
EMILIA H. A. KOUMANS, DOLORES J. KATZ, JEAN M. MALECKI, SAVITA KUMAR, SUSANNE P. WAHLQUIST, MICHAEL J. ARROWOOD, ALLEN W. HIGHTOWER, AND BARBARA L. HERWALDT

Division of Parasitic Diseases, National Center for Infectious Diseases, and Epidemic Intelligence Service, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Georgia; Florida Department of Health, Tallahassee, Florida; Palm Beach County Health Department, West Palm Beach, Florida

Abstract. Before 1995, only one outbreak of cyclosporiasis had been reported in the United States. To identify risk factors for Cyclospora infection acquired in Florida in 1995, we conducted a matched case-control study (24 sporadic cases and 69 controls) and retrospective cohort studies of clusters of cases associated with two May social events (attack rates = 15.4% [8 of 52] and 54.5% [6 of 11]). In univariate analysis of data from the case-control study, consumption of fresh raspberries (odds ratio [OR] = 6.0, 95% confidence interval [CI] = 1.1–31.7) and bare-handed contact with soil (OR = 5.4, 95% CI = 1.4–20.7) were associated with infection; soil contact was also implicated in multivariate analysis. For the events, mixed-fruit items that had only fresh raspberries and strawberries in common had elevated relative risks (3.7 and 4.2), but the confidence intervals overlapped 1.0. The raspberries eaten at the events by sporadic case-patients were imported. Given the cumulative evidence of the three studies and the occurrence in 1996 and 1997 of outbreaks in North America associated with consumption of Guatemalan raspberries, food-borne transmission of Cyclospora was likely in 1995 in Florida as well.

On June 12, 1995, a community hospital laboratory in Boca Raton, Palm Beach County, Florida, reported to the Florida Department of Health that it had identified six cases of infection with Cyclospora species during the previous two weeks. The cases were in adults who had no obvious common exposure and had not recently traveled out of the county. Since the spring of 1992, the laboratory had been examining all stool specimens submitted for testing for ova and parasites for Cyclospora cayetanensis, a recently characterized coccidian parasite.1,2 Before June 12, the laboratory had identified only two other Cyclospora cases, both in persons who had traveled overseas.

The first recognized human cases of infection with Cyclospora, which is acquired by ingestion of oocysts and causes gastroenteritis, were diagnosed in 1977 and 1978 in Papua New Guinea.3 Most of the subsequently reported cases were in persons living or traveling in developing countries. In Nepal, where many of the reported cases have occurred, most cases have been diagnosed during the months of May through August;4,5 the seasonality of cyclosporiasis in Nepal and elsewhere remains unexplained. By the spring of 1995, only two outbreaks in the United States were known to have occurred (one in 1990 and another one in 1995), at least one of which was apparently water-borne6 (Carter R, New York City Department of Health, unpublished data). Direct person-to-person transmission is unlikely because excreted oocysts are thought to require from days to weeks in a suitable environment to become infectious (i.e., to sporulate).1

Cyclospora infection, which responds to treatment with trimethoprim-sulfamethoxazole,7 is identified in stool by such methods as demonstration of blue or green autofluorescence with ultraviolet epifluorescence microscopy, examination of a wet mount under phase-contrast microscopy, or use of modified acid-fast8 or other stains (the oocyst is 8–10 μm in diameter and variably acid-fast).9,10 These procedures are not routine in most microbiology laboratories. Demonstration of sporulation of oocysts provides definitive evidence for the diagnosis.1

METHODS

The policies of the Centers for Disease Control and Prevention (CDC) with regard to human subjects were adhered to in this investigation.

Surveillance. Public health officials in Palm Beach County urged persons with diarrhea to consult a health care provider and sent information about the parasite via facsimile to county practitioners. After training at the community hospital that had diagnosed the original cases, laboratorians at other hospitals in the county and at branch state laboratory A (serves the following counties: Palm Beach [1995 population = 960,268], Martin [1995 population = 115,220], Hendry, Okeechobee, and St. Lucie) were requested to examine all stool specimens that were submitted for parasitologic testing for Cyclospora as well. The branch laboratory began such testing on June 22. The other four state laboratories were trained thereafter and followed suit during the period of July 1 through September 1. On July 25, the state of Florida made Cyclospora infection reportable.

