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An Outbreak of Cryptosporidiosis From Fresh-Pressed Apple Cider

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Background.—Recent waterborne outbreaks have established *Cryptosporidium* as an emerging enteric pathogen, but foodborne transmission has rarely been reported. In October 1993, an outbreak of cryptosporidiosis occurred among students and staff attending a 1-day school agricultural fair in central Maine.

Design.—Environmental/laboratory investigation and cohort study.

Participants.—Attendees of the fair and their household members.

Main Outcome Measures.—Clinical or laboratory-confirmed cryptosporidiosis. Clinical cryptosporidiosis was defined as 3 days of either diarrhea (three loose stools in a 24-hour period) or vomiting.

Results.—Surveys were completed for 611 (81%) of the estimated 759 fair attendees. Among attendees who completed the survey, there were 160 (26%) primary cases. *Cryptosporidium* oocysts were detected in the stools of 50 (89%) of 56 primary and secondary case patients tested. The median incubation period was 6 days (range, 10 hours to 13 days); the median duration of illness was 6 days (range, 1 to 16 days). Eighty-four percent of primary case patients had diarrhea and 82% had vomiting. Persons drinking apple cider that was hand pressed in the afternoon were at increased risk for cryptosporidiosis (154 [54%] of 284 exposed vs six [2%] of 292 unexposed; relative risk, 26; 95% confidence interval, 12 to 59). *Cryptosporidium* oocysts were detected in the apple cider, on the cider press, and in the stool specimen of a calf on the farm that supplied the apples. The secondary household transmission rate was 15% (53/353).

Conclusions.—This is the first large cryptosporidiosis outbreak in which foodborne transmission has been documented. It underscores the need for agricultural producers to take measures to avoid contamination of foodstuffs with infectious agents common to the farm environment.

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CRYPTOSPORIDIUM is an enteric parasite of emerging importance in normal and immunocompromised hosts.[1,2] It causes illness in travelers[3] and in persons living and working in agricultural environments.[4,5] Person-to-person transmission of *Cryptosporidium* infection often occurs, particularly in child care and hospital settings.[6-10] Large waterborne outbreaks have recently occurred,[11-15] but foodborne transmission has only rarely been suggested.[16-20] Large, common-source outbreaks of cryptosporidiosis in which the exposure is known to occur within a narrowly defined time period (ie, "point-source" outbreaks), have not previously been reported. Therefore, determining the precise incubation period of *Cryptosporidium* infection in immunocompetent humans has been difficult.[3]

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BACKGROUND

In October 1993, the principal of an elementary school in central Maine reported an outbreak of enteric illness to the Maine Bureau of Health. A review by the bureau on the same day showed that the outbreak involved two elementary schools and the high school in a rural farming community. Both elementary school principals noted high absenteeism in classes that had attended a 1-day school agricultural fair 8 days previously. The annual fair was organized and staffed by high school students and was attended by students and staff. Elementary students attended either the morning or afternoon session, but not both. The fair consisted of agricultural demonstrations, a petting zoo of farm animals, a hayride, a cider-pressing demonstration, and light refreshments. Two days after the outbreak was reported, *Cryptosporidium* was detected in the stools of three ill children who had attended the fair.

See also p 1597.

We conducted a cohort and environmental/laboratory study to identify the source of the outbreak, to characterize the natural history of cryptosporidiosis, and to determine the risk for secondary household transmission.

MATERIALS AND METHODS

The study population consisted of students and staff who had attended the fair and the household members of primary case patients.

Clinical cryptosporidiosis was defined as 3 days of either diarrhea (ie, three loose stools in a 24-hour period) or vomiting with abdominal cramps; laboratory-confirmed cryptosporidiosis was defined as any gastrointestinal illness in a person with a stool specimen that was positive for *Cryptosporidium* oocysts. Primary cases were defined as laboratory-confirmed or clinical cryptosporidiosis in students or staff following attendance at the fair. Secondary cases were defined as laboratory-confirmed or clinical cryptosporidiosis in household members of primary case patients who did not themselves attend the fair but who became ill within 1 month of onset of illness in the primary case patient.

