SPECIAL SECTION: EMERGING INFECTIONS

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Cyclospora cayetanensis: A Review, Focusing on the Outbreaks of Cyclosporiasis in the 1990s

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Cyclospora cayetanensis, **a coccidian parasite that causes protracted, relapsing gastroenteritis, has a short recorded history. In retrospect, the first 3 documented human cases of *Cyclospora* infection were diagnosed in 1977 and 1978. However, not much was published about the organism until the 1990s. One of the surprises has been the fact that a parasite that likely requires days to weeks outside the host to become infectious has repeatedly caused foodborne outbreaks, including large multistate outbreaks in the United States and Canada. In this review, I discuss what has been learned about this enigmatic parasite since its discovery and what some of the remaining questions are. My focus is the foodborne and waterborne outbreaks of cyclosporiasis that were documented from 1990 through 1999. The occurrence of the outbreaks highlights the need for health care personnel to consider that seemingly isolated cases of infection could be part of widespread outbreaks and should be reported to public health officials. Health care personnel should also be aware that stool specimens examined for ova and parasites usually are not examined for *Cyclospora* unless such testing is specifically requested and that *Cyclospora* infection is treatable with trimethoprim-sulfamethoxazole.**

The intestinal protozoan parasite now called *Cyclospora cayetanensis* has a short recorded history, characterized by periodic rediscovery of the organism and confusion about its identity (figure 1). In retrospect, the first 3 documented human cases of *Cyclospora* infection were diagnosed as recently as 1977 and 1978, and reported in 1979, by Ashford, a British parasitologist who was working in Papua New Guinea [1]. He noted that only 2 of the 3 infected persons were ill and that they excreted "scanty" organisms. He also noted that oocysts of the organism were unsporulated (i.e., immature, with undifferentiated cytoplasm, and thus noninfectious) when excreted and did not begin to sporulate (i.e., to develop sporozoites, the infective units within oocysts), and thus to become clearly recognizable as coccidian parasites, until 8 days (in 1 case) or 11 days (in 1 case) thereafter (figures 2 and 3). In fact, sporulation was so delayed that Ashford had almost discarded the specimens [2]. He remarked that unsporulated oocysts, which is what clinical microbiologists see when examining stool, could easily be misidentified as fungal spores, and thus the organism could be overlooked [1, 2].

Although Ashford correctly deduced that the organism was a coccidian parasite, as are the fellow gastrointestinal pathogens *Cryptosporidium parvum* (figures 3 and 4;table 1) and *Isospora belli* (figure 4), he was unsure how to classify it by genus because he was uncertain how many sporozoites were within each of the 2 sporocysts in sporulated oocysts. His best guess (i.e., 4 sporozoites per sporocyst) was incorrect, as was, therefore, his thought that the organism probably was a species of *Isospora*. In part because the organism remained unclassified and unnamed, Ashford's report in 1979 in a tropical medicine journal [1] went virtually unnoticed for over a decade, just as, presumably, the organism itself had been for much longer.

In the 1980s, the organism was periodically rediscovered in stool specimens by persons who were unaware of Ashford's report and who, like him, were uncertain of the identity of the organism (figure 1). With the exception of a couple of abstracts about some of the cases diagnosed in the 1980s [4, 8], nothing more was published about the organism until the early 1990s. In an abstract in 1986, Soave et al. [8] briefly described 4 cases of infection in travelers to Haiti and Mexico and concluded that the organism was "reminiscent of an unsporulated, coccidian body but a fungal spore could not be ruled out." In an abstract in 1989 [4], Naranjo, Sterling, and colleagues, who were working in Peru, reported having seen "*Cryptosporidium muris*–like objects" (i.e., organisms resembling a large species

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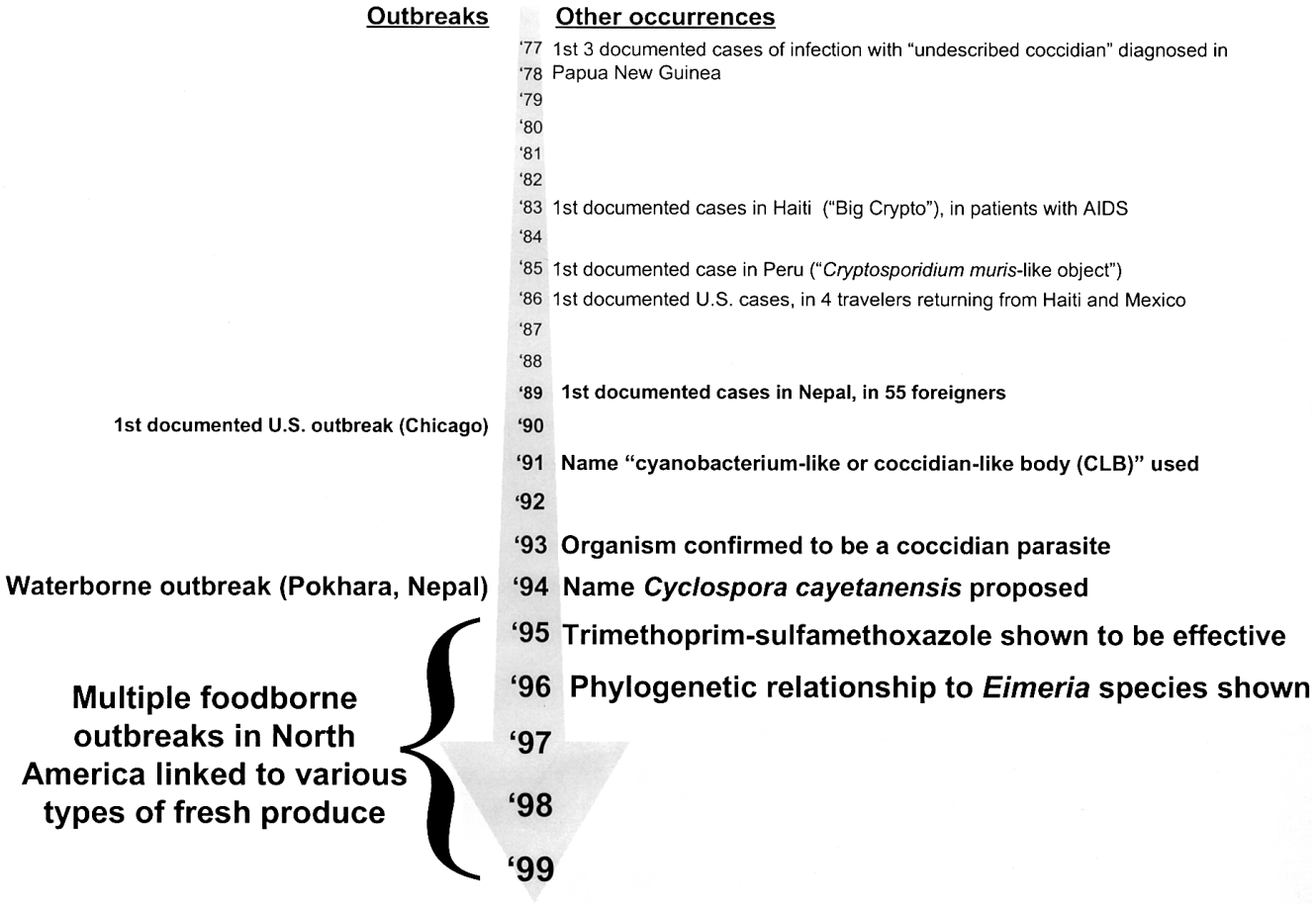

Figure 1. A time line of the increasing activity and expanding knowledge during the brief recorded history of the organism now called *Cyclospora cayetanensis* [1–27]. The time line shows highlights (e.g., not all of the cases diagnosed in the 1980s are shown). The years for the various cases are the years of diagnosis rather than those of publication, which often were much later. With the exceptions of Ashford's article in 1979 about the initial cases in Papua New Guinea [1] and 2 abstracts about some of the cases in the 1980s [4, 8], nothing was published about the organism until the 1990s. Although the first definite cases of cyclosporiasis documented in Nepal were diagnosed in 1989 [11, 12], in retrospect, some cases of gastroenteritis in 1985 [13] might have mistakenly been attributed to *Blastocystis hominis* infection. Cases of cyclosporiasis reportedly began to be noted among foreigners there at about the same time when quinolone antibiotics began to replace trimethoprim-sulfamethoxazole as empirical therapy for travelers' diarrhea [18]. For comparison, it is thought that the first documented human cases of infection with other protozoan parasites date back as follows: for *Giardia lamblia,* to the late 17th century [28]; for *Entamoeba histolytica,* to the late 19th century [29]; for *Isospora belli,* to the early 20th century [30]; and for *Cryptosporidium parvum*, to 1974 [31, 32].

of *Cryptosporidium*), which they thought might be "cysts of an unidentified flagellate," in occasional stool specimens from a total of 3 persons in 1985 and 1987 and during cohort studies initiated in 1988 that focused on *Cryptosporidium parvum*.

The organism discovered by Ashford finally began to emerge from relative obscurity in the early 1990s, when more articles were published about it. Long et al., who had diagnosed, starting in the late 1980s, multiple cases of infection in travelers and persons with AIDS, thought that the organism resembled both a coccidian oocyst (sporocysts were seen) and blue-green algae or cyanobacteria (by electron microscopy, structures resembling photosynthesizing organelles were seen) [14, 15]. In a publication in 1991, they referred to the organism as a "cyanobacterium-like or coccidian-like body (CLB)" [15], which became the interim nomenclature for the organism for several years [11, 12, 40, 46–48].