Laboratory methods. All stool specimens that reportedly were positive for Cyclospora were sent to the Parasitic Diseases Laboratory at CDC for confirmation. Most were sent as acid-fast-stained slides (one as a trichrome-stained slide) prepared from either 10% formalin-fixed or fresh stool specimens. When a formalin-ethyl acetate concentrate was provided, slides were stained at CDC with the Kinyoun carbol-fuchsin modified acid-fast procedure.8 All slides were examined for Cyclospora and Cryptosporidium parvum by viewing ≥ 300 oil-immersion fields. Cyclospora oocysts from one stool specimen placed in 2.5% potassium dichromate were monitored for sporulation.1 Results were requested for the stool specimens that had been submitted to various laboratories for testing for Salmonella, Shigella, and Campylobacter species.

Matched case-control study. Case-patients were residents of Palm Beach or Martin Counties (neighboring counties) who had laboratory-confirmed Cyclospora infection reported to the respective county health department by August.
22, 1995, and reconfirmed by CDC. Potential case-patients were excluded if they had not had at least one gastrointestinal (GI) symptom, were immunocompromised because of acquired immunodeficiency syndrome (AIDS) or cancer, had participated in either social event (see below), had traveled outside the United States within the month before they became ill, or had traveled outside the county for ≥ seven days during the period of interest (the two weeks before onset of illness).

Case-patients suggested specific friends or acquaintances as their potential matched controls. Some controls were selected from a simple random sample of a Southeast regional telephone CD-ROM-generated list of neighbors who lived within a three-block radius of the referent case-patient’s residence. Potential controls were excluded if they had had an acute GI illness during the period of interest for the referent case-patient until the date of the interview, had traveled overnight outside the county during the period of interest, or had traveled outside the United States in the previous six months. Because identifying eligible controls was difficult, two controls who had traveled within the United States were included. Case-patients were matched by age range (Table 1), and adults were also matched by sex.

Telephone interviews using a structured questionnaire took place from July 17 through August 29; we interviewed the parents of children ≤ 10 years of age. We asked about symptoms; coexisting medical conditions; use of medications; travel; and exposures to drinking and recreational water, food (e.g., consumption of fresh fruits and vegetables), animals (i.e., birds, dogs, cats, cows, and sheep) or areas where animal feces may be present (e.g., pet stores, farms, zoos), the environment (e.g., contact with soil); and miscellaneous factors (e.g., exposure to children in diapers). We asked adults two questions about gardening and soil: 1) “In the two weeks of interest, did you do any gardening?” 2) “Did you have contact with your bare hands with any soil or manure?” If the response to the second question was in
the affirmative, we asked about the origins of the soil/manure (e.g., from the ground or from a purchased bag of soil). We asked the parents of children who were case-patients: 1) “Did your child play outside?” 2) “Did your child play in an area outside where she or he touched soil?”

Cohort studies. We conducted retrospective cohort studies among two groups of persons who may have been exposed to Cyclospora during two separate social events that occurred May 19–21 or May 27. To be classified as a laboratory-confirmed case-patient, the diagnosis had to be reconfirmed at CDC. Probable case-patients met the clinical case definition (i.e., diarrhea [three or more loose or watery stools in a 24-hr period] that lasted for seven or more days), with onset of diarrhea by two weeks after the event. Telephone interviews took place from July 2 through July 27. We asked similar questions to those in the case-control study (but no questions about soil exposure because that risk factor had not yet been identified when the cohort studies were conducted) and included questions about ingestion of foods and beverages served at the respective social events. For exposures not related to the events per se, the period of interest was the two weeks before the event of interest.

Statistical methods. In the case-control study, we conducted matched univariate and multivariate analyses with conditional logistic regression (SAS version 6.10 for Windows [Proc Phreg; SAS Institute, Cary, NC]) and calculated 95% confidence intervals (CIs) and etiologic fractions. We used forward stepwise selection of variables in the multivariate analysis. For variables in the cohort studies, we calculated univariate relative risks (RRs), Taylor series 95% CIs, and the corresponding $P$ values.