The incubation period was defined as the time between attendance at the fair and the onset of the first symptoms. Duration of illness was defined as the time between the onset of symptoms and the self-reported resolution of diarrhea or vomiting (whichever persisted longer).

Epidemiologic Investigation

Fair Attendees' Survey.—High school and elementary school students who attended the fair were asked to complete,

with parental assistance, a written questionnaire. Distributed 13 days after the fair, the survey requested information on possible exposures, dates of illness onset and recovery, symptoms, and underlying medical conditions. Possible exposures included all six food and drink items consumed at the fair, petting of farm animals, and participation in the hayride. Nonrespondents were not resurveyed.

Teachers and staff completed the same questionnaire; teachers were asked, in addition, the number of absentees in their class, the activities of their class at the fair, whether the class had visited the cider-pressing demonstration, and if they had noted any evidence of fecal or other contamination of the apples or the cider press.

Household Transmission Survey.—A written questionnaire, directed toward primary case patients, was distributed at the school 5 weeks after the fair. Parents of elementary school students were again asked to assist in completing the questionnaire. The questionnaire addressed whether household members who had not attended the fair had subsequently become ill with vomiting or diarrhea. If household members had been ill, additional information was collected on symptoms and dates of illness. In addition, primary case patients who had reported they were still ill at the time of the fair attendees' survey were asked to provide the date of recovery. Nonrespondents to the household transmission survey were resurveyed 2 weeks later.

We excluded from the analysis of secondary transmission those households without susceptibles (ie, household members who had not attended the fair). In calculating the risk of transmission by age of the primary case patients, we excluded households with more than one primary case because we could not be certain which primary case patient had infected the family member. Fair attendees were not counted as part of the denominator in calculating household transmission rates.

Impact Survey.—Forty-seven case patients who had a home telephone were systematically selected for a telephone survey conducted 2 months after the fair. Every third case patient was eligible after the first case patient had been selected using a random number table. Respondents were asked about the impact of the illness in terms of missed days at work, medical provider visits, emergency department visits, and hospitalizations.

Laboratory and Environmental Investigation

Laboratory Methods.—Specimens were examined at the Maine Health and Environmental Testing Laboratory and the Parasitic Diseases Laboratory of the Centers for Disease Control and Prevention. All specimens were examined for *Cryptosporidium* oocysts by modified acid-fast staining, direct fluorescent antigen detection, or indirect immunofluorescence. Some specimens were tested by two methods. A stool specimen was considered positive if oocysts were detected by any of the methods. The first 12 stool specimens were examined for ova and parasites (including *Cryptosporidium*) and cultured for enteric bacteria (ie, *Salmonella* species, *Shigella, Campylobacter,* and enteropathogenic *Escherichia coli*).

Persons with laboratory-confirmed cryptosporidiosis were asked to submit bimonthly stool samples; stool collection was continued until two sequential stool specimens were negative.

Environmental Investigation.—A high school student had taken a gallon of leftover cider home from the fair for fermenting. Ten days after the fair, we obtained a sample of the partially fermented cider, which we froze and stored. Six weeks later, the cider was thawed and centrifuged. The sediment was resuspended in phosphate-buffered saline (0.01 mol/L; pH 7.2) with sodium azide, and samples were examined by modified acid-fast staining and direct fluorescent antibody staining. Portions of the sediment were processed over discontinuous sucrose gradients and ethyl acetate sedimentations. Swabs obtained from the surface of the portable cider press were placed in modified Stuart's transport medium and examined by direct fluorescent antibody staining.

Two weeks after the fair, we interviewed the three high school students who had pressed the cider and the supervising staff member regarding preexisting illness, the source of the apples, details of the cider-making process, and possible sources of contamination during the pressing operation.

Three weeks after the fair, we obtained stool specimens from the two calves that had been at the fair's petting zoo and from six of the 14 cows and two of the eight calves at the farm that had supplied the implicated apples. Six weeks after the fair, we collected another pooled stool specimen from two calves at the same farm.

