ORIGINAL INVESTIGATION

A Foodborne Outbreak of *Cyclospora cayetanensis* at a Wedding

Clinical Features and Risk Factors for Illness

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Background: *Cyclospora cayetanensis,* a coccidian parasite, has increasingly been recognized as a cause of gastrointestinal tract illness. We describe an outbreak of *Cyclospora* infection following a wedding reception.

Objectives: To investigate and characterize risk factors associated with the outbreak of *Cyclospora* and to describe the observed clinical course and spectrum of illness.

Methods: Retrospective cohort study involving 94 of the 101 guests who attended a wedding reception at a restaurant in Boston, Mass.

Results: Fifty-seven respondents met the case definition of infection; 12 of these had laboratory-confirmed *Cyclospora.* The epidemic curve was consistent with a point source outbreak with a median incubation period of 7 days. Commonly reported symptoms included diarrhea (100%), weight loss (93%), fatigue (91%), and anorexia (90%). The illness had a characteristic waxing and waning course, with 51 persons (89%) reporting recurring symptoms and 35 (61%) reporting illness lasting more than 3 weeks. By univariate analysis, infection was significantly associated ($P<.05$) with consumption of wine and a dessert containing raspberries, strawberries, blackberries, and blueberries. Only the dessert remained significant by stratified analysis with an adjusted relative risk of 2.1 (95% confidence interval, 1.4-3.2).

Conclusions: Findings from this study support a point source outbreak of the newly identified pathogen *C cayetanensis*, with berries as the vehicle of transmission. It suggests that *Cyclospora* may cause severe diarrhea associated with profound anorexia and weight loss, and should be considered in the evaluation of prolonged gastrointestinal tract illness.

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SINCE ITS description in 1986, *Cyclospora cayetanensis*, a coccidian parasite, has been increasingly recognized as a cause of diarrheal illness.[1] Most current knowledge about this parasite is based on studies from Nepal, Haiti, and Peru, regions where it is endemic.[2-6] Although several studies suggest that the organism is waterborne,[2,4,7-11] some case reports have postulated foodborne transmission.[12]

In the United States, sporadic cases of *Cyclospora* infection have been reported among immunocompromised individuals or those with a history of recent travel.[1,13,14] However, the prevalence of reported *Cyclospora* infection has been low,[15] possibly because many physicians are unfamiliar with its clinical features and the laboratory tests required for diagnostic confirmation. Although illness characterized by prolonged cycles of diarrhea and fatigue has been described in Nepal,[2] because of its infrequent recognition in the United States, less information is available regarding clinical illness in this country.

Before 1996, only 3 *Cyclospora* outbreaks had been reported in North America, with drinking water implicated epidemiologically in 2 cases and bare-handed contact with soil in the third.[7,8,16] In May and June 1996, more than 850 cases of laboratory-confirmed *Cyclospora* infection, including both sporadic and event-related clusters, were reported in 20 states east of the Rocky Mountains and 2 Canadian provinces.[17-19]

We describe a large outbreak of *Cyclospora* infection that occurred following a May 1996 wedding reception at a restaurant in Boston, Mass. The objectives of this report were to identify risk factors associated with illness and to describe the clinical course and spectrum of illness among outbreak-associated cases.

RESULTS

Of the 101 guests, 94 completed the questionnaire. Forty-nine (52%) of the respondents were female and 61 (65%) were younger than 50 years. Fifty-seven cases

SUBJECTS AND METHODS

On May 11, 1996, a wedding reception held at a Boston restaurant was attended by 101 guests. In the following weeks, diarrheal illness occurred among several attendees, including one individual in whom *Cyclospora* was confirmed after several weeks of illness. Simultaneously, a second guest was diagnosed as having been infected with *Cryptosporidium* (later confirmed to be *Cyclospora*), and in late May, an outbreak investigation was initiated by the city health department. The investigation included a survey of wedding guests, an environmental inspection of the restaurant, and surveillance among food handlers who prepared and served the implicated meal.

Using a guest list provided by the wedding party, a survey questionnaire designed to ascertain presence and nature of illness, food history, and attendance at other events potentially related to illness was sent to all attendees. A menu was included to facilitate food recall. Respondents who reported ongoing illness at the time the questionnaires were returned were contacted by telephone by health department staff and advised to seek medical attention; this included submission of stool specimens for identification of *Cyclospora*. Guests whose questionnaire had not been returned within 3 weeks were also contacted by telephone and invited to complete the questionnaire by telephone. Of 101 guests, 94 (93%) completed the questionnaire—92 in writing and 2 by telephone contact.