RESULTS

Surveillance and laboratory results. A total of 87 cases in persons with at least one stool specimen positive for Cyclospora had been reported by August 22, 1995, to the Palm Beach or Martin County health department. At CDC, specimens from 45 (51.7%, Figure 1) of the 87 cases were reconfirmed as positive, 36 (41.4%) were not confirmed, and 6 (6.9%) were not examined. By August 22, the community hospital laboratory that had identified the index cases and branch state laboratory A had identified a total of 13 (12 reconfirmed by CDC; one unavailable for testing) and 71 (30 reconfirmed; 42.3%) Cyclospora cases, respectively. One other laboratory in Palm Beach County, one in Martin County, and one in another state had each identified one case, all three of which were reconfirmed by CDC. Sporulation was demonstrated for oocysts from a specimen from a case-patient in the case-control study.

Matched case-control study. We identified 24 laboratory-confirmed case-patients (11 [45.8%] diagnosed by the community hospital laboratory) and selected 69 controls for the case-control study (Figure 1); 34 (49.3%) of the controls were friends/acquaintances of case-patients, and the median case:control ratio was 1:3 (range = 1:1–1:4). The earliest and latest dates of onset of GI symptoms for the case-patients were May 7 and August 13, respectively (Figure 2). The only case-patients who knew each other were a mother and her seven-year-old son; she became ill 30 days later than he did and two days after she submitted her specimen. The three case-patients who lived in Martin County (Figure 3 and Table 1) had not visited Palm Beach County during the period of interest. One case-patient was hospital-
Figure 2. Cases of Cyclospora infection (excluding overseas and domestic travelers), confirmed at the Centers for Disease Control and Prevention, with onset before August 22, 1995, by week of onset of gastrointestinal illness (n = 44); including four persons identified after August 22, seven coinfect patients (Giardia [4], Salmonella [2], and Cryptosporidium [1]), one person who had acquired immunodeficiency syndrome, and one person who had cancer, all of whom were excluded from the studies.

Figure 3. Map of locations of residences in Palm Beach and Martin Counties (southeastern Florida) of the 24 case-patients in the case-control study; - = roads; ■ = residence of one case-patient; □ = residence of two case-patients.
ized because of dehydration. Ten case-patients (41.7%) could not continue their usual activities (e.g., work) and could not do so for a median of five days (range = 1–21 days). The median time from the onset of GI symptoms to laboratory diagnosis was 16 days (range = 2–65 days). The specimens from all 17 case-patients who submitted stool for bacterial culture were negative.

**Univariate analysis.** The only food exposure significantly associated with increased risk for illness was ingestion of fresh raspberries (odds ratio [OR] = 6.0, CI = 1.1–31.7) (Table 2). Five adult case-patients (20.8%) and three controls (4.4%) had eaten raspberries. Four of the five case-patients had onset of diarrhea in the first half of June: two on June 5, one on June 7, and one on June 15; the fifth became ill on July. One other case-patient, who was uncertain about whether he had eaten raspberries and was considered for the purposes of the analysis not to have eaten them, became ill on June 1.

The only nonfood exposure significantly associated with infection was contact with soil or manure (OR = 10.7, CI = 2.3–50.3). The relationship remained significant after reanalysis of the data using only exposure to soil from the ground as the exposure of interest (i.e., reclassifying those with exposure to manure [n = 1], soil from a bag [n = 1], or both [n = 1] as not having exposure to soil from the ground) (OR = 5.4, CI = 1.4–20.7). The association with gardening, irrespective of whether the person had had bare-handed contact with soil, was weaker (OR = 3.5, CI = 1.1–11.6).

The OR associated with drinking unheated, unfiltered (at the faucet) tap water versus exclusively drinking bottled water was 0.9 (CI = 0.3–2.7). The case-patients and controls drank similar amounts of water; the median total number of glasses of water consumed per day by the case-patients was six (range = 0–10) and by the controls was five (range = 0–32; P = 1.0, by matched univariate analysis). Because controls were selected from friends and neighbors of the case-patients, only 11 (15.9%) of the 69 controls were not matched to case-patients by residential water supply. The residences of the case-patients were served by nine different public water systems in Palm Beach and Martin Counties. One utility obtained water from a lake, and the other utilities obtained water from wells; all the utilities filtered their water.

