PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 36, No. 1, p. 18–32

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864

Brent Dixon,* Biljana Mihajlovic, Hélène Couture and Jeffrey M. Farber

Bureau of Microbial Hazards, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada

Qualitative Risk Assessment: *Cyclospora cayetanensis* on Fresh Raspberries and Blackberries Imported into Canada

ABSTRACT

Cyclospora cayetanensis is a protozoan parasite of the human small intestine that may cause diarrheal illness. In Canada and the U.S., foodborne outbreaks of cyclosporiasis have been identified annually since 1995 and have been associated largely with the consumption of imported fresh produce. Epidemiological and traceback studies implicated imported fresh raspberries and blackberries as the sources of the early outbreaks in North America. While many other commodities have been associated with outbreaks of cyclosporiasis in recent years, imported fresh berries continue to be a concern. This qualitative risk assessment discusses the biology and epidemiology of *C. cayetanensis*, potential exposure to the parasite in Canada through the consumption of imported fresh raspberries and blackberries, the severity of symptoms and available diagnostic methods and treatments, methods for food testing, and control methods for minimizing contamination of fresh produce and the potential for foodborne transmission to humans. This risk assessment, and the information gaps with respect to contamination of these berries and the risk to consumers in Canada, may be used in the development of future policies and risk management decisions, as well as in project planning.

INTRODUCTION

Cyclospora cayetanensis is a protozoan parasite of the human small intestine that was first described in 1993 *(79)*. While *C. cayetanensis* is thought to be primarily a waterborne parasite, foodborne outbreaks associated with it have been reported in the U.S. and Canada since the mid-1990s. Epidemiological and traceback studies have clearly implicated imported fresh raspberries as the source of some of the early outbreaks in Canada *(44)*. Also, some epidemiological evidence has linked illnesses in Canada to imported fresh blackberries *(42, 44, 64)*.

This paper follows Codex principles and guidelines for the conduct of microbiological risk assessments. The primary purpose and scope of this paper is to qualitatively

Author for correspondence: Phone: +1 613.957.0904; Fax: +1 613.941.0280; E-mail: Brent.Dixon@hc-sc.gc.ca

assess the health risks associated with *C. cayetanensis* to consumers in Canada, as related to consumption of imported fresh raspberries and blackberries. This includes the determination of information gaps with respect to contamination of these berries in exporting countries and of the risk to consumers in Canada.

HAZARD IDENTIFICATION

Biology of *Cyclospora cayetanensis*

The organism responsible for cyclosporiasis was first recognized as a cause of human illness in New Guinea in the late 1970s. This unidentified human pathogen was later described in travelers to Haiti and Mexico and, in a number of subsequent reports, in travelers and inhabitants in endemic regions around the world in the 1980s and early 1990s *(76)*. The causative agent was finally identified as a coccidian protozoan parasite and was named *Cyclospora cayetanensis* in 1993 *(79)*. While humans appear to be the only host for *C. cayetanensis,* other *Cyclospora* species exist in a variety of mammals, reptiles and myriapods *(76)*. Molecular taxonomy has placed *Cyclospora* within the same family as *Eimeria (84, 92)*.

The life cycle of *C. cayetanensis* is similar to that of other coccidians and has been described in detail elsewhere *(63, 76)*. The transmission stage of *C. cayetanensis*, known as an oocyst, is spherical and 8–10 µm in diameter. Unsporulated (immature) oocysts are shed into the environment with the feces of infected individuals. While the oocysts do not multiply outside the host, they do undergo sporulation, depending upon conditions such as temperature and humidity, becoming mature and viable within 7 to 15 days *(76)*. Unsporulated oocysts in fresh feces have simply a granular (amorphous) cytoplasm and are not infective, while sporulated (infective) oocysts, which have been outside the host for a week or longer, contain two oval shaped sporocysts, each containing two sporozoites *(77, 79, 108)*.

After being ingested, oocysts pass through the stomach and into the small intestine, where they excyst, and the individual sporozoites are released and enter cells lining the gut. Sporozoites then undergo asexual multiplication through several generations, resulting in very large numbers of merozoites, which leave the cell and invade other host cells. Some merozoites develop into male and female gametes that undergo sexual reproduction, producing unsporulated oocysts.