An outbreak-associated case was defined as an individual with diarrhea of 3 or more days in duration with an onset 2 to 14 days following the event, or an individual who had *Cyclospora* identified by modified acid-fast staining of a stool specimen.[20] Diarrhea was defined as 3 or more loose stools in a 24-hour period. Whenever possible, stool samples initially reported positive for *Cyclospora* were forwarded to an experienced parasitologist (P. DeGirolami, MD, Beth Israel Deaconess Medical Center, Boston) for confirmation.

Simultaneously, the restaurant was inspected by the health department, with particular attention directed to the facility's water supply, including the source of drinking water for the function, and food handling practices, particularly methods of preparation and storage of salad and fruit. Water samples were collected from 3 sites in the restaurant using a concentration and microfiltration technique. A sample volume of 380 L of water was obtained from each site and was tested for microbial contaminants including bacteria and parasites such as *Giardia*, *Cryptosporidium,* and *Cyclospora*.

To obtain information about illness and food history among the restaurant employees, a questionnaire was developed and sent to all staff members who worked on May 11. A stool specimen was collected from these employees and sent to an expert in parasitology for detection of *Cyclospora* using modified acid-fast staining. Attendees of 4 other functions held at the restaurant on May 11 were contacted regarding possible illness. Finally, to detect sporadic cases, most hospitals and clinical laboratories in the Boston area were contacted and asked to report all *Cyclospora* cases.

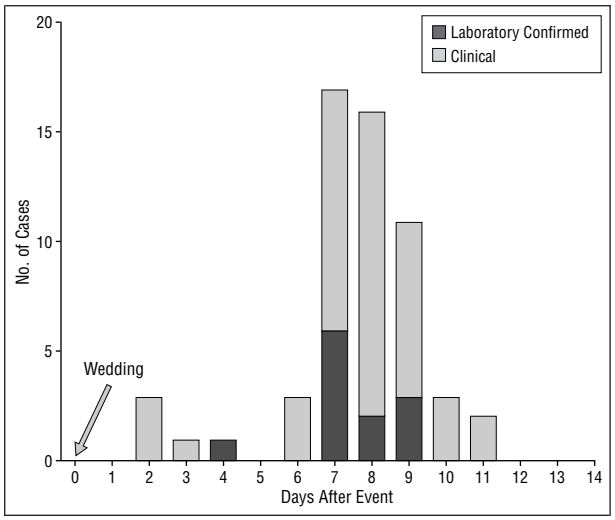
Analysis was performed using SAS software (SAS Institute, Cary, NC).[21] Attack rates for each reported menu item were calculated and *P* values determined using 2-tailed χ^2 or Fisher exact tests. For each food item, a relative risk (RR) and 95% confidence interval (CI) was obtained.[22] An adjusted *P* value, RR, and 95% CI were calculated using 2-tailed Mantel-Haenszel procedures.[23]

were detected, giving an attack rate of 61%. Of these, 12 had *Cyclospora* identified on stool specimens including 1 initially reported as *Cryptosporidium* and on subsequent review confirmed to be *Cyclospora.* Eight of the 12 positive stool samples were available for review and were confirmed as *Cyclospora* (P. DeGirolami, MD). The 12 respondents with positive stool samples were symptomatic. Two respondents reported illness not meeting the case definition.

The epidemic curve was consistent with a point source outbreak with a median incubation period of 7 days, ranging from 2 to 11 days (**Figure**). Illness was not reported among household contacts who did not attend the wedding. All 57 affected respondents reported having had diarrhea, most likely a reflection of the case definition. Bloody diarrhea was not described by any respondent. Weight loss was noted by 53 respondents (93%) (mean, 3.5 kg; range, 0.9-6.75 kg) and debilitating fatigue was experienced by 52 (91%) (**Table 1**). The illness had a characteristic waxing and waning course, with 51 persons (89%) reporting recurring symptoms. Illness was prolonged, lasting more than 3 weeks in 35 people (61%), 2 to 3 weeks in 16 (28%), 1 to 2 weeks in 5 (9%), and less than 1 week in only 1 person. Thirty-eight respondents (66%) sought medical attention. One person who had a history of type 2 diabetes mellitus was hospitalized, and 2 others required intravenous hydration. No other person was known to have an underlying immunosuppressive illness.