**Multivariate analysis.** In multivariate analysis, which was limited by the relatively small size of the study, only bare-handed contact with soil was independently associated with illness (OR = 7.2, CI = 1.5–33.8; etiologic fraction = 43.1%) (Table 2). We could not assess whether consumption of fresh raspberries was an independent risk factor because four of the five case-patients who had eaten raspberries had also had contact with soil. It was not possible to conduct a complete evaluation for interaction between age and soil exposure. For adults, six (35.3%) of 17 case-patients versus eight (15.4%) of 52 controls had had contact with soil (P = 0.10, by matched univariate analysis). For children, six (85.7%) of the seven case-patients versus eight (47.1%) of 17 controls had had contact with soil (P = 1.0, by matched univariate analysis). The only child reported not to have had contact with soil was a three-year-old girl receiving medical care for elevated blood lead levels. The youngest case-patient, a boy 20 months of age whose infection was diagnosed after many weeks of GI symptoms, consumed only prepack-

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**Table 2**

Exposures of participants in the case-control study (n = 93) and cohort studies (social event A, n = 52; social event B, n = 11)*

<table>
<thead>
<tr>
<th>Exposure†</th>
<th>Case-patients (n = 24)</th>
<th>Controls (n = 69)</th>
<th>OR (CI)</th>
<th>P</th>
<th>Etiologic fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil‡</td>
<td>12 (50.0)</td>
<td>16 (23.2)</td>
<td>5.4 (1.4–20.7)</td>
<td>0.02</td>
<td>40.7%§</td>
</tr>
<tr>
<td>Gardening</td>
<td>14 (58.3)</td>
<td>23 (33.3)</td>
<td>3.5 (1.1–11.6)</td>
<td>0.04</td>
<td>41.7%§</td>
</tr>
<tr>
<td>Raspberries</td>
<td>5 (20.8)</td>
<td>3 (4.4)</td>
<td>6.0 (1.1–31.7)</td>
<td>0.04</td>
<td>17.4%§</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>8 (33.3)</td>
<td>48 (69.8)</td>
<td>0.2 (0.06–0.6)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Cohort studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social event A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit cup#</td>
<td>7 (87.5)</td>
<td>27 (61.4)</td>
<td>3.7 (0.5–27.8)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td>6 (75.0)</td>
<td>29 (65.9)</td>
<td>1.5 (0.3–6.5)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Social event B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cake**</td>
<td>5 (83.3)</td>
<td>2 (33.3)</td>
<td>4.2 (0.7–24.9)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td>1.5 (0.5–4.4)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

* OR = odds ratio; CI = 95% confidence interval; RR = relative risk.
† Consumption of strawberries was not associated with illness (OR = 0.6, CI = 0.2–1.7). Consumption of cantaloupe (OR = 0.2, CI = 0.06–0.6), honeydew melon (OR = 0.2, CI = 0.05–1.0), and watermelon (OR = 0.3, CI = 0.10–0.9), all of which were purchased whole (not precut), appeared to be protective. Contact with animals or visits to areas where animals were present were not associated with illness.
‡ Soil from the ground.
§ Etiologic fractions were calculated using univariate ORs (not independent) to assess the possible contribution of consumption of raspberries and contact with soil.
# Consumption of cantaloupe was highly correlated with consumption of watermelon and honeydew melon. If melon consumption (purchased whole or precut) is combined, the OR for soil remains the same, but watermelon consumption becomes protective.
** The cake was covered with fresh strawberries and blueberries and was served with an optional fresh raspberry/strawberry/blueberry sauce.

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aged food, drank four glasses of tap water daily, and played outside where his hands had touched soil. If the soil exposure of two case-patients who had repotted outdoor plants is reclassified to constitute bare-handed contact with ground soil, the multivariate OR increases (OR = 12.4, CI = 2.3–67.6). In addition, it then becomes possible to evaluate the importance of soil contact for adults (OR = 9.0, CI = 1.4–56.5).

**Cohort studies.** A complete line listing of those who attended social event A, which occurred May 19–21 in Boca Raton (Palm Beach County), was never obtained; reportedly, about 120 persons attended. We interviewed 52 persons (43.3% of 120); none had AIDS or was being treated for cancer. Interviews were conducted a median of 50 days (range = 43–68 days) after the catered meal that was held on May 20. The median age of the 52 interviewees was 43 years (range = 10–83 years). A total of 20 interviewed persons (38.5%) had at least one GI symptom. We identified four laboratory-confirmed cases and four probable cases, for an attack rate of 15.4% among the interviewees (Table 1); two of the eight case-patients were married to each other. The median incubation period was seven days (range = 2–10 days) (Figure 2). One case-patient received intravenous rehydration. Of the four laboratory-confirmed case-patients, two submitted stool specimens for bacterial cultures, which were negative.

