerated overnight. The following morning the turkeys were sliced, and the slices were placed into warming ovens at approximately 150°F (66°C) for several hours before being served. Ground portions were prepared from turkey slices.

Laboratory investigation. Fecal specimens were collected by noon, 17 January, from 11 ill and 8 well patients. No *Salmonella*, *Shigella*, or *Campylobacter* spp. or *B. cereus* strains were isolated.

C. perfringens was isolated from fecal specimens from all (19 of 19) nursing-home patients tested (Table 1). Quantitative culture results were similar for ill and well patients: there were $>10^6$ spores per g for 82% (9 of 11) of ill patients and 75% (6 of 8) of well patients, and the median fecal spore count was $>10^6$ for both ill (2.5×10^7/g) and well (4.0×10^6/g) patients ($P = 0.5$, two-tailed rank sum test). However, RPLA testing of fecal specimens from six ill (median spore count, 2.4×10^7/g) and four well (median spore count, 7.5×10^5/g) patients for enterotoxin was positive for all ill and for no well patients ($P = 0.005$, two-tailed Fisher exact test) (Table 1). Enterotoxin was positive by ELISA in specimens from all six ill patients and one of the four well patients ($P = 0.03$). The concentrations of enterotoxin in the positive samples ranged from 1 to 50 μg/g of feces; negative samples had concentrations of essentially zero after correction for background control values.

Acute-phase serum specimens were collected by noon on

TABLE 1. Results of fecal cultures and enterotoxin determinations for patients

Culture group	*C. perfringens* culture			*C. perfringens* enterotoxin assay		
	No. tested	No. (%) positive	No. (%) containing $>10^6$ spores/g	No. tested	No. (%) RPLA positive	No. (%) ELISA positive
Ill patients	11	11 (100)	9 (82)	6	6 (100)	6 (100)
Well patients	8	8 (100)	6 (75)	4	0 (0)	1 (25)

17 January from 10 ill and 4 well patients selected by the nursing staff. (Eight patients had submitted both serum and fecal specimens.) Convalescent-phase serum specimens were collected from the same patients 5 to 6 weeks later. Four (40%) ill patients showed a fourfold serologic titer rise in serum specimens between the acute and convalescent phases, compared with none of the four well patients ($P = 0.5$). As a group, however, ill patients showed a fourfold median reciprocal titer rise in serum specimens from 100 (acute phase) to 400 (convalescent phase) ($P = 0.04$, two-tailed rank sum test), whereas well patients showed no titer rise in serum specimens from the acute-phase median titer of 100.

C. perfringens clinical isolates from all five ill patients and one well patient tested (all had fecal spore counts of $>10^7$/g) produced enterotoxin in vitro. *C. perfringens* isolates from all the 11 ill and 8 well patients were serotype Hobbs 13. *C. perfringens* could not be isolated from cultures of a small amount of remaining turkey.

DISCUSSION

The detection of enterotoxin in fecal specimens in this outbreak is strong supportive evidence that *C. perfringens* was the causative agent. *C. perfringens* enterotoxin is a heat-labile protein of molecular weight ca. 34,000 that is produced during sporulation (12, 23). It is important in the pathogenesis of diarrhea, since it directly damages the intestinal epithelium and inhibits glucose transport (6, 7, 19). Feeding enterotoxin to human volunteers reproduces the symptoms of *C. perfringens* food poisoning (22). Therefore, the detection of enterotoxin probably indicates that symptoms are due to *C. perfringens*. In this investigation, the pattern of enterotoxin detection in ill but not well patients suggested that *C. perfringens* caused the outbreak.

Other evidence supporting *C. perfringens* food poisoning includes the typical clinical symptoms and incubation period (1, 2, 21) and the determination that turkey, a commonly implicated food item (2), was the vehicle. Food preparation histories indicated that the turkeys may not have been completely thawed and may not have reached sufficiently high internal temperatures during cooking to destroy *C. perfringens* spores, which are often present on turkey carcasses (3). The long period of cooling of the whole turkeys at room temperature could have allowed multiplication of vegetative organisms to concentrations sufficient to cause illness.

Quantitative fecal *C. perfringens* spore counts were less helpful in determining the cause of the outbreak, since both ill and well patients had median fecal spore counts of $>10^6$/g. Although fecal counts of $<10^3$ can be found in normal human populations (11, 21), several recent reports have indicated that *C. perfringens* spore counts of $>10^6$/g can also be found in debilitated, institutionalized patients who are neither acutely ill nor involved in a food-poisoning outbreak (24, 26). It is possible, therefore, that the high quantitative fecal counts in our study simply indicated underlying carriage of *C. perfringens* in this nursing-home population and that the true etiology of the outbreak was not identified. Similarly, serotype analysis was inconclusive, since *C. perfringens* of the same serotype was found in specimens from both ill and well patients. Single, predominant serotypes have been reported by other investigators in nursing-home patients (24).