Twenty-five food items were served at the reception, including green salad, lobster bisque, sea scallops, veal, chicken, and a mixed fish platter. By univariate analysis, illness was significantly associated with the consumption of wine and a dessert containing raspberries, strawberries, blackberries, blueberries, and cream (**Table 2**). There was no significant association between illness and drinking water. Only the berry dessert remained significant by stratified analysis with an adjusted RR of 2.1 (95% CI, 1.4-3.2) (**Table 3**). The risk was highest among berry eaters who did not drink wine (crude RR, 3.0; 95% CI, 1.5-6.1). Food traceback involving local, state, and federal authorities revealed that the strawberries originated in California, the blueberries in Florida, the blackberries in Guatemala, and the raspberries in either Guatemala or Chile. Documentation was insufficient to determine a single country of origin for the raspberries.[19]

Inspection of the restaurant revealed no pertinent deficiencies. Extensive review of the facility's plumbing system by the city's inspectional services department identified only minor violations believed to be unrelated to the outbreak. Water samples obtained using concentra-



Incubation period for 57 cases of Cyclospora *infection.*

Table 1. Symptoms Reported by 57 Persons With *Cyclospora* Infection

Symptom	No. (%)
Diarrhea	57 (100)
Weight loss	53 (93)
Fatigue	52 (91)
Anorexia	51 (90)
Weakness	46 (81)
Abdominal pain	43 (75)
Nausea	43 (75)
Bloating	36 (63)
Myalgias	36 (63)
Chills	29 (51)
Subjective fever	22 (39)
Vomiting	21 (37)
Headache	21 (37)

tion and filtration techniques from both faucets in the restaurant preparation kitchen and from a water source located just outside the function room were negative for bacteria and parasites. Surveillance for illness among attendees of 4 other functions held at the restaurant on May 11 revealed no illness among these groups. A mixed-berry dish was among several options at one of these functions; however, it is unclear whether it was ordered. Surveillance for illness among the 11 restaurant employees who had worked at the May 11 reception identified 2 ill persons. Employees at the restaurant routinely ate food at the facility. One waiter reported diarrheal illness that began 7 days after the wedding and was found to have *Cyclospora* on stool testing. However, he was unable to specifically recall food items he had consumed. A second waiter also reported gastrointestinal tract symptoms but a stool sample was negative for *Cyclospora*.

COMMENT

This report describes a large point source outbreak of *Cyclospora* infection. In this outbreak, a dessert containing raspberries, strawberries, blackberries, and blueberries was significantly associated with illness. According to the kitchen staff at the restaurant, the berries had been washed under running water but not soaked before serving. Unfortunately, the mixture of berries precludes the determination of which individual fruit may have been the vehicle of transmission. However, simultaneous with this outbreak, multiple sporadic and event-related cases of *Cyclospora* infection were reported from other geographic areas in North America, with preliminary reports from New Jersey and South Carolina implicating raspberries.[17-19] Berries, particularly raspberries, may be an efficient vehicle as they are difficult to wash, and are rarely cooked. A multistate investigation of *Cyclospora* cases suggested Guatemala was the most likely source of the implicated raspberries.[17,19] The borderline association between wine and illness noted on multivariate analysis is perplexing. However, several types of wine were served, and, for the purpose of analysis, all were grouped together. In addition, recall bias among wine drinkers is difficult to exclude. Our data did not support green salad as a risk factor for illness, although lettuce has been previously implicated as a vehicle of transmission. An investigation of a 1990 outbreak identified *Cyclospora* oocysts on a head of lettuce from which a person had eaten 2 days before becoming ill.[24] Although not implicated as a risk factor for illness in this outbreak, water also has been postulated as a vehicle of transmission for *Cyclospora*. Investigation of an outbreak at a British Military camp at Pokhara, Nepal, demonstrated *Cyclospora* in a 2-L sample of chlorinated filtered water taken from a storage tank, suggesting that like *Cryptosporidium*, *Cyclospora* may be resistant to chlorination.[4] Epidemiological investigation of a 1990 Chicago, Ill, hospital dormitory outbreak implicated tap water from a storage tank that had experienced pump failure, resulting in possible contamination of stagnant water.[7] Potable water was also implicated in a June 1995 outbreak at a New York country club and numerous case reports have suggested contaminated water as a significant exposure.[8-11]