Ultimately, Ortega, Sterling, and colleagues, through research in Peru that they described in a publication in 1993 [5], not only succeeded in demonstrating sporulation of oocysts—thus reconfirming that the organism is a coccidian parasite—but also determined that a fully sporulated oocyst has 2 internal sporocysts, each containing 2 sporozoites (figures 2 and 3). This determination enabled them to classify the organism. The morphological features of sporulated oocysts were thought to resemble most closely the features of parasites in the *Cyclospora* genus. Members of this genus, which was cre-

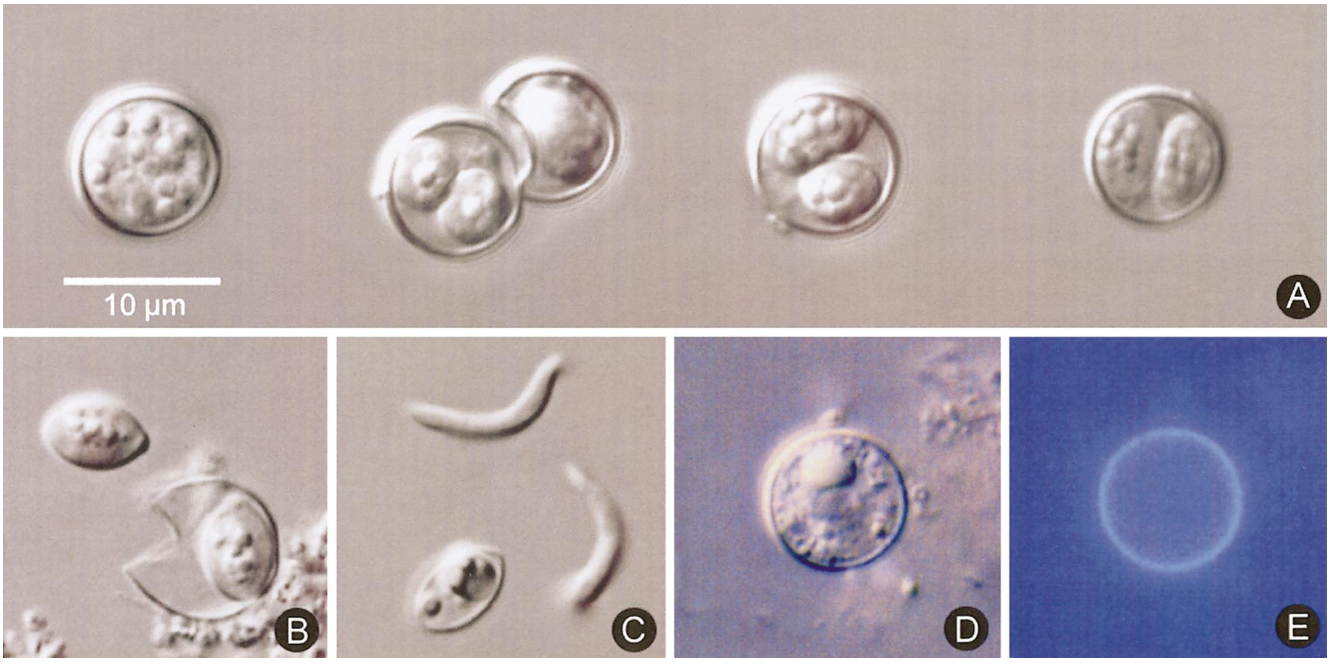

Figure 2. *Cyclospora cayetanensis* oocysts: demonstration of sporulation and autofluorescence in wet preparations of stool specimens [33]. *A, B,* and *C* demonstrate sporulation of oocysts (i.e., formation of 2 internal sporocysts, each containing 2 sporozoites, the infective units) and excystation (i.e., release of sporocysts from oocysts and release of sporozoites from sporocysts), as viewed by differential interference contrast (DIC) microscopy. Oocysts are excreted unsporulated; they usually require at least 1 week under laboratory conditions to sporulate and thus to become infectious. Therefore, clinical microbiologists see unsporulated oocysts when they examine stool specimens. Panel *A* shows, from left to right, the progression from an unsporulated oocyst, which has undifferentiated cytoplasm filled with refractile globules; to an oocyst containing 2 sporoblasts that is adjacent to another sporulating oocyst; to an oocyst containing 2 immature sporocysts; to an oocyst with sporocysts that are more mature. *B* shows an oocyst that has been disrupted mechanically, releasing 1 of its sporocysts. Each sporocyst contains 2 coiled, crescent-shaped sporozoites, although these are not discernible. *C* shows a free sporocyst *(bottom left)* and 2 excysted sporozoites *(top* and *right)*. *D* shows an unsporulated oocyst viewed by DIC microscopy, and *E* shows it viewed by ultraviolet fluorescence microscopy. The oocyst wall of *Cyclospora* is autofluorescent. *Isospora belli* also is autofluorescent (both oocyst and sporocyst walls), whereas *Cryptosporidium parvum* is not (not shown). (Figure courtesy of Michael J. Arrowood.)

ated by Schneider in 1881, were first described by Eimer in 1870 [6]; until the 1990s, the genus had included only species that infect animals (e.g., rodents, insectivores, and reptiles). In 1994, Ortega et al. christened the organism *Cyclospora cayetanensis,* deriving the species name *cayetanensis* from the name of the Peruvian university (Universidad Peruana Cayetano Heredia) where their principal studies had been conducted [6].

Other recent highlights of the short recorded history of *C. cayetanensis* include the following (figure 1). In 1995, trimethoprim-sulfamethoxazole (TMP-SMZ) was reported to have been effective therapy for cyclosporiasis in a double-blind, placebo-controlled trial in Nepal [18], one of the major sites for research on *Cyclospora.* In 1996, the investigation of a multistate outbreak in the United States and Canada that was ultimately linked to Guatemalan raspberries showed that *Cyclospora* is transmissible through food [22]. This fact, which had been suspected previously with regard to an outbreak [20] and individual cases of cyclosporiasis, was reconfirmed in investigations of many subsequent outbreaks (table 2). Also in 1996, phylogenetic analyses, based on the small subunit ribosomal RNA gene, showed that *C. cayetanensis* is as closely related to some species of a different genus of coccidia—namely, *Eimeria*—as some *Eimeria* species are to each other [21]. The unresolved taxonomic issues raised by these and subsequent molecular analyses [36–38] seem almost fitting, given the confusion in the last 2 decades about the identity and characteristics of this newly recognized and persistently enigmatic pathogen (table 3).

Biology of *Cyclospora* and its Effect on Epidemiology

One of the fundamental features of the biology of *C. cayetanensis* and a persistent source of confusion is the fact that *Cyclospora* oocysts in freshly excreted stool are noninfectious. The oocysts are thought to require from days to weeks outside the host, under favorable environmental conditions, to sporulate and thus to become infectious (figures 2 and 3; table 3) [5, 53]; whether the oocysts ever become infectious within a few days or in <24 h is unknown. Sporulation characteristics are one of the many differences between the coccidian parasites

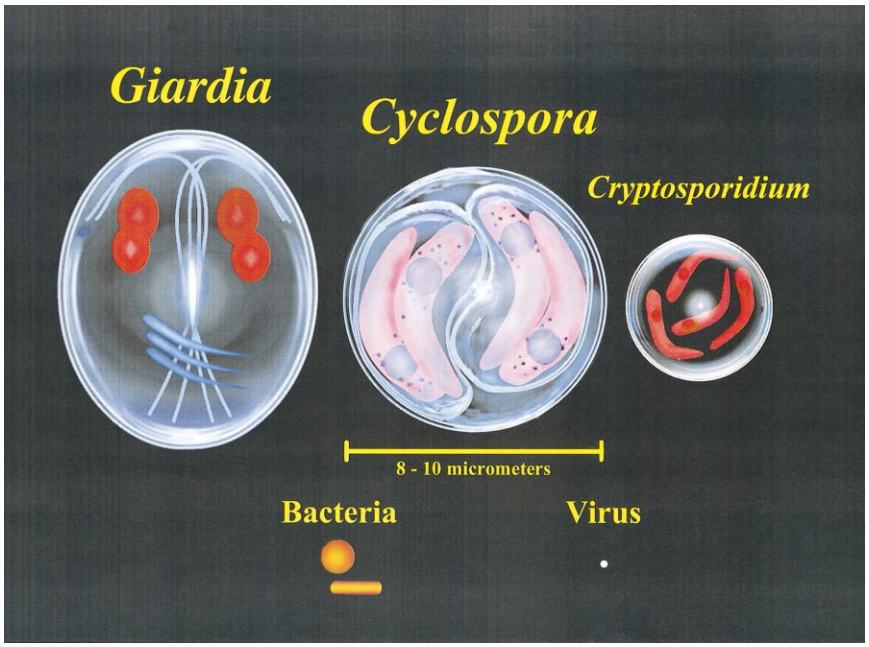

Figure 3. The relative sizes of various microbes. Three protozoan parasites are shown schematically: a *Giardia lamblia* cyst (length ranges from 8 to 19 μm and averages 11–12 μm), a *Cyclospora cayetanensis* oocyst (8–10 μm), and a *Cryptosporidium parvum* oocyst (average dimensions, 4.5 μm × 5 μm). The virus is not drawn to scale. The *Cyclospora* oocyst shown here is fully sporulated—that is, it has 2 internal sporocysts, each containing 2 sporozoites (a total of 4 infective units). Whereas oocysts of *Cryptosporidium,* another coccidian parasite, are fully sporulated and infectious when excreted, *Cyclospora* oocysts sporulate in the environment, days to weeks after excretion. *Giardia,* which is not a coccidian parasite, does not have sporocysts or sporozoites. (Figure courtesy of Dennis D. Juranek.)

Cyclospora and *Cryptosporidium,* which is excreted fully sporulated (table 1).

The biology of *Cyclospora,* particularly the need for excreted oocysts to mature to become infectious, affects its epidemiology. Direct person-to-person transmission through fecal exposure is unlikely to occur with *Cyclospora,* and food or water contaminated with freshly excreted oocysts (e.g., by a chef) shortly before consumption should not cause illness (tables1 and 3). Unfortunately, little is known about the effects of various environmental conditions on the rate of sporulation [5, 53] and on the viability of unsporulated and sporulated oocysts (table 3). The need for oocysts to survive long enough both to sporulate and to be ingested thereafter by a susceptible host suggests that oocysts probably are quite hardy, as coccidian parasites typically are. Whether *Cyclospora* is even more resistant to environmental stresses than *Cryptosporidium,* which does not undergo exogenous sporulation, remains to be determined (table 1).

