Clostridium perfringens Infections Initially Attributed to Norovirus, North Carolina, 2010

Natalie J. M. Dailey,1,5,a Nicole Lee,5 Aaron T. Fleischauer,2,5 Zack S. Moore,5 Edie Alfano-Sobsey,1 Frances Breedlove,1 Andre Pierce,6 Sue Ledford,5 Shermalyn Greene,6 Gerardo A. Gómez,3 Deborah F. Talkington,1 Mark J. Sotir,7 Aron J. Hall,4 and David Sweat6

1Epidemic Intelligence Service, 2Office of Public Health Preparedness and Response, 3National Center for Emerging and Zoonotic Infectious Diseases, and 4National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; 5North Carolina Department of Health and Human Services, and 6Wake County Human Services, Raleigh, North Carolina

We investigated an outbreak initially attributed to norovirus; however, Clostridium perfringens toxicoenteritis was subsequently confirmed. C. perfringens is an underrecognized but frequently observed cause of food-borne disease outbreaks. This investigation illustrates the importance of considering epidemiologic and laboratory data together when evaluating potential etiologic agents that might require unique control measures.

Clostridium perfringens has been implicated as the causative agent in outbreaks, some fatal, and is estimated to cause 1 million or 10% of episodes of food-borne illness attributable to known agents annually in the United States [1–4]. However, because this estimate is based on laboratory-confirmed illnesses and because C. perfringens is reportable only as an outbreak, the true burden of illness might be underestimated. Accurate estimates of food-borne illness burden can aid in determining resource allocation and in implementing targeted prevention and control strategies.

We share one illustration of how C. perfringens-related illnesses might be underrecognized. On 13 February 2010, attendees at a youth conference held in North Carolina during 11–14 February 2010, experienced nausea, vomiting, and diarrhea. The earliest reported onsets occurred at approximately midnight on 13 February. By 10 AM on 13 February, 7 ambulances had been summoned to the conference hotel. We investigated to identify the agent and source of the outbreak and ways to prevent future outbreaks.

MATERIALS AND METHODS

Retrospective Cohort Study

During 19–26 February, we performed a retrospective cohort study by using an Internet-based survey, with solicitations distributed through e-mail to conference attendees and posted on organization websites. The survey covered all organized group meals served before the outbreak, including dinner on 11 February and breakfast, lunch, and a banquet dinner on 12 February (Figure 1). We defined a case of acute gastroenteritis as onset of vomiting or diarrhea (≥2 episodes of loose stools during a 24-hour period) in a conference attendee with onset during 11–14 February 2010. We calculated risk ratios (RRs) and 95% confidence intervals (CIs) to determine relative risk and statistical significance. Analyses were performed with SAS software, version 9.1 (SAS Institute).

Laboratory Testing

We collected 9 unpreserved stool samples from willing ill persons and stored them at 4°C. The North Carolina State Laboratory of Public Health (NCSLPH) extracted norovirus total nucleic acid (TNA) by using the MagNA Pure LC Instrument and the MagNA Pure LC Total Nucleic Acid kit, according to the manufacturer’s instructions (Roche). Per protocol, the Centers for Disease Control and Prevention (CDC) extracted TNA from 6 stool specimens by using the MagMAX-96 Viral RNA Isolation Kit (Ambion). Nucleic acid specimens were subsequently stored at −70°C. Both the NCSLPH and the CDC performed real-time reverse-transcription polymerase chain reaction (PCR) by using the extracted TNA to detect norovirus RNA [5]. CDC TNA extracts were tested by real-time PCR for C. perfringens enterotoxin cpe gene. Direct detection of C. perfringens enterotoxin was performed on the same stool samples by using a commercial kit (Oxoid). Routine bacterial culture was also performed.

Environmental Health Investigation

Environmental health specialists inspected the catering facilities that had provided conference meals before the outbreak.
and interviewed catering company employees regarding illness, absences from work, and food preparation practices.

RESULTS

Among 1020 conference attendees from across North Carolina, 534 (52%) completed the online survey and were included in the retrospective cohort study. Respondents were similar to nonrespondents. Of the 534 respondents, 353 (66%) reported symptoms of gastrointestinal illness; 307 (57%) met the acute gastroenteritis case definition. Seventy percent were aged 15–17 years; age and sex did not differ significantly between ill and nonill persons. Nonill respondents reported close contact with ill persons. Only 15% of ill persons experienced vomiting; only 1% reported fever (Supplementary Table S1). Among respondents with acute gastroenteritis, the earliest symptom onset occurred at midnight on 13 February; 90% reported onset within a period of 12 hours (midnight on 13 February to noon on 13 February), indicating a point-source exposure (eg, contaminated food). Illness lasted <24 hours in the majority of cases. Five persons required transport to the hospital. No deaths occurred.

