Outbreak of Cyclosporiasis Associated with Basil in Missouri in 1999

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During the summer of 1999, an outbreak of cyclosporiasis occurred among attendees of 2 events held on 24 July in different counties in Missouri. We conducted retrospective cohort studies of the 2 clusters of cases, which comprised 62 case patients. The chicken pasta salad served at one event (relative risk [RR], 4.25; 95% confidence interval [CI], 1.80–10.01) and the tomato basil salad served at the other event (RR, 2.95; 95% CI, 1.72–5.07) were most strongly associated with illness. The most likely vehicle of infection was fresh basil, which was included in both salads and could have been grown either in Mexico or the United States. Leftover chicken pasta salad was found to be positive for Cyclospora DNA by means of polymerase chain reaction analysis, and 1 sporulated Cyclospora oocyst was found by use of microscopy. This is the second documented outbreak of cyclosporiasis in the United States linked to fresh basil and the first US outbreak for which Cyclospora has been detected in an epidemiologically implicated food item.

On 11 August and 23 August 1999, local health departments in Missouri were notified of cases of gastroenteritis among people who had attended a birthday party (event A) or a graduation party (event B), respectively. The parties occurred on 24 July in private homes and were catered by establishments in different counties. Stool specimens from attendees were found to be positive for the coccidian parasite Cyclospora cayetanensis, and the Centers for Disease Control and Prevention (CDC) was invited to assist in an investigation.

Both waterborne and foodborne outbreaks of cyclosporiasis, which is treatable with trimethoprim-sulfamethoxazole [1], have been documented previously [2–11]. The implicated food vehicles for US outbreaks have included fresh raspberries, mesclun lettuce, and basil. The mode of contamination of the produce has not been definitively identified for any of the outbreaks. A fundamental feature of the biology of the parasite that affects its epidemiology is that oocysts excreted in feces require days to weeks outside the host to become infectious (i.e., to sporulate) [11–12]. The minimum time in which sporulation can occur is unknown.

METHODS

Epidemiologic investigation. Retrospective cohort studies were conducted; structured questionnaires about symptoms and event-related exposures were used. A case of cyclosporiasis was defined as onset of illness 1–14 days after the events and either (1) 1 positive stool specimen and at least 1 gastrointestinal symptom (i.e., loose or watery stools, nausea, vomiting, stomach cramps, loss of appetite, or weight loss of >2
Outbreak of Cyclosporiasis in Missouri

Figure 1. Fresh produce items common to the implicated foods served at events A and B, which were associated with clusters of cases of cyclosporiasis, and the suppliers of these items. Other common ingredients included garlic, salt, and pepper, which came from different suppliers for each event.

kg) or constitutional symptom (i.e., fever or fatigue); (2) ≥3 loose stools in a 24-h period and at least 1 other gastrointestinal or constitutional symptom; or (3) a total of ≥4 gastrointestinal symptoms.

Statistical analyses. Univariate RRs and 95% CIs (Taylor series approximation) were calculated by use of EpiInfo, version 6.04 (CDC). Multivariate Poisson regression analyses were conducted by use of SAS, version 6.12 for Windows.

Laboratory methods. The Missouri Public Health Laboratory examined wet mounts of stool specimens (both before and after use of a method to concentrate parasites) by means of both ultraviolet fluorescence and bright-field microscopy. Slides stained by use of Kinyoun’s modified acid-fast technique and by use of the trichrome technique were examined; 5 or 6 specimens were also examined by use of the hot safranin staining technique [13, 14]. Eight specimens were tested for Cryptosporidium parvum by EIA; 8 specimens were tested for Salmonella, Shigella, and Campylobacter species and for Escherichia coli O157:H7; and 4 specimens were tested for adenovirus and rotavirus by EIA and for Norwalk virus by use of PCR analysis. Fresh stool specimens were placed in potassium dichromate (final concentration, 2.5%) and monitored by the CDC for sporulation of Cyclospora oocysts [12].