A major constraint on research to address such issues is the limited supply of *C. cayetanensis* oocysts available for study. Currently, infected humans are the only known sources of these oocysts, and methods are not yet available for propagating them in culture or in animals. Research (e.g., to determine whether *Cyclospora* is susceptible to the doses of gamma irradiation permissible for food [23, 55]) has also been constrained by the lack of laboratory methods for assessing the viability and infectivity of *Cyclospora* oocysts, beyond simply whether oocysts sporulate and sporozoites excyst.

Another fundamental issue is how the environment, including water and food, becomes contaminated with *Cyclospora* and whether humans indeed are the only sources of oocysts (table 3). To date, despite some suggestive, mostly anecdotal, evidence [59–62], no natural infection of wild or domestic animals with *C. cayetanensis* per se has been unequivocally documented [43, 52], nor is there definitive evidence of successful experimental infection of any laboratory animal [51, 63]. However, some nonhuman primates have been found to be infected with parasites that are closely related to *C. cayetanensis* [37, 38]. One of the many consequences of the relative ignorance about the host range and natural ecology of *C. cayetanensis* is the difficulty in outbreak investigations of evaluating the plausibility of competing hypotheses about modes of contamination and transmission.

Another intriguing issue is the marked seasonality of *Cyclospora* infection, which varies in different settings (table 3). Although the seasonality defies simple explanation, a partial explanation might be that certain ranges of temperature, humidity, and other environmental factors permit or facilitate sporulation and survival of oocysts. In Kathmandu, Nepal, infection consistently is most common just before and during

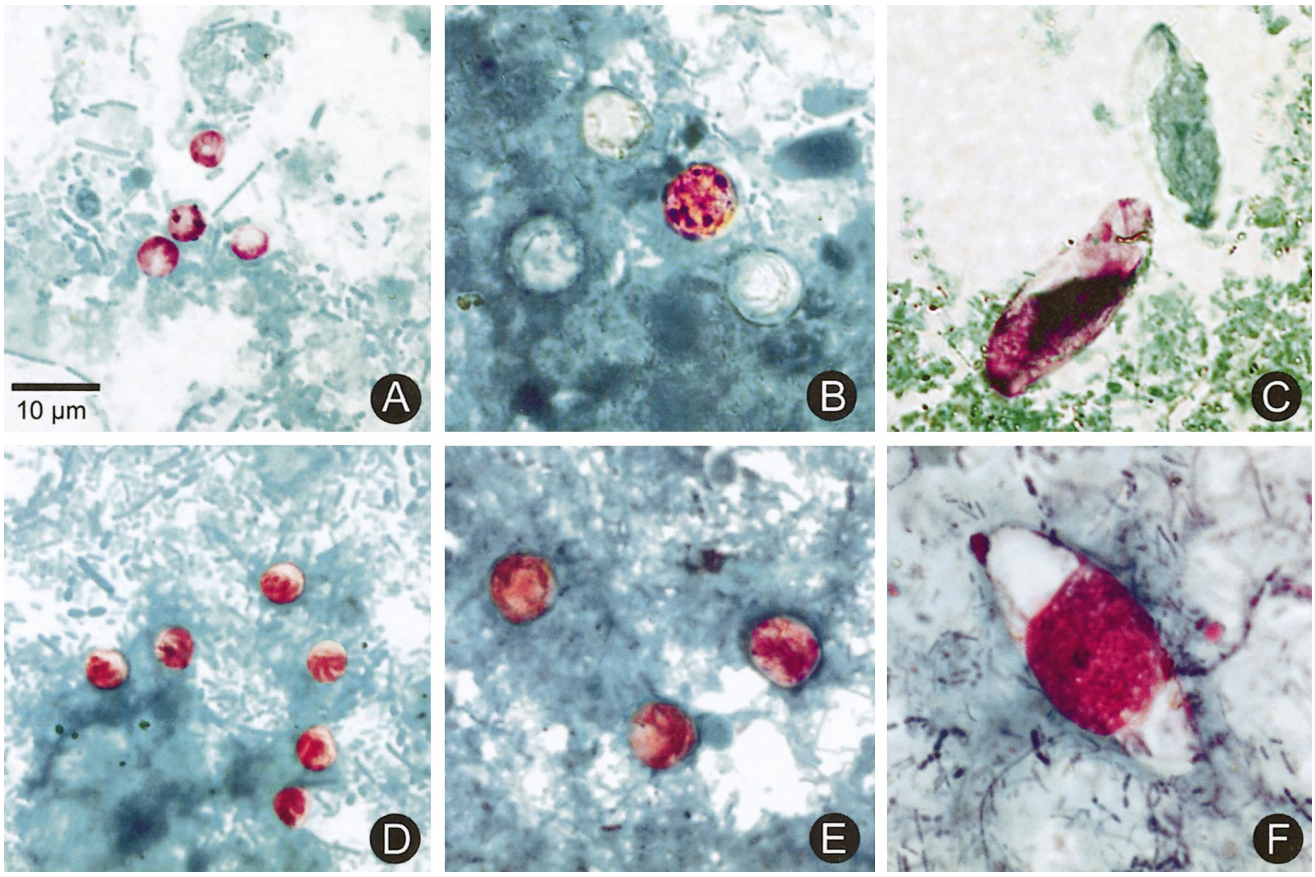

Figure 4. Coccidian parasites from stool specimens stained with modified acid-fast and modified ("hot") safranin techniques [33, 34]. The *upper photographs* show acid-fast–stained oocysts of *Cryptosporidium parvum (A), Cyclospora cayetanensis* (3 unstained oocysts and 1 oocyst that stained acid fast [*B*]), and *Isospora belli* (1 oocyst that stained acid fast [*C, bottom*] and 1 non–acid-fast oocyst that took up the counterstain [*C, top*]). Note that the various parasites differ in size, which highlights the importance of carefully measuring what is found. Note also that the walls of the unstained *Cyclospora* oocysts on the top and lower right in *B* have a hyaline or crumpled-cellophane appearance. The *lower photographs* show the coccidia in the same order in safranin-stained preparations. Note that *Cyclospora* oocysts stain more uniformly with this technique *(E)* than with acid-fast staining *(B)*. (Figure courtesy of Michael J. Arrowood.)

the warm monsoon months and decreases substantially before the rains end [11]. Cases typically are clustered from May through August and peak in June and July [46, 47, 61]. In Guatemala, 1 year of surveillance in outpatient health care facilities showed that cases peaked in June but occurred at elevated rates from May (the month the rainy season began) through August, when the temperature was intermediate, between the highs and lows for the year [44, 45]; limited data were obtained for March and April. On the basis of 1 year of data for a coastal area of Haiti, it appeared that infection was most common during the relatively drier and cooler months in the first quarter of the year [64].

In contrast, in a coastal area near Lima, Peru, that receives <2 cm of rainfall per year [58], the season for infection usually starts in December or January and extends through May and sometimes into June and July [5, 7, 58]. These months include the warmer months for this area, which are cooler than the peak months for infection in Haiti. In Indonesia, cases appear to be most common during the cooler wet season of October through May [65].

Detection of *Cyclospora* in Stool Specimens, Environmental Samples, and Food

Probably the most important thing for health care providers to realize about the diagnosis of *Cyclospora* infection is that stool specimens examined for ova and parasites usually are not examined for *Cyclospora* unless such testing is specifically requested. Even testing for *Cryptosporidium,* which also is not necessarily routine, does not always allow for detection of *Cyclospora. Cyclospora* is not detected when EIA or fluorescent antibody testing is done for *Cryptosporidium,* whereas both organisms are detectable on acid-fast–stained slides (figure 4;table 1). The use of acid-fast staining for parasitologic (vs. bac-

teriologic) applications dates back to a report in 1981 about its use for identification of cryptosporidia [35]; subsequent use of this technique in some laboratories facilitated detection of "big Crypto" (i.e., *Cyclospora*).

Another basic principle is that *Cyclospora* oocysts are easily overlooked, not only because they are relatively nondescript when unsporulated (figure 2), but also because they typically are shed in relatively low numbers, even by nonimmune ill persons. To maximize detection of oocysts, stool specimens may first need to be processed by a method that concentrates parasites [33]. The optimal number of specimens to examine has not been determined. Ultraviolet fluorescence microscopy is a useful technique for screening wet mounts of stool for *Cyclospora* oocysts, which autofluoresce and thus stand out (figure 2); in contrast, *Cryptosporidium* oocysts do not autofluoresce (table 1). If suspect structures 8–10 μm in diameter are found, bright field, phase contrast, or differential interference contrast microscopy should be used to confirm that the structures have the morphological features of *Cyclospora* oocysts (figure 2). Oocysts can also be stained with a modified acid-fast or a modified ("hot") safranin technique (figure 4) [33, 34].

In a comparative study in Guatemala that included 143 positive specimens, the estimated sensitivity of acid-fast staining versus fluorescence microscopy was 78% [66]; comparable data were obtained in a US study that included 18 positive specimens [67]. The diagnosis of *Cyclospora* infection can also be confirmed by demonstrating sporulation of oocysts (figure 2) [5] and by detecting parasite DNA with PCR, an investigational technique [21, 33, 68]. Immunologic assays for detection of antibody to *Cyclospora* in serum are being developed.

Environmental samples (e.g., water or soil) and samples of food are even more difficult to examine than stool specimens, not to mention the problem of getting the relevant samples to examine. The available microscopic and molecular techniques for detecting *Cyclospora* are substantially improving but may not yet be sufficiently sensitive to detect reliably the low levels of contamination (e.g., in food) that probably can initiate infection. One of the major challenges is to separate the oocysts from debris in the sample, without simultaneously losing most of the relatively few oocysts present. Microscopy is often labor-intensive, particularly if the sample has lots of debris, and requires a well-trained microscopist who can distinguish the morphological features of *Cyclospora* from those of impostors and artifacts (e.g., autofluorescent background material), which are even more common in environmental samples than in stool.

PCR has the potential to be more sensitive than microscopy [68], but it cannot distinguish between sporulated and unsporulated oocysts. Another issue with PCR is the need to ensure that the amplicons represent *C. cayetanensis* rather than other species of *Cyclospora* or *Eimeria; Eimeria* species are prevalent in animals and therefore in the environment. Techniques that can be used for species identification include DNA sequence analysis, restriction fragment length polymorphism (RFLP) analysis, and PCR with mismatched primers.