Modes of transmission

While the modes of transmission of cyclosporiasis are not yet fully understood, many of the earlier outbreaks were thought to have resulted from the consumption of fecally-contaminated drinking water. Sturbaum et al. *(107)* demonstrated the potential for transmission as a result of contamination of drinking water or irrigation water with wastewater. The waterborne route continues to be an important mode of transmission, even in chlorinated sources, in endemic countries such as Peru and Nepal *(90)*. Person-to-person transmission (the fecal-oral route) is considered unlikely because of the relatively long sporulation period required before oocysts become infective. Zoonotic transmission has also been suggested, and *Cyclospora*-like organisms, and/or organisms with positive polymerase chain reactions (PCR) for *Cyclospora*, have been observed in the feces of chickens, ducks, dogs, monkeys, chimpanzees and baboons *(18, 61, 76)*. However, to date, there is little more than anecdotal evidence for this method of transmission to humans. Eberhard et al. *(34)* reported that none of the 327 domestic animals tested (including pigs, cattle, horses, goats, dogs, cats, guinea pigs, chickens, ducks, turkeys and pigeons) in Haiti had *Cyclospora*-positive stools, despite living near infected humans. This study suggested that domestic animals do not act as reservoir hosts for *Cyclospora*. Lopez et al. *(61)* reported that baboon-associated *Cyclospora*-like organisms are distinct from, but very closely related to human *Cyclospora*, on the basis of molecular characterization. Eberhard et al. *(33)* reported that *Cyclospora* in baboons, green monkeys and colobus monkeys are all distinct species.

Before 1996, most infections with *Cyclospora* in North America occurred in travelers, and only three small outbreaks had occurred in the U.S. *(43)*. Since then, numerous foodborne outbreaks and cases of cyclosporiasis have been reported in Canada and the U.S. *(9, 22, 25, 26, 28, 38, 42, 43, 44, 76, 86)*. An estimated 90–99% of cyclosporiasis cases in the U.S. are foodborne *(66, 97)*.

Epidemiology

C. cayetanensis has been identified as a cause of diarrheal illness in natives of and travelers to North, Central, and South America, the Caribbean, Southeast Asia, Eastern Europe, U.K., India, and Africa *(76)*. Most of the prevalence data, however, comes from studies done in the endemic regions of Nepal, Haiti, Peru and Guatemala *(11, 76)*. In endemic countries, susceptibility is restricted to the very young and the very old, suggesting that acquired immunity may play a role *(76)*. While most people in developed countries are susceptible to infection, those at greatest risk of infection with *C. cayetanensis* include travelers to endemic regions and immunocompromised individuals.

Bern et al. *(4)* reported that 2.3% of 5,552 surveillance specimens taken from farm workers and family members in Guatemala over a one-year period tested positive for *Cyclospora*. The highest prevalence (6.7%) was observed in June. Among 182 raspberry farm workers and family members examined during April and May, 1997, 3.3% had *Cyclospora* infection. Based on the same sample set, Bern et al. *(5)* confirmed that the prevalence of *Cyclospora* among outpatients in Guatemala was highest in the warmer months, peaking in June, and that cyclosporiasis affected more children than adults. Another study on the prevalence of infection with *C. cayetanensis* among three populations at risk in Guatemala failed to detect oocysts in the stools of raspberry farm workers *(85)*. In Haiti, one study reported that 34% of patients with chronic diarrhea who sought care at an AIDS clinic in Port-au-Prince were infected with *C. cayetanensis (91)*. In another study of HIV-positive adults with chronic diarrhea, 11% had cyclosporiasis *(81)*. Lopez et al. *(59)* reported peak prevalences of infection in both adults and children in a community in Haiti during the month of February.

Two prospective cohort studies were done in Peru (Lima) during 1988–1991. The first study involved children from 1 to 2.5 years of age and reported an 18% prevalence of *Cyclospora* infection, while in the second study, on children from 1 month to 1.5 years of age, the prevalence was 6% *(79)*. A cross-sectional study done in Lima demonstrated that the highest prevalence (2%) was in children 2 to 4 years of age *(62)* and that the prevalence peaked at 3 to 4% in children during the summer months. None of the adults tested in this study were infected with *Cyclospora*.

Several epidemiological studies have been performed in Nepal, where *Cyclospora* is considered endemic *(76)*. In a large study, *Cyclospora* infections were reported in 11% of travelers and expatriates having gastrointestinal symptoms *(47)*. A well documented waterborne outbreak of diarrheal illness associated with *Cyclospora* occurred among British soldiers and dependents in Pokhara, Nepal in 1994 *(90)*.