No event-related exposure was significantly associated with illness. Eating the fruit cup at the catered meal was associated with illness, but not significantly (RR = 3.7, CI = 0.5–27.8) (Table 2); the fruit cup consisted of a hollowed-out cantaloupe, filled with fresh raspberries, strawberries, honeydew melon, watermelon, blackberries, and red grapes. No information is available about whether the fruit had been rinsed.

Social event B, a noncatered dinner party, occurred one week later, on May 27, in an apartment in Palm Beach (Palm Beach County), 20 miles from the location of event A. Twelve adults attended, none of whom had attended the first event or were immunocompromised. Eleven persons were interviewed; the other person, not included in the analyses below, had GI symptoms within two weeks of the event. Interviews were conducted a median of 41 days (range = 41–47 days) after the event. The median age of those interviewed was 54 years (range = 45–75 years) (Table 1). A total of nine (81.8%) of the 11 interviewees had at least one GI symptom; we identified three laboratory-confirmed cases and three probable cases, for an attack rate of 54.5% (Table 1). The median incubation period was six days (range = 2–7 days) (Figure 2). One case-patient submitted stool for bacterial culture, which was negative.

No event-related exposure was significantly associated with illness. The dessert, a home-baked angel-food cake topped with fresh strawberries and blueberries, whipped cream, and an optional fresh raspberry/strawberry/blueberry sauce, was associated with illness, but not significantly (RR = 4.2, CI = 0.7–24.9) (Table 2). Three of the five participants who had eaten the cake remembered eating the sauce; the other two did not remember whether they had eaten it. All fruit had been rinsed in tap water.

**Traceback investigation.** The only foods common to both events were fresh raspberries and strawberries; only raspberries were also implicated in univariate analysis for the case-control study. The raspberries for event A had been purchased from a wholesaler; the distributor who supplied this wholesaler provided raspberries that were from either Guatemala or Chile. Raspberries for event B were purchased at a store belonging to a chain of stores when the chain’s distribution center had raspberries available from both Guatemala and Chile. Based on the typical turnover period for produce and the shelf-life of raspberries, Guatemala was considered the more likely source for the raspberries (Lowdermilk M, Palm Beach County Health Department, unpublished data). In the two weeks before they became ill, all case-patients in the case-control study who had eaten raspberries (including the one who was uncertain whether he had eaten them) had also bought their produce, including all fresh fruit, at stores belonging to the chain. Therefore, at least some of the case-patients might have purchased their raspberries during the same period in late May when the host for event B did. Raspberries reportedly were not shipped from Guatemala to this region of Florida after the end of May.

**DISCUSSION**

Two lines of evidence support the conclusion that one or more outbreaks of cyclosporiasis occurred in Palm Beach and Martin Counties in the spring and summer of 1995. First, the microbiology laboratory at the community hospital that for several years had been examining stool specimens for *Cyclospora* but had not been identifying cases in nontravelers identified 13 cases in May, June, and July (seven before the investigation had received media attention). This finding is noteworthy even though the background prevalence of *Cyclospora* infection in Florida is unknown because no formal surveillance existed before July 1995. Second, in addition to the sporadic cases, clusters of cases were noted that were related to two social events. The case-patients became ill a median of 6–7 days after the events, an incubation period that is consistent with that for cyclosporiasis.13

The cases in our studies were initially diagnosed by five different laboratories, all five of which had at least one case reconfirmed by CDC. Although branch state laboratory A had diagnosed the most cases, only 42.3% of its cases were reconfirmed by CDC. The high percentage of false-positive results apparently was attributable to misidentification of acid fast-staining debris as *Cyclospora*. However, misidentification of acid fast-staining *C. parvum* oocysts (about half as big as *Cyclospora* oocysts), yeast, or pollen grains as *Cyclospora* can occur; a direct immunofluorescent-monoclonal antibody stain for *C. parvum* can be used to distinguish *Cryptosporidium* and *Cyclospora*.14 Polymerase chain reaction methodology for detection of *Cyclospora* in stool15–17 may be useful in future outbreak investigations. The possibilities of both false-negative and false-positive results when using techniques that rely on light microscopy highlight the importance of laboratory training, quality-control procedures, and proficiency testing.