Geographic Distribution, Prevalence, and Clinical Manifestations of Infection

Cyclospora infection has been reported from many countries [9, 10] but appears to be most common in tropical and subtropical areas. In various laboratory surveys conducted during nonoutbreak periods in North America and the United Kingdom, the proportion of stool specimens positive for *Cyclospora* has been <0.5% [48, 69–72], which suggests that the prevalence of infection in the general population is very low. Although occasional unexplained cases of cyclosporiasis in developed countries have been reported, infection typically has been associated with international travel or consumption of imported produce. In a study of German travelers returning from developing countries, 5 (1%) of 469 persons with diarrhea were infected with *Cyclospora* and 13 (3%) with *Cryptosporidium* [73].

The investigations conducted among native populations and foreigners in developing countries have shown that the prevalence of *Cyclospora* infection and the likelihood of having symptoms, if infected, are highly variable. The reasons for the variability are poorly understood but include such factors as the study design and population (e.g., clinic vs. community based), area of the world, sanitary conditions, season, and personal attributes (e.g., age, duration of stay in the area, socioeconomic status, likelihood of prior *Cyclospora* infection, and immune competence) [5, 10, 11, 47, 58, 61, 65, 74].

For example, 1 year of surveillance in outpatient health care facilities in Guatemala showed that infection was most common (1) in the spring, (2) among children aged 1.5–9 years, and (3) among persons with gastroenteritis; 30 (19%) of 160 children in that age group who had gastroenteritis and were evaluated in May or June were infected [44]. In a 2-year, cross-sectional, community-based study in a shanty town near Lima, Peru, the prevalence of infection was highest among children aged 2 to <4 years (~2% were infected for the study period as a whole, with a higher proportion infected during the transmission season) and was 0% among persons aged >11 years, which suggests that immunity develops with repeated exposure [58]. Whereas infected children in developing countries are often asymptomatic or have relatively mild symptoms [5, 58, 65], illness typically is more severe and lasts longer among infected nonimmune adults (e.g., upper and middle class adults in Peru, foreigners in Nepal, and case patients in outbreaks in the United States) [11, 22, 51, 58, 65].

The clinical features and treatment of cyclosporiasis, which are described in table 4, have been addressed through studies in various settings (e.g., among foreigners in Nepal, HIV-infected Haitians, and case patients in outbreaks). Cyclosporiasis, which can be severe in both immunocompetent and immuno-

Table 1. What distinguishes the coccidian parasites *Cyclospora cayetanensis* and *Cryptosporidium parvum?* A list of some of their similarities and differences.

Feature	Description		Possible relevance
	Cyclospora cayetanensis	*Cryptosporidium parvum*	
Similarities			
When the first cases of infection were diagnosed in humans	In retrospect, first diagnosed in 1977 and 1978 (reported in 1979 [1]), but other *Cyclospora* species were previously known to infect animals	First diagnosed in 1974 (reported in 1976 [31, 32]), but *Cryptosporidium parvum* was previously known to infect animals	Although the first human cases were diagnosed at about the same time, much more is known and has been published about *Cryptosporidium*.[a] Little was published about *Cyclospora* before the 1990s.
Acid-fast staining of oocysts (figure 4)	Variably acid fast	Acid fast, as first described in a publication in 1981 [35]	Both parasites are detectable with this technique, but other diagnostic techniques are specific to one or the other
Number of infective units (sporozoites) per sporulated oocyst	4	4	
Completion of life cycle within humans	Yes, except for sporulation	Yes	Neither parasite requires another host to complete its life cycle
No multiplication outside the host (e.g., in water or food)	Does not multiply outside host (sporulation is not the same as multiplication)	Does not multiply outside host	In contrast to bacteria, oocysts do not multiply outside the host, no matter what the conditions are
High level of resistance of oocysts to chlorine and presumably to other halogens (e.g., iodine, bromine)	Deduced from fact that motile sporozoites excyst from oocysts held up to several hours in 100% bleach (infectivity studies have not been done)	Approximately a millionfold more resistant than *Escherichia coli* to chlorine	Both parasites can be associated with waterborne outbreaks, especially if water is not filtered
Differences			
Phylogeny [21, 36–38]	In a clade within the *Eimeria* group	Also a coccidian parasite but in a different phylogenetic group	
Size of oocyst (figures 2–4)	8–10 μm in diameter (intermediate in size between *Cryptosporidium parvum* and *Isospora belli*)	Average width of 4.5 μm and average length of 5 μm	*Cyclospora* oocysts are larger and more easily removed by conventional water filtration
Numbers of organisms in stools of symptomatic nonimmune hosts	Typically excreted in low to modest numbers	Often excreted in somewhat higher numbers	Scarcity of *Cyclospora* oocysts makes it difficult to detect infection and highlights the usefulness of methods to concentrate parasites in stool specimens
Autofluorescence of oocyst wall (by UV fluorescence microscopy [figure 2])	Yes; oocysts of *Isospora* and *Eimeria* species also are autofluorescent	No	UV fluorescence microscopy is a good way to screen stools for *Cyclospora.* Does autofluorescence indicate a characteristic of the oocyst wall that facilitates survival?
Internal morphology of sporulated oocyst: whether sporozoites are inside sporocysts (figure 3)	Each oocyst has 2 internal sporocysts, each of which contains 2 sporozoites, for a total of 4 sporozoites per oocyst	The 4 sporozoites are naked within the oocyst (i.e., not inside sporocysts)	Does the sporocyst wall provide additional protection for *Cyclospora* against stresses in the environment or stomach?
Infectivity of oocysts in freshly excreted stool	Must sporulate outside host to become infectious for next host	Fully sporulated and infectious when excreted (sporozoites can be visualized when oocysts are excreted)	*Cyclospora* is not transmissible person-to-person and must be sufficiently hardy to survive long enough both to sporulate and to be transmitted to next host
Zoonotic potential	Host range unknown, including whether animals are infected and are sources of infection for humans	Infects virtually all commonly known wild and domestic mammals; although several outbreaks among humans have been linked to animals, outbreaks associated with food and water typically have been caused by the "human" (vs. the "bovine") genotype [39]	Watersheds are more likely to be contaminated with *Cryptosporidium* than with *Cyclospora,* even without contamination by infected humans. The public health importance of the non-bovine animal genotypes of *Cryptosporidium parvum* and the non-*parvum* species of *Cryptosporidium* is not yet known. Animal models are available for the study of cryptosporidiosis but not cyclosporiasis.
Location in enterocytes of small bowel	Intracytoplasmic, within a parasitophorous vacuole in apical supranuclear region [40–42]	Intracellular, extracytoplasmic, within a parasitophorous vacuole at lumenal surface of enterocyte	Does the apparent extracytoplasmic location of *Cryptosporidium* make it less accessible to antimicrobial agents?

(continued)

Table 1. ***(Continued)***

Feature	Description		Possible relevance
	Cyclospora cayetanensis	*Cryptosporidium parvum*	
Susceptibility to antimicrobial agents	Treatment with TMP-SMZ leads to both clinical and parasitologic cure [18]	Some antimicrobial agents (e.g., paromomycin) may cause clinical improvement, but no agent has been consistently demonstrated to provide parasitologic cure	The fact that the therapy for cyclosporiasis is different than for some other parasitic diseases (e.g., amebiasis, giardiasis) makes it worthwhile to confirm the diagnosis
Direct person-to-person transmission by fecal exposure	Unlikely, because excreted oocysts must sporulate to become infective	Occurs	
No. of documented *community-wide* waterborne outbreaks in the US[b]	None	Many	Contamination of US water supplies with *Cyclospora* is probably uncommon and/or treatment barriers are effective
No. of documented *large, multistate* foodborne outbreaks in the US (table 2)[b]	Several (see text); usually, contamination of produce at source (vs. where eaten) is most likely, in part because oocysts must sporulate to become infective	None, but several relatively small and localized outbreaks have been documented; the proportion of sporadic cases attributable to foodborne transmission is unknown	Why have widespread foodborne outbreaks of cryptosporidiosis not been documented to date, especially since more contamination of the environment, including produce [43], might be expected with *Cryptosporidium*?
Seasonality of infection in Guatemala, based on 1 y of surveillance in outpatient health care facilities [44, 45]	Markedly seasonal, with most cases occurring May–August, with a peak in June	Less-pronounced seasonality	Seasonality of infection might be one of the reasons outbreaks of cyclosporiasis but not of cryptosporidiosis have been linked to the spring crop of Guatemalan raspberries
Summary of key differences	Not infectious when excreted, not transmissible person-to-person, more easily removed by water filtration, highly seasonal, associated with large foodborne outbreaks	Typically shed in somewhat larger numbers, environmental contamination from both animals and humans, associated with large waterborne outbreaks	Which, if either, organism is more resistant to environmental stresses is not yet known

NOTE. The clinical features of cyclosporiasis and cryptosporidiosis are quite similar, and individual cases of these diseases usually are indistinguishable. Both diseases can cause severe illness in HIV-infected persons. TMP-SMZ, trimethoprim-sulfamethoxazole; US, United States; UV, ultraviolet.

[a] Research on *Cryptosporidium* has been facilitated and motivated by multiple factors (e.g., documentation of more human cases, including more involving HIV-infected persons; availability of animal models; and availability of larger numbers of oocysts for study).

[b] Some outbreaks might have been missed, but the marked contrasts between these parasites with respect to the types of outbreaks that have been documented are noteworthy.

compromised persons, is treatable with TMP-SMZ [3, 18, 80], which also is effective for isosporiasis.

Waterborne Transmission

The transmissibility of *Cyclospora* through water depends on the probability that the source water of interest could become contaminated and that the water treatment, if any, would kill or remove oocysts. *Cyclospora* oocysts, like *Cryptosporidium* oocysts, probably are highly chlorine resistant (table 1), but they should be more easily removed by conventional filtration because they are about twice as big as *Cryptosporidium* oocysts (figures 3 and 4).