Leftover chicken pasta salad from event A, which had been frozen and thawed, was examined for Cyclospora oocysts by the CDC and the US Food and Drug Administration (FDA). An aliquot of ~50 g (~3/8 cup) was suspended in 50 mL of water and agitated (60–100 rpm in an orbital shaker for 30 min). The eluted fraction was centrifuged, treated with ethyl acetate (at a ratio of 1:5), sedimented (at 2000 g for 10 min), resuspended in water, sieved through a glass wool column, and split into 2 fractions (fraction A for microscopical analysis and fraction B for PCR analysis). Fraction A was clarified by 2 successive gravity sedimentations (3–4 min each), and the pooled supernatant was centrifuged (3200 g, 10 min). The pellet was resuspended in water (700 µL) and transferred to microscope slides (10 µL/slide) for examination by means of both ultraviolet fluorescence and bright-field microscopy.

Fraction B was concentrated by centrifugation, and a portion was spotted onto FTA filters (Fitzco) for direct use in PCR analysis as a DNA template for the amplification reactions [15]. A second type of PCR analysis involved extracting DNA from 500-µL aliquots of concentrated sediment by use of selected components from the FastDNA kit (BIO 101) [16]. Nested PCR amplification was performed with primers modified from ones described elsewhere [17]. The restriction sites EcoRI and BamHI were removed from the 5’ end, and bases matching the C. cayetanensis small subunit (SSU) rRNA coding region were added. Thus primers CYCF1 (5′-ATTACCCAATGAAAACAGTTT-3′) and CYCR2 (5′-TGCAGGAGAAGCCAAGGTAGG-3′) were used for the first step of nested PCR to amplify a fragment of 640 bp. This preamplified fragment was nested with primers CYCF3 (5′-GCCTTCCGCGCTTCGCTGCGT-3′) and CYCR4 (5′-TCGTCTTCAAAACCCTACTG-3′) for amplification of a fragment of 296 bp from the C. cayetanensis SSU rRNA coding region. DNA sequencing and analysis of the fragment amplified in the first step of nested PCR were then performed [18].

Environmental and source investigations. To determine the sources of the basil served at the events, we interviewed staff and obtained shipping documents from the country club, restaurant, and the basil distributor they had in common (distributor X; figures 1 and 2). To investigate how basil was grown...
and handled, we visited distributor X in September 1999, distributor Y (figure 2) in October, and the 2 farms that could have supplied basil in October (the US farm) and December (the Mexican farm).

RESULTS

Epidemiologic investigation: event A. Event A was a birthday party catered by a country club. Of the 46 attendees, 45 (97.8%) were interviewed, 32 (71.1%) of whom met the case definition (table 1); 9 (28.1%) of 32 had laboratory-confirmed cyclosporiasis. The CDC demonstrated the sporulation of *Cyclospora* oocysts in specimens from the 2 people who submitted fresh stool. The median incubation period was 7 days (range, 3–14 days; figure 3).

Six of the 22 food items that were served were found to be significantly associated with illness by univariate analyses (table 2). The chicken pasta salad was the item most strongly asso-
Table 1. Age, sex, and symptom data for attendees of 2 events in Missouri (events A and B) associated with clusters of cases of cyclosporiasis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Event A</th>
<th>Event B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case patients (n = 32)</td>
<td>Non–case patients (n = 13)</td>
</tr>
<tr>
<td>Age, y, median (range)a</td>
<td>46.5 (28–73)</td>
<td>42 (38–66)</td>
</tr>
<tr>
<td>Female sex, n (%)b</td>
<td>16 (50.0)</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-reported diarrhea, n (%)</td>
<td>32 (100)</td>
<td>0</td>
</tr>
<tr>
<td>No. of stools per d, median (range)d</td>
<td>6 (2–40)</td>
<td>0</td>
</tr>
<tr>
<td>Constant severity, n (%)</td>
<td>13 (40.6)</td>
<td>0</td>
</tr>
<tr>
<td>Duration of illness, d, median (range)f</td>
<td>11.4 (1–18)</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue, n (%)</td>
<td>31 (96.9)</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia, n (%)</td>
<td>30 (93.8)</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss, n (%)</td>
<td>30 (93.8)</td>
<td>0</td>
</tr>
<tr>
<td>kg Lost, median (range)g</td>
<td>3.2 (0–6.8)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea, n (%)</td>
<td>28 (87.5)</td>
<td>0</td>
</tr>
<tr>
<td>Cramps, n (%)h</td>
<td>22 (68.8)</td>
<td>0</td>
</tr>
<tr>
<td>Fever, n (%)i</td>
<td>18 (56.3)</td>
<td>0</td>
</tr>
<tr>
<td>Temperature, °C, median (range)j</td>
<td>37.8 (37.2–38.9)</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting, n (%)j</td>
<td>10 (31.3)</td>
<td>0</td>
</tr>
<tr>
<td>Hospitalization, n (%)</td>
<td>1 (3.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

