An Outbreak of Cryptosporidiosis From Fresh-Pressed Apple Cider

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Background—Recent waterborne outbreaks have established Cryptosporidium as an emerging etiologic 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 and 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 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 (31%) of 56 primary and secondary cases 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 86% had vomiting. Persons drinking apple cider that was hand pressed in the afternoon were at increased risk for cryptosporidiosis (154 [64%] of 248 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 call on the farm that supplied the apples. The secondary household transmission rate was 15% (9/63/0).

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.

CRYPTOSPORIDIUM is an etiologic parasite of emerging importance in normal and immunocompromised hosts. It causes illness in travelers and in persons living and working in agricultural environments. Persons in-person transmission of Cryptosporidium infection often occurs, particularly in child care and hospital settings. Large waterborne outbreaks have recently occurred, but foodborne transmission has only rarely been suggested. 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 immunocompromised humans has been difficult.

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 of 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 9 days previously. The annual fair was organized and staffed by high school and 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, an insect-generating 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.

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 diarrhea (one loose stool 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 case were defined as laboratory-confirmed or clinical cryptosporidiosis confirmed within 1 month of attending the fair. Secondary case 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 a

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with parental assistance, a written ques-
tionnaire. Distributed 15 days after the
fair, the survey requested information on
possible exposures, dates of illness onset
and recovery, symptoms, and underlying medical conditions. Possible exposures in-
cluded all six food and drink items con-
sumed at the fair, petting zoo ani-
mals, 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-drinking area of the fair, 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. Par-
ents of elementary school students were
again asked to assist in completing the
questionnaire. The questionnaire ad-
dressed whether household members
who had not attended the fair had sub-
sequently 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 attended survey’s were asked to
provide the date of recovery. Nonrespon-
ents to the household transmission sur-
vey were resurveyed 2 weeks later.
We excluded from the analysis of sec-
ondary transmission those households
without accessible individuals (i.e., house-
hold members who had not attended
the fair). In addition, we excluded from
analysis of transmission by age of the
primary case patient, we excluded house-
holds with more than one primary case
because we could not be sure which
primary case patient had introduced the
agents. Fair attendees were not counted as
part of the denominator in calculating
household transmission rates.

Impact Survey—Forty-seven case pa-
tients who had a home telephone were
systematically selected for a telephone
survey conducted 2 months after the fair.
Every third case patient was eligible af-
ter the first case patient had been se-
lected using a random number table. Re-
spondents 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 Pre-
vention. All specimens were examined for Cryptosporidium oocysts by modi-
fied acid-fast staining, direct fluorescent
antigen detection, or indirect fluores-
cence. Some specimens were tested by
two methods. A stool speci-
men was considered positive if oocysts
were detected by any of the methods.
The first 12 stool specimens were ex-
amined for ova and parasites (including
Cryptosporidium) and cultured for en-
teric bacteria (i.e., Salmonella species,
Shigella, Campylobacter, and enter-
opathogenic E. coli). Persons with laboratory-confirmed cryptosporidiosis were asked to submit
monthly stool samples; stool collection
was continued until two sequential stool
samples 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 fer-
mented cider, which we froze and stored.
Six weeks later, the cider was thawed
and centrifuged. The sediment was re-
suspended in phosphate-buffered saline
(0.01 mol/L, pH 7.2) with sodium azide,
and samples were examined by modi-
fied acid-fast staining and direct fluo-
rescent antibody staining. Portions of
the sediment were processed over dis-
continuous sucrose gradients and ethyl
acetate sedimentations. Swabs obtained
from the surface of the culture press were
placed in modified Stuart’s transport
medium and examined by di-
rect fluorescent antibody staining.
Two weeks after the fair, we inter-
viewed the three high school students
who had pressed the cider and the su-
pervising staff member regarding pre-
existing illnesses, the source of the apples,
details of the cider-making process, and
problems of contamination dur-
ing the pressing operation.
Three weeks after the fair, we ob-
tained 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
imprinted apples. Six weeks after the fair,
we collected another pooled stool speci-
men from two calves at the same farm.