In June 1994, a waterborne outbreak occurred among British expatriates in a military detachment in Pokhara, Nepal (figure 1) [17]. Twelve of 14 persons became ill (unknown incubation period); specimens from 6 of 8 ill persons were positive for *Cyclospora.* The water supply was a mixture of river and municipal water, which was chlorinated and piped from a sealed storage tank to the homes in the camp. During the outbreak, structures morphologically consistent with *Cyclospora* oocysts were found in a 2-L sample of chlorinated water from the tank. Although the mode of contamination of the water was not identified, it is plausible to think that a river in a country where *Cyclospora* infection is endemic could become contaminated.

Consumption of untreated water was identified as a risk factor for cyclosporiasis in a case-control study in 1992 among travelers and expatriates at 2 outpatient clinics in Kathmandu, Nepal [47]. Although only 26 (28%) of the 93 cases were explicable by this exposure, structures morphologically consistent with *Cyclospora* oocysts were found in tap water from the home of a case patient who had drunk untreated tap water.

In the United States, several isolated cases of cyclosporiasis possibly associated with exposure to drinking or recreational water or to sewage have been reported [70, 71, 81]. The first documented US outbreak of cyclosporiasis (figure 1; table 2), which occurred in a physicians' dormitory in Chicago in 1990 [16], might have been waterborne and has raised questions about the modes of transmission of *Cyclospora* in the United States. The outbreak was detected because the clinical laboratory that examined stool specimens routinely examined acid-fast–stained slides when looking for parasites. The epidemiologists who investigated the outbreak concluded that it was waterborne and hypothesized that stagnant water in the bottom

Table 2. Documented outbreaks of cyclosporiasis in the United States and Canada in the 1990s [16, 19, 20, 22–27, 49].[a]

Month and year of outbreak[b]	Location	No. of clusters[c]	No. of cases[d]	Vehicle[e]	Source of vehicle	Comment
June or July 1990	Illinois	1	21	Water or food (if food, unidentified vehicle)		If waterborne, related to repair of water pump on 5 July; if foodborne, related to party on 29 June
May–June 1995	New York	1[f]	32	Possibly food		Fresh raspberries of unknown source were among the fruits served
May 1995	Florida	2	38	Raspberries?	See comment	Guatemala was a possible source
May–June 1996	US, Canada	55	1465	Raspberries[g,h]	Guatemala	
March–April 1997	Florida	—[i]	—[i]	Mesclun	Peru (or US)	If evidence from December 1997 outbreak also is considered, most likely source of mesclun was Peru
April–May 1997	US, Canada	41	1012	Raspberries[h]	Guatemala	
June–July 1997	Washington, D.C. area[j]	57	341	Basil	Multiple possible sources	See text about possibility that basil was contaminated locally
Sept 1997	Virginia	1	21	Fruit plate	See comment	Fruit plate might have included raspberries (non-Guatemalan) but not blackberries
Dec 1997	Florida	1	12	Salad (mesclun)	Peru (mesclun)	If evidence from March 1997 outbreak also is considered, most likely vehicle was mesclun
May 1998	Ontario	13	315	Raspberries[h]	Guatemala	
May 1998	Georgia	1	17	Fruit salad?	Undetermined	Multiple combinations of many fruits were served
May 1999	Ontario	1	104	Dessert (berry)[h]	See comment	Implicated dessert included fresh Guatemalan blackberries, frozen Chilean raspberries, and fresh US strawberries
May 1999	Florida	1	94	Probably fruit, most likely a berry[h]	See text	Multiple combinations of many fruits were served
July 1999	Missouri	At least 2	64	Basil	Mexico or US	

NOTE. US, United States.

[a] See text for further description of the outbreaks. Some of the outbreaks that are listed separately might have been related (i.e., the outbreaks in New York and Florida in 1995 and those in Florida and Ontario in 1999). Some potential outbreaks were not included in the list.

[b] If the outbreak included both cluster-related and sporadic cases, the months listed are those in which the events (e.g., parties) associated with the clusters of cases occurred. Implicated produce might have been harvested in the previous month.

[c] A cluster of cases of cyclosporiasis was defined as ⩾2 cases among persons with similar exposures (e.g., attended same party). At least 1 case per cluster had to be laboratory confirmed.

[d] The case counts are inexact because of underrecognition and underreporting of cases and partial investigations of some outbreaks (e.g., not all event attendees were interviewed). Both laboratory-confirmed and clinically defined cases are included. If the outbreak included sporadic cases that were not related to events, those are included in the case counts.

[e] The produce was served fresh.

[f] The cases were related to exposures, on various days, at a country club.

[g] Raspberries were not served at 1 event at which blackberries that reportedly were from Guatemala were served (whether the blackberries were fresh is unknown) [22].

[h] See text for discussion of the possible role of blackberries.

[i] The investigation that implicated mesclun focused on persons who ate at a restaurant in Tallahassee in mid-March (case count, 29; 14 other cases, including 2 cases involving persons who had eaten salads containing mesclun at another local restaurant, were not included in the main investigation). Other identified cases that might have been linked to mesclun and might have been related to this initial cluster of cases were associated with eating at a restaurant elsewhere in Florida in early April (case count, 5) and with being on a cruise ship that departed from Florida on 29 March (257 of 783 persons who completed a questionnaire had been "ill," 77 of 249 respondents in a case-control study had had diarrhea, and 45 of the 77 met the case definition).

[j] The outbreak occurred in the Northern Virginia–Washington, D.C.–Baltimore metropolitan area [24, 25].

of either of 2 storage tanks in the dormitory contaminated the water supply, which was municipal water, after a broken water pump was fixed on 5 July and the tanks were refilled [16]. *Cyclospora* oocysts were not detected when small-volume, post-outbreak water samples were examined by ultraviolet fluorescence microscopy.

The water in the storage tanks might have been vulnerable to contamination because the tanks were covered only with canvas and were in a penthouse area with unscreened, broken windows. However, the ultimate source of the oocysts and the means by which a water tank in a Chicago building could have become contaminated are unclear, especially given the uncertainty about whether animals are infected with *C. cayetanensis*. In addition, if the outbreak was related to fixing the water pump on 5 July, that would make the median and mode incubation periods very short—i.e., 2 days (range, 12 h to 7 days) and 1 day, respectively. Although other cases with short incubation periods have been reported [11, 22, 23, 81], the median incubation period in most foodborne outbreaks has been ~7 days [20, 22, 23, 26, 27]. Whether the median incubation period is shorter with high inocula is unknown.

An alternative hypothesis is that the outbreak was foodborne, as many subsequent US outbreaks have been (table 2), and that it was associated with a catered party held in the dormitory on 29 June. If so, the median and mode incubation periods would be 8 and 7 days, respectively. Party attendance was associated with illness, but attendance was highly correlated with consumption of tap water in the dormitory. Details about the ingredients of the food items served at the party (e.g., a fruit salad) and food-specific attack rates were not obtained

Table 3. Facts and remaining questions about *Cyclospora cayetanensis.*

Facts	Remaining questions
C. cayetanensis is a coccidian parasite (phylum Apicomplexa, family Eimeriidae) that is as closely related to some *Eimeria* species as they are to each other [21, 36–38]	Do *Cyclospora* species other than *C. cayetanensis* infect humans? How much genetic and antigenic diversity is there within *C. cayetanensis* [50], and do the variants behave differently (e.g., differ by sporulation rate, infectivity, and clinical manifestations)?
In retrospect, the first documented human cases of *Cyclospora* infection were diagnosed in 1977 and 1978 in Papua New Guinea [1]. The first documented common-source waterborne and foodborne outbreaks occurred in the 1990s (figure 1, table 2).	Was *Cyclospora* an important although unrecognized cause of human illness and outbreaks before 1977?
Except for sporulation, the parasite completes its life cycle, including asexual and sexual stages, in humans [40, 41, 51]. Although no animals have been unequivocally documented to be infected with *C. cayetanensis* per se [43, 52], some nonhuman primates are infected with closely related parasites [37, 38].	Are animals infected with *C. cayetanensis* per se, and are they sources of infection for humans?
Cyclospora oocysts in freshly excreted stool are not infectious. Thus, direct person-to-person transmission through fecal exposure is unlikely. Oocysts become infectious (i.e., sporulate) in the environment, days to weeks after excretion. The rate of sporulation, which usually takes at least 1 week under laboratory conditions, is influenced by environmental factors. For example, storage of oocysts at 4°C or 37°C (vs. at 22°C–32°C) slows sporulation [5, 53, 54]. Preliminary data suggest that oocysts do not sporulate after exposure to −20°C for 24 h or to 60°C for 1 h [54].	What is the minimum time required for sporulation (e.g., does it ever occur within a few days or even in <24 h)? Are there ranges of temperatures and humidities that are favorable for sporulation? Is the air temperature or the water temperature more important? What is the impact of fluctuating environmental conditions on the rate of sporulation?
To maintain transmission, *Cyclospora* must survive in the environment long enough both to sporulate and to be ingested thereafter by a susceptible host. A moist environment is probably more conducive to survival than a dry one [15, 54]. Methods to assess viability (beyond sporulation and excystation) are not yet available.	How long do unsporulated and sporulated oocysts survive under various natural conditions (e.g., at specific temperatures and humidities) and within feces or other hygroscopic organic matter on dry indoor surfaces (e.g., on kitchen counters)?
Cyclospora can be transmitted through food or water contaminated with either sporulated oocysts or unsporulated oocysts that have time to sporulate before consumption. Food or water contaminated with unsporulated oocysts shortly before consumption should not cause infection. No documented outbreaks have been associated with cooked or commercially frozen food.	What are the pathways by which oocyst-laden feces reach foods or water that serve as vehicles of infection for humans? What conditions would be required to kill oocysts in food or water during routine commercial handling or processing—for example, what exposure times at what temperatures, disinfectant concentrations, or doses of irradiation [55]?
Asymptomatic infection occurs and probably is most common in settings in which *Cyclospora* infection is endemic. Reinfection has been documented [42, 46, 47].	What factors affect whether infection remains asymptomatic? Does immunity develop with repeated exposure? If so, what constitutes and how effective is the immune response? How long does immunity persist in the absence of reexposure?
Shedding of oocysts, with or without symptoms, can be protracted [5, 11, 16]. The possibility that *Cyclospora* causes ascending infection of the biliary tract (e.g., acalculous cholecystitis) in patients with AIDS has been raised [56].	Does a "typhoid Mary"–type carrier state exist? Does asymptomatic shedding ever continue for many months or years? Does extraintestinal infection occur in areas adjoining the gastrointestinal tract (e.g., in the gall bladder, bile ducts, pancreatic ducts), and does it help maintain a carrier state? (*Cyclospora talpae,* which infects moles, infects the liver and bile ducts [57].)
Infection is seasonal, but the specifics of the seasonality vary around the world	Where is *Cyclospora* during "off" seasons? What "carries" *Cyclospora* to the next season (e.g., low levels of transmission, chronically infected persons, unidentified animal reservoir hosts, or forms of the organism that can resist environmental stresses) [58]? Do environmental or other triggers initiate the onset of a new season (e.g., by providing conditions hospitable for sporulation and survival of excreted oocysts)? If so, what are they? Do specific vehicles become available in the relevant season that facilitate transmission to large numbers of persons in a short period?