a Differences between case patients and non–case patients were not statistically significant (P > .05), as determined by use of the Kruskal-Wallis test.
b Differences between case patients and non–case patients were not statistically significant, as determined by use of the χ² test.
c Differences between case patients and non–case patients were statistically significant, as determined by use of Fisher’s exact test for event A and the χ² test for event B, unless otherwise indicated.
d For event B, case patients and non–case patients.
e Attendees were asked whether their diarrhea was constant or was less severe on some days than others. For event B, non–case patients.
f The longer duration of illness for case patients for event B might be attributable to the fact that the interviews for event B were started 11 days later than the interviews for event A (on 12 August vs. 23 August). The durations are minimums because 11 (34.4%) case patients from event A and 16 (53.3%) case patients from event B were still ill when interviewed.
g For event A, case patients. For event B, case patients and non–case patient.
h Differences between case patients and non–case patients for event A were statistically significant, as determined by use of the χ² test.
i For event A, case patients. For event B, case patients.
j Differences between case patients and non–case patients were statistically significant for event A only, as determined by use of Fisher’s exact test.
k One person received iv fluids.

Associated with illness by univariate analyses and the only item associated with illness by multivariate analyses. On 26 July, the host of the party served leftover pasta salad and rolls to 3 people who had not attended the party. They developed symptoms consistent with the case definition a median of 7 days (range, 6–7 days) later; they were not tested for Cyclospora infection.

The leftover pasta salad was found to be positive for Cyclospora DNA by use of both types of PCR analysis, and 1 sporulated Cyclospora oocyst was identified by use of ultraviolet fluorescence and bright-field microscopy (figure 4). DNA sequence analysis of the amplified fragment showed 100% similarity with the C. cayetanensis SSU rRNA (GenBank accession number AF111183) [19].

The fresh produce in the salad included basil, red onions, and red bell peppers. Red onions and red bell peppers were also served fresh in other foods that were not associated with illness. Although green leaf lettuce was placed around the salad bowl, this lettuce was not included with the leftovers taken by the host, and other food items served on this lettuce were not associated with illness. Therefore, the basil was the ingredient most likely to have caused illness.

None of the 7 chefs involved with event A had traveled overseas or been ill in the previous month. However, 3 chefs became ill a median of 6 days (range, 5–8 days) after the event, and their stool specimens tested positive for Cyclospora. The CDC demonstrated sporulation of oocysts in the specimen obtained from the 1 chef who submitted fresh stool. One of the chefs who became ill had prepared and tasted the salad. The other 2 ill chefs did not recall tasting the salad or other items that contained fresh basil on other days that they were at work.
Figure 3. Dates of onset of symptoms among case patients who attended event A (A) or event B (B), which were associated with clusters of cases of cyclosporiasis.

Epidemiologic investigation: event B. Event B was a graduation party catered by a restaurant. Of the 58 attendees, 55 (94.8%) were interviewed, 30 (54.5%) of whom met the case definition (table 1); 8 (26.7%) of 30 had laboratory-confirmed cyclosporiasis. The median incubation period was 7 days (range, 5–13 days; figure 3).