In a number of surveys of stool samples submitted to clinical laboratories in the U.S. and the U.K., *Cyclospora* was identified in 0.1–0.5% of samples *(108)*. From 1993 to 1998, only 44–66 laboratory reports of *C. cayetanensis* were made in England and Wales each year, with most cases occurring in June and July *(13)*. In the U.S., from 1997 to 2009, a total of 370 laboratory-confirmed cases of cyclosporiasis were reported *(41)*. Approximately 70% of these cases were in residents of Connecticut and Georgia. Positive stool specimens were collected in all months of the year, with a peak in June and July. Approximately half of the cases reported during 2004–2009 were associated with international travel, known outbreaks, or both.

In Canada, Brennan et al. *(6)* reported an estimated prevalence of 0.5 cases per 1,000 stool examinations, based on a survey of 80,000 stool samples submitted to three parasitology laboratories in Ontario between June 1993 and June 1995. In an analysis of reported cases in Ontario, the provincial public health laboratory found an average range of 0 to 10 cases per quarter (three-month period) each year starting in 1994, with the exception of known outbreak periods (58). In 1996, 1997 and 1998 where there were documented outbreaks, the number of cases in the second quarter, April to June, was as high as 110 cases. Cyclosporiasis became a notifiable disease in Canada in 2000. The annual number of cases of cyclosporiasis, and the rates per 100,000, reported in Canada from 2000 to 2012 are shown in *Table 1 (12)*. In a recent publication on the burden of foodborne illness in Canada, Thomas et al. *(109)* reported that, while 71% of cyclosporiasis cases are travel related, an estimated 2,450 cases per year are domestically acquired foodborne illnesses.

The first outbreak known to originate in North America occurred at a hospital in Chicago in 1990. The outbreak was linked to a contaminated roof top water tank that supplied drinking water to staff *(48, 114)*. Two more outbreaks occurred in the U.S. during the spring and summer of 1995 *(50, 108)*. One occurred in New York and was originally thought to be waterborne, although it is now apparent that berries may have been involved. The second outbreak was associated with the consumption of fresh produce in Florida.

As summarized by Herwaldt et al. *(43)*, a total of 1,465 cases of diarrheal illness due to *Cyclospora* infection were reported in the U.S. and Canada during May and June of 1996. Cases were distributed across 20 states, the District of Columbia and two Canadian provinces (Ontario and Quebec, representing 13% of the total number of cases). The only exposure that was consistently epidemiologically linked to *Cyclospora* infection was fresh raspberry consumption. Traceback studies of 29 events for which adequate information was available, found the country of origin in every case was Guatemala. The only event in which raspberries were definitely not served was an event in Ontario where Guatemalan blackberries were part of a mixed fruit dish *(43, 64)*. In response to the 1996 outbreak, the Guatemalan Berry Commission stipulated that only farms classified as "low risk" could export fresh raspberries to North America, starting on April 22, 1997. These farms implemented more stringent control measures for improving sanitation, water quality and employee hygiene. Specifically, these measures included the use of microbiologically filtered water for pesticide application and for washing of hands and equipment; monitoring programs for intestinal infections in farm workers; mandatory use of toilets and handwashing stations on farms; improved identification of shipments for easier traceback, etc. *(26)*. Despite these efforts, 1,012 cases of diarrheal illness due to cyclosporiasis were again reported in the spring of 1997 in the U.S. and Canada *(44)*. Fresh raspberries were the only food common to all events and epidemiologically linked to cyclosporiasis. Traceback investigations of 31 of 33 events, for which there was good traceback information available, demonstrated that the raspberries definitely (eight events) or possibly (23 events) came from Guatemala. For two events, the raspberries were traced back to one Chilean and one Californian source. The one Canadian cluster, which occurred in Ontario, did not show a link to raspberries, but suggested a link to blackberries.

Early in March, 1997, clusters were linked to meals at restaurants in Florida, and on a cruise ship in which there were 220 cases, including eight Canadians. The suspect food in all cases was mesclun (mixed greens) traced to Peru and Arizona. A separate outbreak during June and July, 1997, comprised of at least 25 clusters and approximately 185 cases,

Table 1. Annual number of cases of cyclosporiasis, and rates per 100,000, reported in Canada from 2000 to 2012 *(12)*

Year[a]	Reported cases[b]	Rate (per 100,000)[c]
2000	28	0.12
2001	71	0.30
2002	81	0.34
2003	78	0.32
2004	137	0.43
2005	208	0.65
2006	157	0.48
2007	175	0.53
2008	151	0.45
2009	222	0.68
2010	214	0.65
2011	141	0.42
2012	109	0.32
Total	**1,772**	–

[a]Cyclosporiasis was added to the Canadian notifiable disease list in 2000.