Statistical Analysis

Chi-square tests were used for evaluating differences in proportions. Relative risks (RRs) and confidence intervals (CIs) were calculated using the method of Greenland and Robins.[21] We performed age-specific analyses after categorizing age into three groups: 5 to 9 years, 10 to 19 years, and 20 years and older. For calculating the duration of oocyst excretion and assessing group differences in time to clearance, we used the SAS Lifetest procedure.[22] A two-tailed P value of .05 was considered significant in all analyses.

RESULTS

The first survey was completed for 611 (81%) of the estimated 759 students and staff who attended the fair. Among the respondents, 230 persons (38%) complained of gastrointestinal symptoms following the fair and 160 primary cases (26%) were identified; 33 cases were laboratory-confirmed. None of the respondents reported illnesses or medical therapy known to be associated with immune dysfunction.

The number of absentees from the three affected schools and the number of incident primary cases by the number of days since the fair are shown in Figure 1. Primary case patients were 5 to 66 years old, 54% were male, and 56% were younger than 10 years.

Epidemiologic Investigation

Clinical Illness Among Primary Case Patients.—The clinical details of illness reported by primary case patients are shown in the Table. Of the 160 primary case patients, 106 (66%) reported both vomiting and diarrhea, 19 (12%) reported diarrhea without vomiting, and 26 (16%) reported vomiting without diarrhea. Forty-eight percent of case patients reported a recurrence of vomiting or diarrhea after the initial resolution of symptoms. There were no substantial or statistically significant differences in symptoms among case patients by age or by the amount of cider consumed.

The median incubation period was 6 days (range, 10 hours to 13 days; interquartile range, 5 to 7 days). The median duration of illness was 6 days (range, 1 to 16 days; interquartile range, 4 to 9 days) (Figure 2). Neither incubation period nor duration of illness was affected by the age or sex of the case patient or by the amount of cider consumed.

Exposures.—A total of 576 respondents answered the question addressing whether they drank cider at the fair. Of the 284 respondents who reported drinking cider in the afternoon, 154 (54%) met the case definition; in comparison, six (2%) of 292 who did not report drinking cider in the afternoon met the case definition (RR=26; 95% CI, 12 to 59). All laboratory-confirmed primary case patients and 121 (95%) of 127 clinically defined primary case patients reported drinking cider in the afternoon. No other exposures, including the drinking of apple cider in the morning or consuming other food items at the fair, were associated with cryptosporidiosis.