Two of the 12 food items that were served were significantly associated with illness by univariate analyses (table 2). The tomato basil salad was the item most strongly associated with illness by univariate analyses and the only item significantly associated with illness by multivariate analyses. The fresh produce in the tomato basil salad, which was served on a tray lined with green leaf lettuce, included basil, red onions, tomatoes, and shallots. Slices of the red onions were served in a bowl next to the salad and, reportedly, not many were eaten. The only other food item with fresh basil was the vegetable pasta salad, which was significantly associated with illness only by univariate analyses. This salad also contained pesto sauce that had been prepared 2 days earlier by another chef, green onions, and red bell peppers, and it was served on a tray lined with green leaf lettuce. Several other food items that were not significantly associated with illness were also served on this lettuce. The pasta salad was divided into halves, which were served at event B and at another event on 24 July. Reported, no one who attended the other event became ill.

None of the 3 chefs involved with event B had traveled overseas or been ill in the previous month. However, the chef who prepared the food reportedly became ill 1 week later; he was not tested for Cyclospora infection. Although all 3 chefs tasted the pasta salad, only the chef who prepared the food became ill; none of the chefs tasted the tomato basil salad.

Environmental and source investigations. Of the fresh produce common to the implicated foods at events A and B (i.e., basil, green leaf lettuce, and red onions), basil was the only item for which the country club and restaurant had a common local distributor (distributor X; figures 1 and 2). The country club received 2 0.45-kg bags of basil on 23 July, and the chef recalled using the basil received that day.

The restaurant received 2 0.45-kg bags of basil from distributor X on 24 July, the date the salads were prepared. The chef recalled using the freshest basil available for the tomato basil salad. The vegetable pasta salad was garnished with basil from the same shipment, but not necessarily the same bag, that was used for the tomato basil salad. The pesto sauce for the pasta salad had been made 2 days earlier with basil from a local farm.

During the week before the events, local distributor X in Missouri received basil from distributor Y in another state. The only sources that could have supplied distributor Y during the week before the events were a Mexican farm, which provided 2 shipments of basil to distributor Y that could have been served at the events, and a US farm, which provided 1 such shipment. Which of the 2 farms provided the basil that was served could not be determined. However, the Mexican farm provided 15-fold more basil than did the US farm to distributor Y during the period of interest (figure 2).

Both farms obtain agricultural water from wells. On the Mexican farm, basil plants are watered and fertilized by drip irrigation. On the US farm, a sprinkler system is used to irrigate the basil plants; fertilizer is applied as granules. On both farms, pesticides and fungicides are mixed with water and sprayed directly onto the plants. Two samples of well water and a basil plant from the US farm had \( <3 \) organisms/g (the most probable number) of Escherichia coli when tested by the FDA. Two samples of well water from the Mexican farm had no detectable E. coli when tested by a Mexican laboratory, and a basil plant was negative for E. coli when tested by the FDA.

On both farms, basil is harvested by people wearing gloves. The basil is repackaged at distributor Y by people working with bare hands. Once the basil is repackaged, it is no longer distinguishable by source, and shipments (but not individual bags) may commingle product from different sources. The relevant shipments from Mexico and the United States would have taken
3–8 days and 7 days, respectively, to reach the events in Missouri, including 1–5 days after reaching distributor Y (figure 2).

DISCUSSION

We investigated 2 clusters of cases of cyclosporiasis that were associated with separate events held on the same day but in different counties in Missouri. The clinical illness was consistent with cyclosporiasis. The most common symptoms were diarrhea, which waxed and waned in intensity; fatigue; anorexia; and substantial weight loss. Other notable features included a week-long incubation period and a long duration of illness. Stool specimens from people at both events were reconfirmed by the CDC as being positive for *Cyclospora*. The stool testing done for the presence of other microbes had negative results.