(P. Huang, personal communication). Seven of the 21 case patients, including at least 1 (possibly 2) with laboratory-confirmed infection, did not attend the party, whereas all recalled having consumed tap water (P. Huang, personal communication). Whether those who did not attend the party had similar food exposures elsewhere is unknown. However, relatively few cases were identified involving persons unassociated with the dormitory: 2 (2%) of 131 respondents who worked in an adjacent building met the clinical case definition (they were not included in the main study), and 2 patients in the Chicago area were identified through laboratory-based surveillance, 1 of whom was interviewed and had become ill on 8 July.

Clearly, the relative merits of the different possible modes of transmission of the outbreak are difficult to evaluate retrospectively. The uncertainty about what caused this early outbreak highlights the difficulties inherent in investigating outbreaks that are caused by poorly understood, newly recognized pathogens and that occur among persons with multiple types of exposures in common.

Documented Foodborne Outbreaks in the 1990s

General Comments

In the 1990s, at least 11 definite and probable foodborne outbreaks of cyclosporiasis, affecting at least ~3600 persons, were documented, all of which occurred in North America (table 2). The numbers of foodborne outbreaks and cases are

Table 4. Clinical characteristics and treatment of *Cyclospora* infection.

Variable	Description
Incubation period	Variable, but averages 7 days [20, 22, 23, 27]
Asymptomatic infection	Occurs and probably is most common in settings where *Cyclospora* infection is endemic
Prodromal illness	Up to several days of flu-like symptoms is common
Symptoms associated with gastroenteritis in nonimmune persons	Abrupt or gradual onset of watery diarrhea, with frequent, sometimes explosive, stools; anorexia; nausea; vomiting; abdominal bloating and cramping; weight loss, which can be substantial; fatigue; and body aches [11, 22, 47]. In a large US outbreak [22], the 4 most common symptoms were diarrhea, anorexia, fatigue, and weight loss, all of which occurred in >90% of case patients. Fever, if present, usually is low-grade [22]. Malabsorption of D-xylose and increased excretion of fecal fat occur [11, 40, 46].
Severity of illness among untreated, nonimmune persons	Usually not life-threatening but not trivial either (e.g., frequent bowel movements, substantial weight loss, remitting-relapsing symptoms, persistent fatigue); complications can include malabsorption, Reiter's syndrome,[a] and possibly Guillain-Barré syndrome [75]
Duration of symptoms among untreated, nonimmune persons	Often prolonged but ultimately self-limited, with remitting-relapsing symptoms lasting up to several weeks or months. Fatigue and malaise are typically 2 of the most persistent symptoms. In 2 clinic-based studies among infected foreigners in Nepal, duration of illness was prolonged (mean duration, 43 days [range, 4–107] in one study [11] and median duration of 7 weeks [interquartile range, 4–9] in the other study [47]). The median durations of diarrheal disease for case patients in several US outbreaks were 10 days (range, 1–60) [22], 10.5 days (range, 1–42), 11.4 days (range, 1–18) [27], 21 days (range, 1–47), and 24 days (range, 1–27) [27]; the data do not account for the fact that some persons were still ill when interviewed or had already been treated.
Duration of shedding of oocysts	Although cessation of symptoms and excretion of oocysts typically occur within a few days to 1 or 2 weeks of each other [8, 11, 47], some untreated persons excrete oocysts for >1 month after symptoms resolve [11, 16] or have symptoms for several weeks longer than oocyst excretion is documented [47]. Untreated young children (aged ⩽2.5 years; maximum of 28% had diarrhea when evaluated) in cohort studies in Peru shed organisms for a mean of 22–23 days (range, 7–70) [5]. Among treated patients, excretion of oocysts usually stops during therapy or by several days to 1 week after therapy [18, 58, 76, 80].
Histopathology	Sporozoites, after excystation from oocysts, invade enterocytes of the small bowel, where they reproduce both asexually and sexually [40, 41, 51]. Histologic abnormalities include acute and chronic inflammation, disruption of surface epithelium, partial villous blunting, and crypt hyperplasia [46, 51]. Some inflammatory changes can persist after the infection is cured [42].
Reinfection	Can occur [42, 46, 47] but appears to become less common after repeated exposure
Treatment	TMP-SMZ for 7–10 days (longer, if symptoms persist) [18]. Adult dosage: 160 mg TMP plus 800 mg SMZ (1 double-strength tablet) orally twice daily. In a double-blinded, placebo-controlled trial among Peruvian children, a 3-day course of TMP (5 mg/kg/d) plus SMZ (25 mg/kg/d) decreased the duration of oocyst excretion, but too few symptomatic children were treated to address the effect on duration of diarrhea [58].
Alternative treatments	Not yet identified (see below about ciprofloxacin for HIV-infected persons). Limited data suggest that the following drugs are ineffective [10, 11, 46, 77–79]: albendazole,[a] TMP, azithromycin, nalidixic acid, norfloxacin, tinidazole, metronidazole, quinacrine, tetracycline, and diloxanide furoate. Approaches to consider for treatment of patients who cannot tolerate TMP-SMZ therapy include observation and symptomatic treatment; use of an antibiotic whose effectiveness against *Cyclospora* is unknown or has been surmised from limited data; or desensitization to TMP-SMZ (for selected patients who require treatment, have been evaluated by an allergist, and do not have a life-threatening allergy).
Infection in HIV-infected persons	Occurs [3, 14, 41, 56, 71] and can range from asymptomatic to severe; the possibility that ascending infection of the biliary tract (e.g., acalculous cholecystitis) occurs in some patients with AIDS has been raised [56]. Therapy with TMP-SMZ (see adult dosage above) is effective [80], and secondary prophylaxis with 1 double-strength tablet thrice weekly prevents symptomatic recurrences [3, 80]. In a small randomized, controlled trial comparing oral TMP-SMZ (9 patients) and ciprofloxacin (11 patients) for treatment of and secondary prophylaxis for *Cyclospora* infection in HIV-infected Haitians, ciprofloxacin (500 mg twice daily for 7 days as therapy and thrice weekly for 10 weeks as secondary prophylaxis) was moderately effective, although not as effective as TMP-SMZ [80]. These results suggest that ciprofloxacin might be an alternative for patients who cannot tolerate TMP-SMZ. However, the results should be confirmed in other patient populations because substantial anecdotal experience among immunocompetent patients has suggested that ciprofloxacin is ineffective as therapy for cyclosporiasis.

NOTE. SMZ, sulfamethoxazole; TMP, trimethoprim; US, United States.
[a] B. Connor, personal communication.

inexact because of underrecognition and underreporting of cases, uncertainty about whether some of the documented clusters of cases were part of the same outbreak (e.g., the clusters in New York and Florida in 1995 and those in Canada and Florida in 1999; table 2), and uncertainty about the mode of transmission for some outbreaks (e.g., the 1990 outbreak in Chicago). Even so, the number of documented foodborne outbreaks caused by *Cyclospora,* including high-profile, multistate outbreaks in the United States and Canada, is unprecedented in the experience with other protozoan parasites, such as *Cryptosporidium* (table 1).

Several types of fresh produce have been the vehicles of the foodborne outbreaks of cyclosporiasis. The produce has included, but probably has not been limited to, raspberries, mesclun (a mixture of young salad greens of various types, also known as spring mix, field greens, baby greens, and gourmet salad mix), and basil. More than 1 outbreak caused by each of these vehicles has been documented, which has strengthened the evidence and conclusions. For some of the outbreaks, the vehicle or its source could not be identified. For the outbreaks for which trace-back investigations to determine the source of the produce were conducted, a foreign country was always

found to be one of the possible sources. The outbreaks highlight the fact that the supply of fresh produce in the United States has become increasingly international [82]. Unfortunately, the mode of contamination of the produce was not determined for any of the outbreaks, although the possibility that contaminated water played a role is a strong consideration for at least some of the outbreaks [22, 23, 54].

Another commonality among the outbreaks has been the complexities of the investigations, including the difficulties posed by *Cyclospora* being the etiologic agent and fresh produce the type of vehicle. For example, the long incubation period (~1 week) for cyclosporiasis leads to delays in detection of cases and outbreaks, which usually are not reported to public health officials until several weeks after the relevant exposure [23]. Such delays not only complicate epidemiological investigations but also make it unlikely that leftovers of the epidemiologically implicated fresh produce will be available for testing. Determining which produce item was the vehicle of an outbreak can be difficult because fresh produce is often served as garnishes or in other relatively inconspicuous ways that are easily overlooked or not remembered, and several types of produce are often served together (e.g., in a fruit salad).

The short shelf life and lack of brand-name recognition of fresh produce are two of the factors that complicate trace-back investigations to determine where it was grown. The limited knowledge about the biology and epidemiology of *Cyclospora* hinders evaluations of the plausibility of various modes of contamination (e.g., the plausibility that oocysts from a particular source could have sporulated and remained viable if the contaminated produce was held under particular sets of conditions until ingestion).