[b]Some provinces/territories did not report cases during certain years.

[c]Whenever provinces/territories did not report cases, the population of those provinces/territories were removed for rate calculations.

occurred in the Washington, D.C. area and was associated with eating basil products sold by a gourmet food store *(86)*. It was subsequently determined that a local food handler was infected and may have been responsible for contamination of the basil.

In the spring of 1998, Canada allowed the importation of fresh Guatemalan raspberries from "low risk" farms after a team was sent to Guatemala to observe the farm practices. On June 2, the Toronto Public Health Department was notified of a confirmed case of cyclosporiasis linked to an event at a hotel in early May *(110)*. By the end of the subsequent investigation, a total of 192 cases of cyclosporiasis were identified in southern Ontario, primarily in the Toronto area. Fresh raspberries were the only food item common to all 13 events and were epidemiologically linked to cyclosporiasis. Traceback investigations linked the raspberries to exporters in Guatemala. In June, 1998, Guatemala voluntarily stopped shipping fresh raspberries to Canada, and in September, 1998, the Canadian Food Inspection Agency (CFIA) imposed an import restriction on these berries. This restriction remained in effect until December 1999, when the CFIA lifted the import restriction for the fall crop only.

In the spring of 1998, the U.S. restricted the importation of fresh raspberries but allowed the importation of fresh Guatemalan blackberries. These two types of berries are often grown under the same conditions on the same farms. Despite this, no outbreaks of cyclosporiasis were reported that year, suggesting that blackberries were not as likely to be contaminated, or possibly do not stay contaminated, with *Cyclospora* parasites. The U.S. allowed fresh raspberries to be imported in the fall of 1998 and, as in past years, no outbreaks linked to the fall harvest were reported.

In May of 1999, outbreaks were reported in Ontario and in Florida. The implicated vehicle for the outbreak in Ontario was a dessert that included fresh Guatemalan blackberries, frozen Chilean raspberries and fresh U.S. strawberries *(42)*. Guatemalan blackberries were accepted without restriction in Canada and the U.S. at the time. Fresh raspberries from Guatemala were not accepted for importation into Canada during that time, while the U.S. permitted importation only from Guatemalan farms meeting strict standards. The evidence that the blackberries caused illness was suggestive but not conclusive, given that the dessert included multiple types of berries *(42)*. The Florida outbreak was associated with consumption of fruit, probably berries, from an undefined source.

In 2000, outbreaks of cyclosporiasis associated with the consumption of fresh berries were reported in Atlanta, GA (May) and Philadelphia, PA (June). Following the outbreak in Philadelphia, the presence of *C. cayetanensis* was demonstrated by PCR in cake filling containing imported raspberries *(45)*.

Since 2000, foodborne outbreaks of cyclosporiasis have continued to be reported annually in North America and have been associated with a variety of fresh produce, including Thai basil from the U.S., cilantro from Mexico, snow peas from Guatemala, and basil from Peru and Mexico *(28, 29)*. In August 2012, the CFIA decided to revise its import policy by lifting the import restriction on Guatemalan cultivated fresh blackberries for the period corresponding to March 15 to August 14 of each year.

The distinct seasonality in foodborne outbreaks of cyclosporiasis is only partially explained by environmental factors such as temperature and rainfall *(42)*. In North America, most foodborne outbreaks have been reported in the spring to early summer and have been associated with the consumption of raw imported fruits and vegetables. A list of foodborne outbreaks of cyclosporiasis in North America is shown in *Table 2*.