The median incubation period of 7 days seen in this outbreak is longer than that observed for most bacterial causes of foodborne illness; however, it is consistent with other reports of *Cyclospora* infection. Shlim et al[3] described 5 foreign residents who became ill with *Cyclospora* 2 to 11 days after their arrival in Katmandu. In the Chicago outbreak, symptoms occurred 1 to 7 days after drinking implicated water; however, the exact time of exposure in this outbreak was speculative.[7] A long incubation period may hinder detection and investigation of outbreaks, because individuals often relate their illness to more recent events. In many countries, seasonality of *Cyclospora* infection has been described. In Nepal, most cases occur between May and July[2] (coinciding with the rainy season), and in Peru, cases usually occur between January and July.[6] For reasons that remain unclear, the majority of *Cyclospora* cases reported to date in North America have occurred between May and August.[7,8,15-19,25]

Like other reported *Cyclospora* outbreaks, this episode was characterized by a relatively high attack rate (61%), a fact that is likely to have facilitated its detec-

Table 2. Food Items Consumed at the Wedding and Their Association With *Cyclospora* Infection*

	Eaten			Not Eaten			
Food Items	Ill	Not Ill	AR, %	Ill	Not Ill	AR, %	RR (95% CI)
Lamb chop appetizers	24	16	60	33	21	61	0.98 (0.71-1.37)
Cheese	23	12	66	34	25	58	1.14 (0.82-1.58)
Crackers	15	14	52	42	23	65	0.80 (0.54-1.19)
Bread	31	17	65	26	20	57	1.14 (0.82-1.59)
Sea scallop with bacon	35	18	66	22	19	54	1.23 (0.87-1.74)
Feta cheese and spinach triangles	24	16	60	33	21	61	0.98 (0.71-1.37)
Coconut shrimp	25	17	60	32	20	62	0.97 (0.70-1.34)
Champagne toast	50	30	63	7	7	50	1.25 (0.72-2.17)
Lobster bisque	51	30	63	6	7	46	1.36 (0.74-2.51)
Salad	53	32	62	4	5	44	1.40 (0.66-2.97)
Chicken with madeira sauce	21	11	66	36	26	58	1.13 (0.81-1.57)
Veal, artichokes, and madeira sauce	12	10	55	45	27	63	0.87 (0.57-1.33)
Mixed fish grille	28	18	61	29	19	60	1.00 (0.73-1.40)
Mushrooms	15	8	65	42	29	59	1.10 (0.77-1.57)
Potatoes	44	33	57	13	4	77	0.75 (0.54-1.04)
Carrots, asparagus, and red pepper	46	28	62	11	9	55	1.13 (0.73-1.75)
Wedding cake	41	26	61	16	11	59	1.03 (0.72-1.49)
Berries and cream†	47	17	73	10	20	33	2.20 (1.30-3.73)
Coffee	40	21	66	17	16	52	1.27 (0.87-1.86)
Wine†	31	12	72	26	25	51	1.41 (1.02-1.96)
Water	27	11	71	30	26	54	1.33 (0.97-1.82)

**AR indicates attributable risk; RR, relative risk; and CI, confidence interval.*
†*Significant by univariate analysis (P<.05).*

Table 3. Stratified Analysis of Food Items Significantly Associated With *Cyclospora* Infection*

		Eaten			Not Eaten				
Food Item	Stratification Food Item	Ill	Not Ill	AR, %	Ill	Not Ill	AR, %	Crude RR (95% CI)	Adjusted RR (95% CI)
Berries and cream	Wine	25	6	81	6	6	50	1.6 (1.0-2.6)	2.1 (1.4-3.2)
	No wine	22	11	67	4	14	22	3.0 (1.5-6.1)	. . .
Wine	Berries and cream	25	6	81	22	11	67	1.2 (0.9-1.6)	1.3 (1.0-1.8)
	No berries and cream	6	6	50	4	14	22	2.3 (0.8-6.3)	. . .