Definite and Possible Fresh Raspberry–Associated Outbreaks in 1995–1998

In the spring of 1995, 2 small outbreaks, which might have been related, were documented in the United States: one was associated with a country club in New York and the other with 2 social events in Florida (table 2) [19, 20]. Detection of the outbreaks was facilitated by the unusual occurrence of having stool specimens from the case patients tested by laboratories that routinely looked for *Cyclospora* when examining stool for parasites. The outbreak in New York was initially thought to be related to consumption of water from coolers on the country club's golf course [19]. However, reevaluation of the data, prompted by subsequent foodborne outbreaks, suggested that the outbreak might have been associated with fruit (R. Carter, personal communication). A specific food vehicle could not be definitively implicated, in part because members of the country club shared multiple exposures and meals, at which fruits were served in various combinations. Fresh raspberries of undetermined source were among the fruits served.

In the Florida outbreak [20], foods containing mixtures of various fresh fruits were the items most strongly associated with illness for the 2 social events that were linked to clusters of cases. The commonalities between the 2 events limited the fruits of interest to raspberries, for which Guatemala was a possible source, and strawberries. Raspberries, but not strawberries, were significantly associated with illness in univariate analyses of the data from the case-control study, which included sporadic cases from the outbreak period. Although the findings were inconclusive, in retrospect, the outbreak in Florida appears to have been a harbinger of the outbreaks in subsequent years that were associated with Guatemalan raspberries; the outbreak in New York also might have presaged the later outbreaks.

The outbreak that brought cyclosporiasis to prominence in North America and that definitively established that *Cyclospora* is transmissible through food occurred in the spring of 1996 in the United States and Canada and was linked to a third country, Guatemala, which was the source of the implicated fresh raspberries (table 2) [22]. Probably not coincidentally, New York and Florida (i.e., states with laboratorians experienced at detecting *Cyclospora*) were the first to report cases in the outbreak; in mid-May, the Centers for Disease Control and Prevention (CDC) was informed of sporadic cases in these states. At the end of May, clusters of cases related to events in Texas and Canada were reported to the CDC.

Although the true size of the outbreak is unknown, ultimately 1465 cases were reported to the CDC, which was more cases of cyclosporiasis than had ever previously been documented in the world. The cases were reported by 20 states, the District of Columbia, and 2 Canadian provinces. Approximately half of the cases ($n = 725$) were cluster related; 55 clusters (i.e., mini-outbreaks), associated with events from 3 May through 14 June, were reported. The other half of the cases ($n = 740$) were sporadic cases that appeared to be outbreak related.

Having 55 event-related clusters to investigate made it possible to look for commonalities concerning what was served at the various events and thus to overcome the limitations of individual clusters. Raspberries definitely or probably were served at all of the events except 1, at which blackberries that reportedly were from Guatemala were served (table 2). In addition, consumption of raspberries was strongly associated with cyclosporiasis, both in retrospective cohort studies of clusters of cases and in case-control studies of sporadic cases [22, 83, 84]. The trace-back data implicated Guatemala as the source of the raspberries, even though most of the raspberries shipped in the United States during the outbreak period were from elsewhere [22, 23]. Raspberries had been introduced into Guatemala in the late 1980s as an export crop, and the volumes exported to the United States markedly increased in the mid-1990s [83]. Most of the 1996 spring crop went to North America.

The lack of commonalities in the distribution system for the implicated raspberries (e.g., in US ports of entry) suggested that the berries were contaminated in Guatemala, probably on

multiple farms (no single farm could account for the entire outbreak) with a common practice or attribute. Although the specific mode of contamination is unclear, the mode would need to be able to account for contamination of large numbers of berries because the outbreak lasted well over a month and the cases were widely distributed geographically. Given that raspberries generally are kept cool after they are picked, which would retard sporulation, and have a relatively short shelf life, the raspberries probably were contaminated with oocysts that already had sporulated or were well along in the process of sporulating. The leading hypothesis is that the raspberries were contaminated through exposure to water, specifically when the berries were sprayed with insecticides, fungicides, and fertilizers that were mixed with water.

In this scenario, if humans are the only sources of the organism, then, presumably, infected persons either indirectly or directly contaminated the water, which ultimately led to contamination of the raspberries. In fact, surveillance from April 1997 through March 1998 in outpatient health care facilities in Guatemala showed that the seasonality of human cyclosporiasis overlapped with Guatemala's spring export season for raspberries; in contrast, cases of cryptosporidiosis were less seasonal (table 1) [44, 45]. Four of 164 workers from 3 raspberry farms who were tested in April and May 1997 were found to be infected with *Cyclospora,* 3 of whom were asymptomatic [44].

Research to explore possible modes of contamination has been constrained by the difficulties entailed in detecting low levels of *Cyclospora* and by the limited numbers of samples (e.g., of water, soil, and berries, and from farm workers and animals) from Guatemalan farms that have been available for testing. Given the short shelf life of fresh produce, the long incubation period for cyclosporiasis, and the delays inherent in diagnosing and reporting cases of infection, leftover raspberries from the various events attended by the case patients generally were unavailable for testing when the clusters of cases were detected. Thus, Guatemalan raspberries were implicated as the vehicle of the outbreak on the basis of epidemiological and trace-back evidence, rather than on the basis of detection of contaminated raspberries.

By the time Guatemalan raspberries were definitively implicated, Guatemala's spring 1996 export season had already essentially ended. During the subsequent fall and winter (i.e., the next major export season for Guatemalan raspberries), no outbreaks of cyclosporiasis were documented. To prepare for the 1997 spring export season, the Guatemalan Berry Commission instituted control measures on farms, which focused on improving employee hygiene, sanitation, and the quality of water used in agriculture. The Commission specified that only farms they classified as low risk in such regards could export fresh raspberries to the United States in the spring (starting 22 April 1997) [23].

Despite the control measures, another multistate, multicluster outbreak associated with Guatemalan raspberries occurred in April and May 1997 [23]. This suggests that some farms did not fully implement the control measures or instituted them too late, or that the measures were ineffective or not directed against the true mode of contamination. The 1996 and 1997 outbreaks were remarkably similar with respect to when they occurred, the numbers of reported cases and clusters, and the fact that cases were widely distributed geographically (table 2). Although the bulk of the cases in the 1997 outbreak were associated with events held in May, some case patients had been exposed in early April to raspberries grown in late March. The fact that the rainy season in Guatemala in 1997 did not begin in earnest until May or June, depending on the area, indicates that the onset of the outbreak was not dependent on heavy rainfall. The outbreak ended shortly after exportation of fresh Guatemalan raspberries was voluntarily suspended at the end of May 1997, despite the continued availability of raspberries from other sources. The US Food and Drug Administration (FDA) allowed shipments of raspberries to resume in mid-August 1997.

In the spring of 1998, the FDA did not permit importation of fresh Guatemalan raspberries into the United States, whereas importation into Canada continued. Thus, an intervention trial was inadvertently conducted. The outcome was that a multicluster outbreak linked to Guatemalan raspberries occurred in Ontario, Canada, thus continuing the pattern of consecutive years of springtime raspberry-associated outbreaks (table 2) [26].

This series of outbreaks not only conclusively established that *Cyclospora* is transmissible by food but also provided other insights. Having many cases and clusters of cases to investigate provided opportunities to build on the foundation laid by the clinical studies conducted in Nepal [11, 47] and to define further the clinical manifestations and incubation period of cyclosporiasis (table 4). The high attack rates for illness that were noted, sometimes despite consumption of few raspberries (e.g., a lemon tart garnished by 1 raspberry), suggested that the infective dose of oocysts is low or the number of oocysts per berry was high or both. A human-volunteer study is being conducted by investigators at the CDC and the University of North Carolina at Chapel Hill to determine, among other things, the infective dose of *Cyclospora.* The fact that some of the raspberries that were eaten reportedly had been washed [22, 23] suggests that washing produce does not eliminate—although it may reduce—the risk of acquiring *Cyclospora* infection. The fact that some *Cyclospora* oocysts remain on produce after washing has been confirmed with experimentally contaminated lettuce [43].

Other Fresh Fruit–Related Outbreaks in 1997–1999

Other fruit-related outbreaks of cyclosporiasis, which were unrelated to Guatemalan raspberries but for which the specific

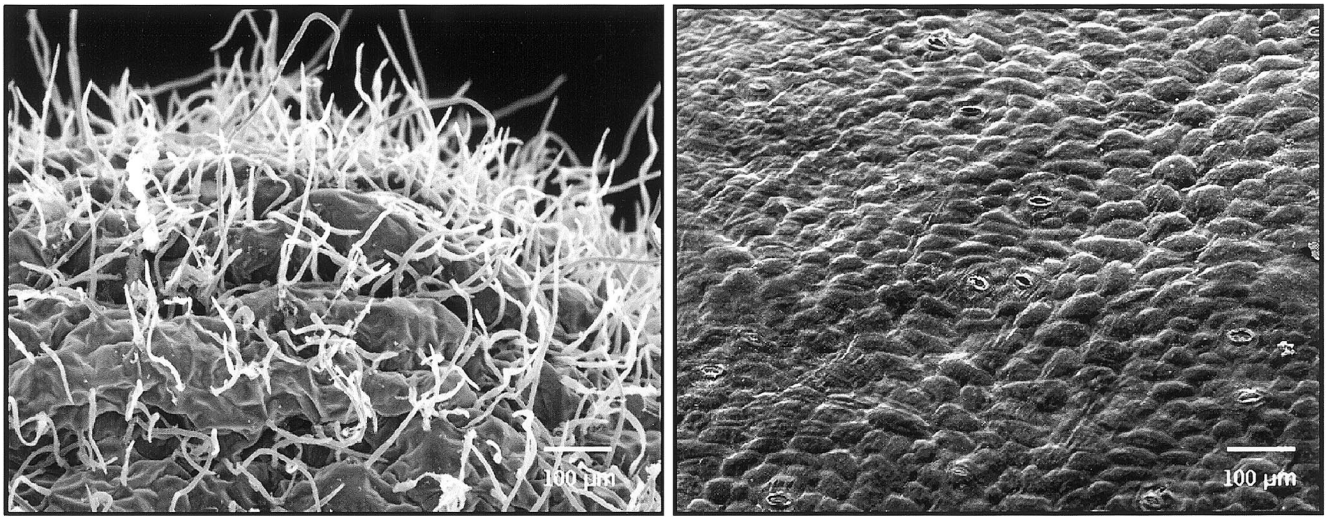

Figure 5. The contrast between the surface of a raspberry *(left)* and a blackberry *(right),* as viewed by scanning electron microscopy. Note the uneven surface of the raspberry, replete with crevices and hairs. (Figure courtesy of the Seattle District of the US Food and Drug Administration and the Fred Hutchinson Cancer Center; the photograph of the raspberry is reprinted with permission of Academic Press, London [85].)

vehicles were unclear, were documented in North America in 1997, 1998, and 1999 (table 2). The outbreak investigations were inconclusive because mixed-fruit items had been served.