Statistical Analysis

Chi-square tests were used for evalu-
ating differences in proportions. Rela-
tive risks (RRs) and confidence in-
tervals were calculated using the
method of Greenland and Robins.23
We performed age-specific analyses after
categorizing age into three groups: 5 to
19 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.24 A two-
tailed P value of 0.05 was considered sig-
nificant in all analyses.

RESULTS

The first survey was completed for
611 (85%) of the estimated 750 students
and staff who attended the fair. Among
the respondents, 350 persons (58%) com-
plained of gastrointestinal symptoms fol-
lowing the fair and 140 primary cases
(36%) were identified; 35 cases were
laboratory-confirmed. None of the re-
spondents reported illnesses or medical
therapy known to be associated with
immune dysfunction.

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

Epizootiologic Investigation

Clinical Illness Among Primary Case
Patients. The clinical details of illnesses
reported by primary case patients are
shown in the Table. Of the 105 primary
case patients, 100 (96%) reported both
cramping and diarrhea, 112 (124%) reported
diarrhea without vomiting, and 85 (89%)
reported vomiting without diarrhea.
Forty-eight percent of case patients re-
ported a recurrence of vomiting or di-
arrhea 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, 0 to 14 days; interquar-
tile range, 3 to 7 days). The median
duration of illness was 6 days (range, 1
to 16 days; interquartile range, 4 to 9
days) (Figure 2). No secondary cases of
ilness was affected by the age or sex of the
case patient or by the amount of cider con-
sumed.

Exposure. A total of 576 respon-
dents answered the question address-
ing the impact of the illness at the fair.
Of the 254 respondents who reported
drinking cider in the afternoon, 164 (64%)
met the case definition; in comparison, six
(26%) of 230 who did not report drink-
ing cider in the afternoon met the case
definition (RR=6.95; 95% CI, 2.12 to 69). All
laboratory-confirmed primary case pa-
ients and 121 (95%) of 126 clinically defined primary case patients were drinking cider in the afternoon. Another exposure, including the drinking of apple cider in the morning or consuming other food items at the fair, were asso-
ciated with cryptosporidiosis.
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 (0/29; 0% [0.3%]) was less than the attack rate among those who drank 2 cups (0/111; 0.9% [3.2%]; RR:1.3) or more than 2 cups (2/128; 1.6% [5.6%]; RR:1.8) per day.

**Incubation Period, Duration of Illness, and Symptoms of Cryptosporidiosis Reported by Laboratory Confirmed, Clinically Defined, and All Primary Cases.**

<table>
<thead>
<tr>
<th>Laboratory Confirmed (n=30)</th>
<th>Clinically Defined (n=27)</th>
<th>Total (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median incubation period, d</td>
<td>5 (IQR 2-10)</td>
<td>6 (IQR 3-13)</td>
</tr>
<tr>
<td>Median duration of illness, d</td>
<td>17 (IQR 10-48)</td>
<td>13 (IQR 8-35)</td>
</tr>
<tr>
<td>Diarrhea, %</td>
<td>94 (31)</td>
<td>81 (30)</td>
</tr>
<tr>
<td>Median No. of loose stools per 24 h</td>
<td>6 (IQR 2-12)</td>
<td>7 (IQR 2-15)</td>
</tr>
<tr>
<td>Nausea, %</td>
<td>93 (32)</td>
<td>83 (30)</td>
</tr>
<tr>
<td>Vomiting, %</td>
<td>74 (25)</td>
<td>80 (30)</td>
</tr>
<tr>
<td>Median frequency of vomiting per 24 h</td>
<td>7 (IQR 3-15)</td>
<td>3 (IQR 1-5)</td>
</tr>
<tr>
<td>Body aches, %</td>
<td>65 (22)</td>
<td>50 (19)</td>
</tr>
<tr>
<td>Fever, %</td>
<td>70 (24)</td>
<td>59 (22)</td>
</tr>
<tr>
<td>Mean body temperature, °C</td>
<td>38.5</td>
<td>38.2</td>
</tr>
</tbody>
</table>