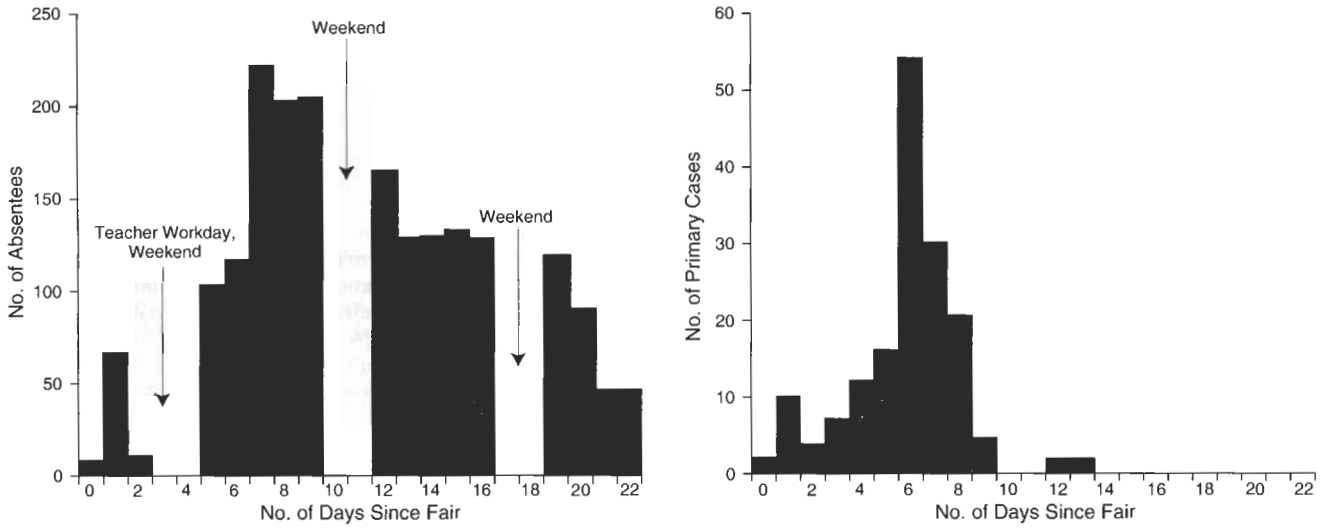

Figure 1.—School absentees (left) and onset of illness in primary case patients (right), by number of days since the school fair.

Incubation Period, Duration of Illness, and Symptoms of Cryptosporidiosis Reported by Laboratory Confirmed, Clinically Defined, and All Primary Case Patients

	Primary Cases		
	Laboratory Confirmed (n=33)	Clinically Defined (n=127)	Total (n=160)
Median incubation period, d (range, h or d)	6 (1 d-8 d)	6 (10 h-13 d)	6 (10 h-13 d)
Median duration of illness, d (range, d)	8 (1-14)	6 (3-16)	6 (1-16)
Diarrhea, %	94	81	84
Median No. of loose stools per 24 h	6	7	6
Abdominal cramps, %	96	97	96
Nausea, %	91	83	84
Vomiting, %	94	80	82
Median frequency of vomiting per 24 h	7	3	4
Body aches, %	52	50	50
Fever, %	70	58	60
Mean body temperature, °C*	38.5	38.2	38.3

*Among those with documented fever.

Among persons who drank apple cider in the afternoon, there were no substantial or significant differences in attack rate by age or sex. A dose-response relationship was evident; the attack rate among those who drank 1 cup (approximately 112 mL) or less of cider in the afternoon (67/138 [49%]) was less than the attack rate among those who drank 2 cups (69/111 [62%]; RR=1.3) or more than 2 cups (12/18 [67%]; RR=1.4) (χ^2 for trend, P=.02).

Household Transmission.—The household transmission survey was completed for 135 (84%) of 160 primary case patients. One primary case patient was excluded from the analysis of secondary transmission because she lived alone. We identified 53 secondary cases, 17 of which were laboratory-confirmed. Thirty-seven (33%) of 112 households with one primary case and four (36%) of 11 households with two primary cases reported one or more secondary cases. Among household members of primary case patients, secondary cases occurred in 21 (20%) of 106 persons less than 20 years of age and in 32 (13%) of 247 persons 20 years of age and older. Secondary cases occurred a median of 8 days (range, 1 to 24 days) following the onset of illness in the primary case patient. All secondary case patients experienced diarrhea and 60% had vomiting.

Each primary case patient who transmitted cryptosporidiosis to one or more household members did so to a mean of 1.3 persons. Among the 112 primary case patients living in households with no other primary cases, 25 (37%) of 68 primary case patients aged 5 to 9 years transmitted the infection to one or more household members, compared with 10 (28%) of 36 primary case patients aged 10 to 19 years, and two (25%) of eight primary case patients aged 20 years or older.

Impact of the Outbreak.—A telephone survey was completed for 45 (96%) of the 47 primary case patients chosen, representing 44 separate households. Fourteen respondents (31%) reported that one or more adults in the household had missed work because of cryptosporidiosis in the primary case patient. Thirteen primary case patients (29%) had visited a private medical office, seven (16%) had been evaluated in a hospital emergency department, and three (7%) had been hospitalized for a mean of 3 days.

Laboratory and Environmental Investigation

The five bushels of apples used for the morning pressing had been purchased from a commercial producer; the five bushels of apples used in the afternoon had been gathered by high school students from uncultivated trees on the edge of a pasture where cows had recently been grazing. The students had harvested the apples on the day before the fair by shaking the branches of trees over a farm truck and by collecting apples from the ground. The apples were stored overnight in clean wooden boxes on school grounds and were sprayed with municipal water from a hose on the morning of the fair. The municipal water was supplied by a surface water source that was chlorinated but not filtered. The cider was pressed in 4-L batches from 9 AM until 2:45 PM, except for a 30-minute break at 11:45 AM.