The epidemiological evidence and the detection of *Cyclospora* in 1 of the epidemiologically implicated food items indicate that this outbreak was foodborne. The most likely vehicle of infection was fresh basil, which was not washed before event A but was before event B. Both of the implicated food items (1 per event) contained fresh basil, and the same distributor (i.e., distributor X) supplied the establishments with the basil that most likely was used. Although both of the implicated items also included red onions and green leaf lettuce, they came from different distributors, and details about how they were served suggest they were not to blame (figure 1).

We do not know whether the outbreak was substantially larger than the 2 clusters of cases that we investigated, which comprised 62 case patients among the attendees of the 2 events. We attempted to identify more cases by having country club staff members contact members of the club (~15% were contacted), asking other establishments that could have received basil from the same shipments received by the country club and restaurant whether they had received reports of illness, and informing local laboratories and physicians of the outbreak. Although we did not find additional cases through these means, we learned of 3 additional people who had laboratory-confirmed cases of cyclosporiasis and had eaten at the restaurant; 2 of the 3 people recalled eating food that did or could have contained fresh basil.

The true size of the outbreak would have depended in part on...
on where and how the basil was contaminated, how much was contaminated, and whether the basil was served fresh at the times and places (besides events A and B) that it was eaten. Although we were unable to determine where and how contamination occurred, it is unlikely that the basil was contaminated at the homes where the events were held, which were in different counties; at the catering establishments, which also were in different counties; or at distributor X, which handled the bags of basil but not the basil itself. Additionally, it is unlikely that basil contaminated shortly before consumption would have caused illness, because *Cyclospora* oocysts would not have had sufficient time to become infectious (i.e., to sporulate).

We think it is more likely that the contamination occurred further back in the distribution chain that was common to the basil served at the 2 events—that is, on 1 of the 2 possible source farms or at some point in the distribution chain up to and including distributor Y (figure 2). One of the possibilities is that the basil was contaminated with sporulated oocysts through contact with water on the farms, such as when pesticides and fungicides mixed in water were sprayed onto the plants. Consideration is being given to evaluating the water supplies further. Another possibility is that the basil was contaminated with unsporulated oocysts when handled by an infected person, who could have been asymptomatic. The basil was handled barenhanded on the Mexican farm when basil plots were weeded and by distributor Y during the repacking process. However, the further down the path from farm to table that the contamination occurred, the less time that the oocysts, if unsporulated when contamination occurred, would have had to sporulate. We could not determine whether contamination occurred on a farm and, if so, which one. However, the fact that the Mexican farm supplied 15-fold more basil than did the US farm to distributor Y during the week before the events increases the probability, but does not prove, that the basil served at the events was grown in Mexico.

The fresh basil served in the implicated food items represented a small fraction of what the 2 possible source farms supplied to the distribution chain during the period of interest and of what distributor Y shipped to distributor X (figure 2). The fact that the vegetable pasta salad that was made by the restaurant and was garnished with basil from the same 0.9-kg shipment, but not necessarily from the same bag, that was used for the tomato basil salad (the implicated item) was not associated with illness at event B or at the other event at which it was served (figure 2) suggests that some but not all of the basil in the 0.9-kg shipment to the restaurant and, presumably, in the larger shipments further back in the distribution chain was contaminated.

This outbreak in Missouri in 1999 is the second documented outbreak of cyclosporiasis that has been associated with consumption of fresh basil. The first occurred in 1997 in the Northern Virginia–Washington, D.C.–Baltimore, Maryland, metropolitan area [7, 8]. Mexico was one of the possible sources of the basil in the 1997 outbreak as well. However, the possible source farms were different in the 2 outbreaks.

The outbreak in 1999 is the first US outbreak for which *Cyclospora* has been detected in an epidemiologically implicated food item. Generally, leftovers of fresh produce are not available for testing once an outbreak investigation begins. Fortunately, the hostess of event A froze the leftover chicken pasta salad. Contamination was detected by means of both PCR and microscopy, but the level of contamination is not known. The continued occurrence of foodborne outbreaks of cyclosporiasis highlights the importance of further elucidating the biology and epidemiology of *Cyclospora* and of identifying the modes of contamination of produce and effective measures to prevent outbreaks.

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**References**