*Among those who drank apple cider in the afternoon.*

**Impact of the Outbreak.** A telephone survey was conducted for 41 (96%) of the 43 primary case patients, 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 (30%) had visited a private medical office, 10 (23%) had been evaluated at a hospital emergency department, and the other 19 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 unirrigated trees on the edge of a pasture, where cattle had recently been grazing. The apples were harvested on the day before the fair by slopping 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 2:30 PM, except for a 30-minute break at 11:45 AM. Cryptosporidiosis oocytes were detected in the left-offer apple cider and on swabs from the surface of the portable cider press. Based on the ethyl acetate sediment, the cider contained 500 oocytes per liter. Based on the sucrose gradient fraction, the cider contained

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*Cryptosporidiosis From Apple Cider—Miller et al.*
Symptoms and time to clearance of eczema 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 re-infection.

Household transmission of Cryptosporidium infection has previously been studied among families of child care center attendees, but not among older age groups. Tsuji et al. (Note 1) suggested that 31% of household members infected in a child care center outbreak shared 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 shifting contacts. In our household population, we found that transmission of cryptosporidium to family members was common; younger children were more likely to transmit infection than were older children. Transmission was more likely to occur during the acute phase of the primary patient's illness, and that attack rates were higher among shifting 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 levels of Salmonella in the feces of the infected calves were inadequately washed before pressing.

The high response rates in this study minimized potential selection bias. However, the clinical end definition may have been misclassified as unclassified those case patients who were mildly ill or were ill for less than 4 days and who did not undergo laboratory testing. This may have biased the overall estimate of duration of illness and symptoms prevalence upward. A solely laboratory-based case definition is less desirable because relatively few persons undergo laboratory testing and those who did were self-selected. The high proportion of tested subjects who had laboratory-confirmed cryptosporidium 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 no evidence of Cryptosporidium in tap water, shellfish, or in only our stool specimens. Furthermore, the attack rate was low (2%) among persons who did not drink the afternoon cider. This suggests that there was little or no disease among the community who minimal water used to wash the apples was not a source of contamination, and that the presence of other infectious agents was unlikely to have caused the

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

- 37% to 78% oocysts per liter. Assuming that many oocysts captured during the flush-class cycle were not detectable by the recovery methods used, the actual concentration was probably much higher.

The pooled stool samples from two calves at the farm that supplied the implicated apples contained Cryptosporidium oocysts, but no oocysts were identified in specimens from the two calves at the potting shed.

**Human Oocyst Excretion.** Cryptosporidium oocysts were detected in stool samples of 30 (69%) of 43 primary case patients and 17 (94%) of 18 secondary case patients tested. The median time to the first stool examination was 6 days after the onset of symptoms. No bacterial pathogen was detected in the stools of primary or secondary case patients; one primary case patient had not uncommon Giardia lamblia infection. An isolate from the case patient was identified as Cryptosporidium parvum by the Centers for Disease Control and Prevention Parasite Diarrhea 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 remained for a median of 26 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**

Cryptosporidiosis is a major cause of acute diarrhea worldwide. This point-source outbreak provided an opportunity

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- 20
- 17
- 14
- 11
- 8
- 5
- 2

No. of Primary Case Patients

- 30
- 15
- 10
- 5
- 2

Duration of Illness, d

- 20
- 17
- 14
- 11
- 8
- 5
- 2

No. of Primary Case Patients

- 30
- 15
- 10
- 5
- 2

Duration of Illness, d
estimated rate of secondary household transmission. However, we did not evalu-
ate the background rate of enteric dis-
eases in control households without rats, and we did not test case patients for viral
pathogens. Furthermore, we did not test
exposed persons for asymptomatic in-
fec tion. Our estimate of duration of ocucyt
shedding should be interpreted with cau-
tion, since we only performed follow-up
laboratory testing of case patients ap-
proximately every 2 weeks.

This outbreak demonstrates the po-
tential for large foodborne outbreaks of
cryptosporidiosis and underscores the
need for increased awareness of the
potential for foodborne transmission of
cryptosporidiosis with common to the farm
environment.

Previous outbreaks of di-
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