Cryptosporidium oocysts were detected in the leftover apple cider and on swabs from the surface of the portable cider press. Based on the ethyl acetate sediment, the cider contained 500 oocysts per liter. Based on the sucrose gradient fraction, the cider contained

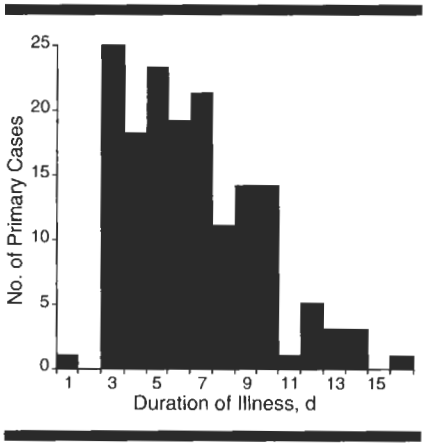

Figure 2.—Duration of illness among primary case patients.

375 to 750 oocysts per liter. Assuming that many oocysts ruptured during the freeze-thaw cycle and were not detectable by the recovery methods used, the actual concentration was probably much higher.

The pooled stool specimen from two calves at the farm that supplied the implicated apples contained *Cryptosporidium* oocysts, but oocysts were not identified in specimens from the two calves at the petting zoo.

Human Oocyst Excretion.—*Cryptosporidium* oocysts were detected in the stools of 33 (89%) of 37 primary case patients and in 17 (89%) of 19 secondary case patients tested. The median time to the first stool examination was 6 days after the onset of symptoms. No bacterial pathogens were detected in the stools of primary or secondary case patients; one primary case patient had concomitant *Giardia lamblia* infection. An isolate from a case patient was identified as *Cryptosporidium parvum* by the Centers for Disease Control and Prevention Parasitic Diseases Laboratory; the oocysts reacted to cryptosporidial monoclonal antibodies and were used to successfully infect a neonatal calf.

Twenty-two primary case patients and 12 secondary case patients submitted sequential stool samples for analysis. No case patients had a positive stool examination after having had a negative test result. Oocyst excretion continued for a median of 28 days (range, 10 to 65 days; interquartile range, 23 to 42 days) from the onset of symptoms to the first negative test. There were no significant differences in duration of stool oocyst excretion by age, sex, or primary vs secondary case status.

COMMENT

Cryptosporidium is a major cause of acute diarrhea worldwide.[23] This point-source outbreak provided an opportunity to document transmission of *Cryptosporidium* infection via an unusual vehicle and to further describe the natural history of this emerging enteric parasite in immunocompetent hosts.

The number of oocysts needed to cause symptomatic cryptosporidiosis in humans is not precisely known, but 10 to 50 oocysts have been shown to cause infection in infant macaques.[24] Anecdotal evidence suggests that cryptosporidiosis can be acquired by consuming raw sausage, offal, and dairy products,[16-20,25] but this is the first report to document a large foodborne outbreak of cryptosporidiosis.

Although most studies suggest that vomiting occurs in approximately half of cryptosporidiosis cases,[3,7,26] vomiting was reported in 63% of children with cryptosporidiosis in a clinic-based study from Switzerland[8] and in 82% of the primary case patients in our cohort. The explanation for differences in reported rates of vomiting in different studies is speculative, but may include differences in the vehicle, the size of the infecting inoculum, or characteristics of the organism or host. The lower prevalence of vomiting among secondary case patients (60%) suggests that a lower inoculum in secondary case patients or characteristics of the cider vehicle may have contributed to vomiting. The similarity in symptoms among case patients of varying ages in this cohort suggests that age may not alter the manifestations of illness in humans to the extent that it does in other species.[23] Little is known about subspecies differences in virulence, but it is possible that different subspecies could be responsible for the distinct clinical presentations seen in different outbreaks.[27]

Previous estimates of incubation period have been hampered by the absence of exact information on the time of ingestion. The estimate of incubation period obtained from this study was similar to the estimate reported by Jokipii and Jokipii,[3] but their study subjects were travelers who did not know precisely when they became infected. We believe that this study provides the most methodologically sound estimate to date of the distribution of incubation period in immunocompetent hosts.