**AR indicates attributable risk; RR, relative risk; and CI, confidence interval.*

tion. The Centers for Disease Control and Prevention reported an attack rate of 58% (37 persons) following a luncheon attended by 64 persons in Charleston, SC, in May 1996.[18] During a June 1992 outbreak, 87% (12/14) of residents of a British military camp developed diarrhea due to infection with *Cyclospora*.[4] Although the infective dose is unknown, 2 persons with laboratory-confirmed *Cyclospora* in the Boston outbreak with particularly severe illness, reported eating a second berry desert, suggesting a dose-response relationship. Attempts to quantify the amount of dessert consumed by individual guests were unsuccessful, because of poor recall.

No evidence of secondary transmission was found in this outbreak. Because the waiter who was infected with *Cyclospora* ate food at the restaurant, and had an onset of illness 7 days after the wedding, it seems likely that he was infected at the same time as the wedding guests rather than being the source of the outbreak. Because *Cyclospora* is excreted as an oocyst that does not become infectious until it sporulates, person-to-person spread is unlikely.[6]

Despite the fact that most of those affected were otherwise healthy, significant morbidity occurred. All subjects in our series reported diarrhea, partially a reflection of the case definition. However, cases of *Cyclospora* infection without significant diarrhea or with constipation have been reported.[25] Most outbreak-associated cases (61%) had prolonged illness lasting more than 3 weeks, with frequent recurrence of symptoms being experienced by most people. This prolonged cyclical course is consistent with the pattern of illness described in previously reported case series.[2,7] In a study of immunocompetent patients with *Cyclospora* infection in Nepal, a similar duration of illness (43 days) was noted.[3] Although less prolonged illness (median, 5 days) was reported in a Chicago outbreak, the length of illness may have been underestimated owing to the investigation methods used and the relapsing nature of the infection.[7] Other frequently reported symptoms in our cohort included debilitating fatigue, significant weight loss (3.5 kg), and anorexia. Although not specifically asked of all respondents, several reported dyspepsia that persisted after other symptoms resolved. *Cyclospora* is thought to affect primarily the small bowel, based on the finding of abnormal D-xylose absorption in a small number of patients who have

been studied,[26,27] and the demonstration of coccidia within jejeunal epithelial cells.[26] In addition, biopsy specimens from the small bowel of affected persons have shown epithelial disruption, villous atrophy, and crypt hyperplasia, changes similar to tropical sprue.[27] These findings may explain the presence of significant upper gastrointestinal tract symptoms observed in our series and others.

Although two thirds of the 57 affected respondents sought medical attention, follow-up information regarding eventual clinical outcomes and response to antimicrobial therapy is unavailable from our investigation. The efficacy of a 7-day course of a combination of trimethoprim and sulfamethoxazole was demonstrated by Hoge et al[28] in a placebo-controlled trial of an expatriate population in Nepal.

This investigation has several limitations, including 2 sources of potential recall bias. First, attendees completed the questionnaire approximately 3 weeks after the event and may have had difficulty with accurate food recall. To minimize this, a menu for prompting was provided as part of the survey questionnaire. Second, high-profile media attention received by nationwide *Cyclospora* cases shortly after this outbreak may have contributed to recall bias. However, because most questionnaires were completed and returned before this publicity, its effect is likely to be minimal. Misclassification is also possible as only 12 of 57 cases had laboratory confirmation of *Cyclospora* infection. However, the 12 cases are most likely to be an underrepresentation of the actual number for several reasons. First, unfamiliarity with *Cyclospora* and the methods for its detection resulted in many guests, particularly those with illness of less than 3 weeks' duration, not having an appropriate diagnostic test performed. The sensitivity of modified acid-fast stool staining in the diagnosis of *Cyclospora* infection is unknown and may be decreased if a laboratory is unfamiliar with the organism. In addition, data are unavailable on whether persons infected with *Cyclospora* shed the organism continuously or intermittently. Finally, some respondents had diagnostic tests pending when they completed the questionnaire, so subsequent positive results would not have been included. However, our use of a strict case definition should have minimized misclassification bias.

Findings from this outbreak investigation support a point source epidemic of the emerging pathogen *Cyclospora*, with berries as the vehicle of transmission. Our data suggest that infection with *Cyclospora* has the potential to cause significant morbidity and should be considered in individual cases of gastrointestinal tract illness characterized by prolonged and relapsing symptoms. Our findings, along with those of others, also suggest that this organism has the potential to cause foodborne outbreaks characterized by relatively long incubation periods and high attack rates.[17,18] These facts underscore the importance of developing improved public health surveillance systems to detect this and other newly recognized pathogens.

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