Table 2. Foodborne outbreaks of cyclosporiasis in North America

Date	Location	No. cases	Implicated food (Origin)	References
May, 1995	Florida	38	raspberries (possibly Guatemala)	(55)
May–June, 1995	New York	32	fruit suspected	(42)
May, 1996	Boston, MA	57	berries	(38)
May, 1996	Charleston, SC	38	raspberries (Guatemala)	(9)
May–June, 1996	U.S. (multi-state)/ Ontario, Quebec	1,465	raspberries (Guatemala)	(43, 64, 65)
Mar. –Apr., 1997	Florida	220	mesclun (Peru)	(42)
Apr. –May, 1997	U.S. (multi-state)/ Ontario	1,012	raspberries (Guatemala)	(25, 44)
June–July, 1997	Washington, D.C.	185	basil	(86)
Sept., 1997	Virginia	21	fruit plate	(42)
Dec., 1997	Florida	12	mesclun (Peru)	(42)
May, 1998	Ontario	192	raspberries (Guatemala)	(110)
May, 1999	Ontario	104	blackberries suspected	(42)
May, 1999	Florida	94	berries	(42)
July, 1999	Missouri	62	basil (Mexico or U.S.)	(60)
July, 1999	Vancouver, BC	15	unknown	(7)
May, 2000	Atlanta, GA	19	raspberries and/or blackberries suspected	(16, 40)
June, 2000	Philadelphia, PA	54	raspberries	(16, 45)
Jan., 2001	New York, NY	3	unknown	(16)
Jan.–Feb., 2001	Florida	39	unknown	(16)
May, 2001	Vancouver, BC	17	Thai basil (U.S.)	(46)
Dec., 2001–Jan., 2002	Vermont	22	raspberries likely	(16, 40)
Apr.–May, 2002	Massachusetts	8	unknown	(16)
June, 2002	New York	14	unknown	(16)
June–July, 2003	Vancouver, BC	10	cilantro suspected	(8)
Feb. 2004	Texas	38	unknown	(16, 76)
May, 2004	Tennessee	12	unknown	(16)

Table 2. Foodborne outbreaks of cyclosporiasis in North America (cont.)

Date	Location	No. cases	Implicated food (Origin)	References
May–June, 2004	Pennsylvania	96	snow peas (Guatemala)	(16, 24)
June, 2004	British Columbia	9	cantaloupe or basil suspected	(88)
Mar.–May, 2005	Florida	582	basil (Peru)	(16, 75)
Apr., 2005	Massachusetts	58	unknown	(16)
Apr., 2005	Ontario	40	basil (Peru)	(87)
May, 2005	South Carolina	6	unknown	(16)
May, 2005	Massachusetts	16	unknown	(16)
June, 2005	Quebec	148	basil (Mexico)	(68)
June, 2005	Connecticut	30	basil suspected	(16)
June, 2006	Minnesota	14	unknown	(16)
June, 2006	New York	20	unknown	(16)
July, 2006	Georgia	3	unknown	(16)
May–July, 2007	British Columbia	29	organic basil (Mexico)	(98)
Mar., 2008	Wisconsin	4	sugar snap peas likely	(16)
July, 2008	California	45	raspberries and/or blackberries likely	(16)
Oct.–Dec., 2008	British Columbia, Ontario	11	unknown	(88)
June, 2009	District of Columbia	34	unknown	(16)
May, 2010	Sarnia, ON	210	pesto	(23)
June, 2011	Florida	12	unknown	(16)
July, 2011	Georgia	100	unknown	(16)
June–July, 2012	Texas	16	unknown	(16)
June, 2013	Iowa, Nebraska	161	bagged salad mix (Mexico)	(15, 16)
June–July, 2013	Texas	38	cilantro (Mexico)	(15, 16)
June–Sept., 2013	British Columbia, Ontario, Nova Scotia	25	leafy greens suspected	(88)
July, 2013	Wisconsin	8	berry salad suspected	(15, 16)
Apr.–Aug., 2014	British Columbia, Ontario, Quebec	85	blackberries, cilantro	(89)
June, 2014	Michigan	14	unknown	(16, 17)
June–July, 2014	Texas	26	cilantro (Mexico)	(16, 17)
July, 2014	South Carolina	13	unknown	(16)

EXPOSURE ASSESSMENT

Potential sources of *Cyclospora* contamination

Cyclosporiasis outbreak investigations have not identified a single point of contamination, although a number of links in the food chain have been put forward as possible sources. The evidence available suggests that raspberry or blackberry contamination may occur during pre-harvest and/or post-harvest activities such as growing, irrigation, fumigation, harvesting, packaging or transporting. *Cyclospora* is not found naturally in or on fruits or any other foods. Rather, produce is contaminated with the oocysts shed in human feces. Farm activities involving contact of berries with fecally-contaminated water, and non-hygienic handling, are the most probable sources of contamination. Irrigation or fumigation using contaminated water, and contact with fecally-contaminated hands or equipment during harvest, packaging, transport, and food preparation, are the specific activities of concern. One of the most likely sources of contamination identified is the water used to mix insecticides and fungicides that are then sprayed directly on the raspberry plants *(67)*. Irrigation is not likely to be a vehicle of transmission when the water does not come into direct contact with the raspberries, such as with drip irrigation.