Our analysis included 307 persons with acute gastroenteritis and 227 who did not meet the case definition. In the bivariate analysis, those who had eaten the banquet dinner on 12 February had an increased risk for acute gastroenteritis (RR, 2.8; 95% CI, 1.7–4.5). Respondents reported that the chicken appeared to have been inadequately cooked.

On 14 February 2010, the NCSLPH detected norovirus RNA in stool specimens from 5 of 9 ill conference attendees who submitted samples. However, subsequent norovirus testing of these specimens conducted at the CDC and the NCSLPH determined all were negative for norovirus. On 24 March 2010, the CDC detected \( C. \) \( \perfringens \) enterotoxin in 8 of 9 specimens tested and the \( \text{cpe} \) gene in 5 of 6 TNA specimens tested. Positive cultures were confirmed as \( C. \) \( \perfringens \), but colony counts were unreliable because of the length of time between collection and culture.

Environmental health specialists noted that the chicken served at dinner on 12 February was seared on 11 February, chilled to 3–4°C, and then baked in an oven on the day of the event. Oven air temperature and chicken internal temperature were not documented. No other concerns were identified during inspections. No employees were absent because of illness around the conference dates for any of the food service companies involved. No food items were available for testing.

DISCUSSION

This investigation illustrates the importance of considering epidemiologic, environmental, and laboratory data together when evaluating potential etiologic agents. We initially considered norovirus the most likely cause of this outbreak because of the time of year during which it occurred; initial positive laboratory results supported this hypothesis. However, we considered alternative causes because the findings of our epidemiologic investigation were inconsistent with norovirus infection. Norovirus outbreaks are characterized by \( \geq 50\% \) of patients experiencing vomiting, mean or median duration of illness lasting 12–60 hours, and a mean or median incubation period of 24–48 hours [6, 7]. Person-to-person transmission is frequently observed, and fever can be common [6]. By contrast, only 15% of ill attendees reported vomiting, illness lasted <24 hours, and incubation periods ranged from 1 to 24 hours, findings more consistent with \( C. \) \( \perfringens \) toxin-induced illness. The low incidence of fever and the absence of secondary cases served as further evidence against norovirus infection. Confirmation of \( C. \) \( \perfringens \) was delayed by the initial suspicion of norovirus and detection of norovirus RNA in samples, which could not be confirmed. The reason for initial detection of norovirus is unknown. Hypotheses include laboratory contamination at the time of initial testing or degradation of the specimen before testing. Other investigations of \( C. \) \( \perfringens \) infection have been complicated by initially positive norovirus results [4], underscoring the
problem of asymptomatic norovirus infection and the need to collect an adequate number of stool specimens during an outbreak [8, 9].

C. perfringens is underrecognized as a cause of food-borne outbreaks, probably because the available methods of testing, PCR of the cpe gene and toxin detection, are not widely available. All 50 states perform norovirus testing (J. Vinje, PhD, CDC; personal communication, 2010). However, only 24 (55%) of 44 state public health laboratories provided biotoxin testing, and 7 (16%) of 44 facilitated testing elsewhere (unpublished data, APHL Food Safety Survey, Association of Public Health Laboratories, 2007). The NCSLPH did not offer such testing during this outbreak. Public health laboratories might be less likely to offer testing for C. perfringens, because of the expense. PCR, although less expensive, necessitates semiquantitative anaerobic culture, which requires extensive training and resources and only detects whether the strain of C. perfringens possesses the cpe gene. Only toxin detection kits, which are expensive and expire quickly, can determine whether toxin was elaborated in the intestine. States may contact the CDC to request testing of specimens when they suspect C. perfringens during an outbreak investigation.

The catering company involved in this outbreak reported that factors associated with this outbreak are common in the catering industry. Although outbreaks of C. perfringens toxin-induced illness are known to be associated with inadequately cooking meats followed by slow cooling, precooking or searing meats before full cooking is used to preserve food texture. This outbreak illustrates a need to reiterate to the catering industry the importance of following searing with immediate complete cooking or documented adequate and timely cooling. Full cooking of the chicken after slow cooling could have killed any C. perfringens organisms that had proliferated. However, based on survey responses, we hypothesize that cooking on the day of the banquet was insufficient to kill live C. perfringens organisms that had proliferated. We suspect these live organisms were consequently ingested and released toxin in the small bowel.

By identifying outbreaks caused by C. perfringens and other toxigenic bacteria, public health officials can have an accurate view of the burden of disease associated with different agents that cause food-borne illness. They can then focus on implementing targeted food-handling interventions (eg, time and temperature control measures) and educating food handlers about safe food-handling practices for prevention of future outbreaks. Public health officials should consider diagnostic testing for bacterial toxins when epidemiologically indicated during outbreak investigations.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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References