In our study, both ill and well patients had probably been exposed to the implicated food item. The inability to identify a suitable, unexposed comparison group is a common problem in epidemiologic investigations of cohorts of institutionalized or otherwise related people. In outbreak settings it is often most appropriate to collect clinical specimens from persons on the basis of illness status, since it may take several days to completely survey the population at risk to implicate a food item, and delay in obtaining specimens may reduce the chances of finding a pathogen. For this outbreak, in which most of the patients were exposed to the implicated food item, the strategy of collecting specimens from both ill and well patients set the stage for finding that fecal excretion of large amounts of *C. perfringens* may be common in persons exposed to sufficient numbers of organisms whether or not they become ill. Enterotoxin was measurable only in specimens obtained from those who did become ill. To our knowledge, this is the first demonstration in a *C. perfringens* outbreak that enterotoxin production is a suitable marker for illness. This finding is analogous to that in *Clostridium botulinum* infection in infants, when the organism can be recovered from fecal specimens from asymptomatic infants but the botulinum toxin is present only in specimens from those with symptomatic botulism (25).

The interpretation of the serologic results in this investigation is less certain. As a group, the 10 ill patients tested showed a fourfold median titer rise of anti-enterotoxin antibodies in serum, but only four patients in the group showed an individual fourfold titer rise. No well patients who had eaten turkey showed a fourfold titer rise in serum, suggesting that infection (gastrointestinal tract colonization with *C. perfringens*) without disease does not lead to a serologic response against enterotoxin. Thus, serologic testing is specific but not very sensitive for diagnosing *C. perfringens* food poisoning. It may be of some value in the diagnosis of individual cases, but may be more useful in outbreak settings in which groups of ill and well patients can be compared.

The results of this investigation may also shed light on the pathogenesis of *C. perfringens* diarrhea. Although both ill and well nursing-home patients exposed to *C. perfringens* had high fecal spore counts, indicating sporulation of the organism in the intestinal tract, only those with measurable fecal enterotoxin were ill. Several possible mechanisms could explain this finding. First, enterotoxin may not have been produced in amounts greater than those necessary for spore coat formation in infected patients who did not become ill. The concentrations of free enterotoxin in these

patients might have been below the detection limits of the RPLA test or ELISA, which are in the range of 50 to 250 ng of enterotoxin per g of feces (9), as well as below the levels which cause illness. Others (12, 14) have suggested a similar mechanism to explain the differences in pathogenicity among *C. perfringens* strains. Since all isolates in this outbreak were of the same serotype, variations in the amount of enterotoxin produced in different patients might have been due to undetermined host environmental factors.

An alternative explanation for our results is that enterotoxin was not produced at all in infected patients who did not become ill. This explanation implies that enterotoxin production might not be necessary for sporulation to occur: a possibility suggested recently by Goldner et al. (8). Our data cannot distinguish between these two explanations. However, one clinical isolate from a patient in our study was capable of producing enterotoxin in vitro, although none was detected in the feces of that patient, suggesting that the organism infecting the patient had not lost the ability to produce enterotoxin. A third possibility is that enterotoxin may be inactivated in some way, perhaps by binding to intestinal tract antibodies, in exposed persons who remain well.

The use of enterotoxin as a marker for *C. perfringens* diarrhea in epidemiologic investigations may aid in correctly identifying the cause of food-borne outbreaks. Diagnostic laboratories may find the available commerical RPLA kits for enterotoxin to be technically easier and less time-consuming and to allow better maintenance of proficiency, despite sporadic test demand, than current quantitative spore count methods. The standard epidemiological criteria for determining whether an outbreak is due to *C. perfringens* might be expanded to include detection of *C. perfringens* enterotoxin in fecal specimens from ill but not well patients.

ACKNOWLEDGMENTS

We thank Noelle Howland, Tom Hubble, Susan Freisem, and microbiology laboratory personnel, Vermont Department of Health, for technical assistance; Charles Hatheway, Botulism Laboratory, Centers for Disease Control, for serotyping the *C. perfringens* isolates; and both Charles Hatheway and Robert A. Gunn, Division of Field Services, Epidemiology Program Office, Centers for Disease Control, for critically reviewing the manuscript.

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