Seroprevalence rates range from 25% to 35% in Europe and North America, suggesting that the infection is common even in industrialized countries.[23] A recent study in Wisconsin found seropositivity rates of 44% among dairy farmers and 24% among persons who had never worked on a farm.[4] It has been suggested that primary infection results in a degree of acquired immunity.[23,28] Assuming that our rural study subjects had substantial previous environmental *Cryptosporidium* exposure, the similarity in attack rates, symptoms, and time to clearance of oocysts between young persons and those who were older (and who were therefore more likely to have been previously infected) suggest that previous *Cryptosporidium* infection may not substantially alter the clinical course or provide long-term immunity against symptomatic reinfection.

Household transmission of *Cryptosporidium* infection has previously been studied among families of child care center attendees,[29,30] but not among older age groups. Tangermann et al[6] found that 31% of household members of children infected in a child care outbreak reported diarrhea, compared with only 3% of household members of controls. In these studies, the incidence of diarrhea in household members was higher among adult than among sibling contacts. In our school-aged population, we found that transmission of cryptosporidiosis to family members was common, that younger children were more likely to transmit infection than were older persons, that transmission was more likely to occur during the acute phase of the primary case patient's illness, and that attack rates were higher among sibling than among adult household contacts.

Our environmental investigation led us to hypothesize that the farm-collected apples were contaminated by calf feces on the ground before or during harvest. The inoculum was probably disseminated throughout the batch of cider because contaminated apples were inadequately washed before pressing.

The high response rates in this study minimized potential selection bias. However, the clinical case definition may have misclassified as uninfected those case patients who were mildly ill or were ill for less than 3 days and who did not undergo laboratory testing. This may have biased the overall estimate of duration of illness and symptom prevalence upward. A solely laboratory-based case definition was less desirable because relatively few persons underwent laboratory testing and those who did were self-selected. The high proportion of tested subjects who had laboratory-confirmed cryptosporidiosis suggests that the specificity of our clinical case definition was high.

We had no reports of other enteric illnesses in the community and we found another pathogen, *Giardia*, in only one stool specimen. Furthermore, the attack rate was low (2%) among persons who did not drink the afternoon cider. This suggests that there was little enteric disease circulating in the community, that municipal water used to wash the apples was not a source of contamination, and that the presence of other infectious agents was unlikely to have biased the

estimated rate of secondary household transmission. However, we did not evaluate the background rate of enteric disease in control households without cases, and we did not test case patients for viral pathogens. Furthermore, we did not test exposed persons for asymptomatic infection. Our estimate of duration of oocyst shedding should be interpreted with caution, since we only performed follow-up laboratory testing of case patients approximately every 2 weeks.

This outbreak demonstrates the potential for large foodborne outbreaks of cryptosporidiosis and underscores the need for increased awareness of the potential for contamination of foodstuffs with pathogens common to the farm environment. Previous outbreaks of cider-borne salmonellosis and *E coli* O157:H7 infection have been associated with apples that were apparently contaminated with animal manure.[31,32]

Because *Cryptosporidium* infection can result in debilitating or life-threatening illness in immunocompromised persons, preventing contamination of foodstuffs with this common organism is especially important. Although *Cryptosporidium* is resistant to common disinfectants,[33] it is rendered noninfective by pasteurization.[34] Immunosuppressed persons should consider avoiding unpasteurized beverages. Foodstuffs should not be harvested from pastures where farm animals graze. All raw or unprocessed foodstuffs should be thoroughly washed and brushed before consumption; those with evidence of fecal contamination should be discarded. Massachusetts has recently mandated washing and brushing of apples used for cider production,[35] and Maine cider producers are currently formulating voluntary sanitary guidelines. Such voluntary and regulatory actions may be needed to prevent future foodborne cryptosporidiosis outbreaks.

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