An outbreak at an inn in Virginia in late September 1997 is the only outbreak to date to have been documented during this time of year. Illness was associated with a fruit plate, which probably included raspberries, which might have been imported but not from Guatemala. Thus, the outbreak was caused either by non-Guatemalan raspberries or by a fruit other than raspberries or blackberries.

An outbreak at an establishment in Georgia in mid-May 1998 is of interest because it occurred during the spring that the United States did not import Guatemalan raspberries (importation of Guatemalan blackberries continued). Although no specific vehicle was implicated, the investigators concluded that some type of fruit most likely was to blame (J. Koehler, personal communication). Multiple combinations of many fruits were served at several meals and snacks on the day of the event. Raspberries and blackberries of undetermined sources were among the fruits served.

Two outbreaks in May 1999 in Ontario, Canada (end of May), and in Florida (mid-May) might have been related to each other and probably were associated with consumption of fruit. During the spring of 1999, both Canada and the United States imported Guatemalan blackberries. Canada did not allow importation of fresh Guatemalan raspberries; the US FDA permitted importation from several farms that met stricter standards than had been used in the past. The implicated vehicle for the outbreak in 1999 in Canada was a dessert that included fresh Guatemalan blackberries, frozen Chilean raspberries, and fresh US strawberries [49]. The evidence that the blackberries caused illness (i.e., they were served fresh, were the berries most strongly associated with illness in multivariate modeling, and were from Guatemala) was suggestive but not conclusive, given that the dessert included multiple types of berries.

The investigation of the outbreak in Florida was complicated by the fact that the outbreak was associated with a multiday, multimeal convention at which many different combinations of fresh produce were served often. The investigation showed that the vehicle probably was some type of fruit, most likely a berry. The berries that were served included non-Guatemalan raspberries (both foreign and domestic sources were possible), imported blackberries (Guatemala was one of the possible sources), strawberries, and blueberries.

The possibility that blackberries rather than raspberries caused illness was also raised for some of the cases in the 3 outbreaks in 1996–1998 that were attributed to raspberries, particularly for the 1 cluster (of 55) in the 1996 outbreak that was associated with an event at which raspberries were not served [22]. For some of the clusters in all 3 outbreaks, blackberries and raspberries were served together and thus were difficult or impossible to distinguish in the analyses [22, 23, 26]. Therefore, it was noteworthy that no large outbreak linked to Guatemalan blackberries was uncovered in the United States in the spring of 1998, when Guatemalan raspberries were not being imported. Unfortunately, the vehicle of the small outbreak in May 1998 in Georgia was not determined. Overall, the evidence concerning Guatemalan blackberries from the outbreak investigations in the 1990s, although suggestive, was not as strong as that against raspberries, despite the facts that Guatemala exports more blackberries than raspberries and some farms grow both berries. Whether and how the different structures of the berries (figure 5) [86] or other factors influence the

risk for contamination with *Cyclospora* or for its adherence to the produce is unknown.

Outbreaks in 1997 Associated with Fresh Mesclun

Not all of the US outbreaks of cyclosporiasis have been linked to berries or other fruits. Mesclun is one of the other vehicles associated with outbreaks. Multiple outbreaks probably caused by mesclun occurred in Florida in 1997, including at least 1 outbreak that began in March and another that occurred in December (table 2).

For the first outbreak, the investigation focused on persons who ate at a particular restaurant in Tallahassee in mid-March. Other identified clusters of cases that might have been related to the outbreak in Tallahassee were associated with eating at another restaurant elsewhere in Florida in early April and with being on a cruise ship that departed from Florida in late March. Mesclun was implicated in the investigation in Tallahassee, but its source could have been either Peru or the United States. For the outbreak in early December, which was associated with a catered dinner in Orlando, the investigation implicated a salad that included mesclun. Although which of the salad ingredients was to blame could not be determined, the trace-back investigation of the mesclun was straightforward and led solely to Peru. If the evidence from the March and December outbreaks is combined, then the vehicle of both outbreaks most likely was mesclun and its source most likely was Peru. The timing of the outbreaks, particularly the one(s) in March and April, is consistent with the seasonality of cyclosporiasis in Peru [5, 7, 58].

Outbreaks in 1997 and 1999 Associated with Fresh Basil

Two outbreaks linked to fresh basil have been documented (table 2), including 1 that occurred from mid-June through mid-July 1997 in the Northern Virginia–Washington, D.C.–Baltimore metropolitan area [24, 25] and another that occurred in late July 1999 in Missouri [27]. Thus, these outbreaks occurred somewhat later in the year than the outbreaks linked to raspberries.

For the 1997 outbreak, where and how the basil became contaminated are unknown [24, 25]. However, this was the only outbreak of cyclosporiasis to date for which the possibility of contamination by a local US food handler was a serious consideration. In general, consumption of food contaminated with unsporulated oocysts does not cause infection if the food is eaten before the oocysts have time to sporulate. However, features of this outbreak made local contamination at least a possibility.

First, despite intense media attention, all cases in this large, multicluster outbreak (table 2) were linked to a particular chain of gourmet food stores, even though it received only ~10% of the distributor's basil, which came from multiple international and domestic suppliers and went to many other customers. Second, there were opportunities for contamination to occur locally because the implicated basil was intensely handled (e.g., when the leaves were removed from the stems and pesto sauce was made); multiple food-handlers were ill, including 1 who became ill (unknown cause) before the outbreak began. Third, oocysts might have had sufficient time thereafter to sporulate because the interval from when the basil was handled until it was eaten averaged at least several days (and included time at room temperature), which is a longer-than-usual time interval for fresh produce–related outbreaks. It is not known whether this interval, which is somewhat shorter than documented sporulation times under laboratory conditions [5, 53], was sufficiently long for sporulation to occur and whether the food ingredients (e.g., in pesto sauce) could have accelerated sporulation. An alternative hypothesis is that the basil was contaminated before it reached the company but that all of the documented cases were linked to the company because its food-handling practices (e.g., suboptimal refrigeration) facilitated completion of sporulation.

The basil implicated in the outbreak in Missouri in 1999 probably was not contaminated locally [27]. The 2 main clusters of cases in the outbreak were associated with parties catered by establishments in different counties, and the implicated food items were eaten relatively soon after they were prepared. The 2 possible sources of the basil were a Mexican farm and a US farm. Different Mexican farms were among the possible sources of the basil in the 1997 outbreak.

Although *Cyclospora* has occasionally been detected on various types of produce in various countries [12, 43, 61], the outbreak in Missouri in 1999 was the first US outbreak for which *Cyclospora* was found in an epidemiologically implicated food item. Typically, food items containing fresh produce are no longer available for testing when investigations begin. In this outbreak, *Cyclospora* was detected by both microscopy and PCR in frozen leftovers from one of the parties [27].

Conclusions

Certain questions naturally follow from discussion of the outbreaks of cyclosporiasis, particularly the foodborne ones. The issues include why the outbreaks were first documented recently (figure 1); why they have been documented in North America but, to date, not in Europe [87]; why they have been linked repeatedly to particular types of fresh produce; and why foodborne outbreaks of comparable scale have not been documented yet with the related parasite *Cryptosporidium parvum* (table 1).

Only partial answers to these questions are known, some of which have already been discussed briefly. The timing and location of the documented outbreaks reflect factors related to both the occurrence and the recognition of outbreaks. When

and where the outbreaks have occurred reflect in part the increased importation of fresh produce in general [82] and the exportation trends and patterns for the implicated produce in particular (e.g., for Guatemalan raspberries, as discussed above). Although some outbreaks presumably have gone unnoticed, the outbreaks documented in the United States were recognized because of such factors as the availability of improved techniques for examining stool specimens and the increasing awareness that cyclosporiasis should be included in the differential diagnosis of protracted gastroenteritis and that documented cases should be reported to health officials [26].

Whether certain types of fresh produce are more likely than others to be associated with outbreaks and whether the physical structure or other characteristics of the produce are important are unknown. Similarly, the reasons most of the documented US outbreaks of cyclosporiasis have been foodborne whereas most of the recognized outbreaks of cryptosporidiosis have been waterborne are poorly understood (table 1). Overlap between the seasonality of human infection where the produce was grown (e.g., of *Cyclospora* vs. *Cryptosporidium* infection in Guatemala) and the growing and harvest seasons for the implicated produce may be important.

This series of "why?" questions naturally leads to "what next?" questions. Will more outbreaks of cyclosporiasis, perhaps linked to additional fruits, vegetables, and herbs, be documented in the near future? Will the modes of contamination of the food be identified and effective prevention measures implemented? The occurrence of the past outbreaks and concern about the potential for future outbreaks underscore the need for better understanding of the biology and epidemiology of *C. cayetanensis*, an enigmatic and seemingly unlikely foodborne pathogen.

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A Supplemental Reading List appears in the electronic version of this article, immediately after the References.

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Note Added in Proof In the spring of 2000, 2 social events held in different states in the United States were each associated with a cluster of cases of cyclosporiasis. For one event, the implicated food item contained raspberries but not other produce. For the other event, no food item could be implicated, but a dessert that included raspberries in a mixture of berries could account for most of the cases. A particular farm in Guatemala was one of the possible sources of the raspberries that were served at the 2 events and was the only possible source common to both events.