Ours is the first investigation to suggest that contact with soil may be a risk factor for *Cyclospora* infection. We included questions about contact with soil in the case-control study because such contact can result in transmission of an-
other coccidian parasite, *Toxoplasma gondii.* Soil contact is also important for transmission of the so-called soil-transmitted helminths (e.g., *Ascaris lumbricoides* and *Trichuris trichiura,* which, like *Cyclospora*, are not immediately infectious after excretion (i.e., require an extrinsic developmental period). Whereas the definitive animal host for *Toxoplasma* is the cat (and a relevant exposure is contact with soil contaminated with cat feces), the animal reservoir(s) for *Cyclospora cayetanensis*, if any, has not yet been identified. Although soil contact is a plausible mode of transmission for *Cyclospora*, further research is needed to test the hypothesis that this contact is an important mode of transmission, to determine how soil could become contaminated, and to identify other risk factors for infection (contact with soil could account for 43.1% of the cases in the case-control study).

In the case-control study, we could not assess whether consumption of fresh raspberries was independently associated with infection because only one case-patient who had eaten raspberries had not also had contact with soil; we do not have an explanation for the apparent protective effect of consumption of cantaloupe. Although no item served at the social events was significantly associated with illness, both the fruit cup (event A) and the dessert (event B) included raspberries in mixtures of fruits that were associated with increased RR s. Consumption of these fruit items (versus of other items) accounted for the greatest proportion of case-patients from these events (seven [87.5%] of eight case-patients from event A and five [83.3%] of six from event B ate these items). The raspberries served at both events and those eaten by four of the five case-patients in the case-control study who ate raspberries may have been purchased during the same period (the last two weeks of May) and may have been grown in the same country (Guatemala). Although consumption of raspberries may have accounted for event-related illness, only five case-patients (20.8%) in the case-control study (none of the seven children) recalled having eaten raspberries. Possible explanations include poor recall and other vehicles or modes of transmission for some cases.

The cumulative evidence from the case-control study and two cohort studies and the possible commonality of the source of the raspberries suggest that *Cyclospora* may have been food-borne. This conclusion is all the more likely, given that consumption of Guatemalan raspberries was implicated as the cause of large outbreaks of cyclosporiasis that occurred in the United States and Canada in the spring months of 1996 and 1997. Viewed with the benefit of hindsight, the cases of cyclosporiasis in Florida in 1995 likely were a harbinger of these multistate outbreaks in 1996 and 1997. The cases that were recognized during all three outbreaks probably represented only a small fraction of all that occurred. Increased awareness of and testing for *Cyclospora* may lead to identification of additional cases and outbreaks and to a better understanding of the risk factors for transmission. The experience gained by laboratorians and medical and public health staff in Florida in 1995 prepared this state to play a key role in the detection and investigation of the multistate outbreak associated with consumption of raspberries that occurred in 1996, as well as of outbreaks of cyclosporiasis in Florida in 1997 that were associated with consumption of other fresh produce (i.e., mesclun lettuce).

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Authors’ addresses: Emilia H. A. Koumans, Division of STD Preven- tion, National Center for HIV, STD and TB Prevention, Centers for Disease Control and Prevention, Mailstop E-02, 1600 Clifton Road NE, Atlanta, GA 30333. Dolores J. Katz, Department of Ep- idemiology and Public Health, University of Miami School of Med- icine, Box 016069 (R-669), Miami, FL 33101. Jean M. Malecki, State of Florida Department of Health and Rehabilitative Services, Palm Beach County Health Department, PO Box 29, 826 Evernia Street, West Palm Beach, FL 33401. Savita Kumar, State of Florida Department of Health and Rehabilitative Services, Palm Beach County Health Department, 1050 15th Street West, Riviera Beach, FL 33404, Susanne P. Wahlenquist and Michael J. Arrowood, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F-13, 4770 Buford Highway NE, Chamblee, GA 30341-3724. Allen W. Hightower and Barbara L. Herwaldt, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F-22, 4770 Buford Highway NE, Chamblee, GA 30341-3724.

Reprint requests: Barbara L. Herwaldt, Division of Parasitic Dis- eases, Centers for Disease Control and Prevention, Mailstop F-22, 4770 Buford Highway NE, Chamblee, GA 30341-3724.

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