The intensity and uniformity of contamination on raspberries is largely dependent on the mode of contamination. Because of the high attack rates among persons who ate raspberries, Osterholm *(80)* suggested that the dose response for cyclosporiasis may be very low and that implicated raspberry lots may be uniformly contaminated. This author concluded that this type of contamination may indicate an environmental reservoir such as water or animal vectors, rather than direct contact with infected workers. Contamination by means of water (e.g., application of pesticides) may result in more raspberries being contaminated with fewer oocysts per berry. However, direct contamination of raspberries from the hands of food handlers must not be ruled out, and may result in smaller numbers of heavily-contaminated berries. If the latter non-uniform contamination pattern predominates, sampling plans involving small numbers of raspberry samples will be ineffective.

Prevalence on foods

A number of surveillance studies world-wide have reported the presence of *Cyclospora* on a variety of fruits and vegetables. *Cyclospora*-like organisms have been found on green leafy vegetables and other fresh produce items in Nepal *(39, 100, 101)*. In a survey of vegetables collected from markets in Peru, Ortega et al. *(75)* reported the presence of *Cyclospora* on lettuce and two types of herbs. Tram et al. *(111)* found *Cyclospora* spp. oocysts on all seven types of herbs tested, including lettuce, collected from farms and markets in Vietnam. These authors also detected oocysts in irrigation water on farms and in "sprinkling water" used to keep produce fresh in the markets. *Cyclospora* spp. was also reported on lettuce and other fresh produce items in Egypt *(1, 36)* and Costa Rica *(10)*. Anh et al. *(2)* reported *Cyclospora* spp. on water spinach collected in Cambodia.

Although a variety of fruits and vegetables, including raspberries, blackberries, mesclun and basil, have been epidemiologically implicated in outbreaks in North America, until recently, no large-scale surveillance studies have been done for *Cyclospora* or other foodborne protozoan parasites on fresh produce in Canada or the U.S. *(30)*. Raspberries, in particular, are difficult to test, as their surface is covered in tiny hairs *(94)* that may trap the oocysts and make them difficult to wash off without damaging the fruit. This difficulty in washing off the oocysts also increases the probability of infection in consumers. In contrast, blackberries are more washable than raspberries and therefore less likely to remain contaminated with oocysts, because of their considerably smoother, hairless surface.

In a collaborative study with FoodNet Canada (Public Health Agency of Canada), Dixon et al. *(30)* tested 544 samples of ready-to-eat packaged leafy greens obtained at retail in Ontario for the presence of *Cyclospora, Cryptosporidium* and *Giardia*. These authors reported a PCR-prevalence of 1.7% for *Cyclospora,* and *Cyclospora*-like oocysts were identified by microscopy in five of the nine PCR-positive samples. More recently, these authors tested samples of retail fresh berries and found a small number of *Cyclospora* PCR-positives on imported raspberries, blackberries, and strawberries *(14)*. However, none of these positive samples were associated with any illness outbreaks or cases.

Food testing methodology

As with other foodborne protozoan parasites, no standard methods are available for detection of *C. cayetanensis* in foods. Many of the same methods used in clinical diagnoses, however, are now being evaluated and adapted to testing of foods. Detection is very much dependent on the methods used for elution and concentration of the oocysts. Most published methods involve agitation of the fruits or vegetables in water *(2, 10, 75, 101, 111)* or in buffer, for elution, often in combination with detergents. The subsequent concentration of oocysts generally involves centrifugation and/or flotation.

Most surveillance studies have involved the use of microscopy (e.g., bright-field, differential interference contrast, epifluorescence, and acid-fast staining) for the detection of *Cyclospora* spp. oocysts on foods *(2, 10, 75, 95, 101, 111)*. PCR methods have also been useful in detecting the presence of *C. cayetanensis* on foods *(52, 56, 71, 106)*.

The viability of oocysts is also an important consideration in the risk management of foodborne cyclosporiasis. In the absence of animal models or *in vitro* cultivation for *C. cayetanensis*, it is very difficult to determine the true infectivity of oocysts; however, their viability may be determined by use of a sporulation assay followed by excystation of the oocysts, or by electrorotation *(76)*.

Importation of fresh raspberries and blackberries into Canada

The total quantities of fresh raspberries and blackberries imported into Canada from all countries for each of the years 2011 to 2013 are shown in *Table 3*. The quantities of fresh raspberries and blackberries imported into Canada in 2013 by country of origin are shown in *Table 4*.

Control measures

Control measures to reduce the risk of foodborne cyclosporiasis include farm-level controls, post-harvest disinfection, and consumer-level controls *(26)*. Worker hygiene and water quality on farms, particularly in endemic

Table 3. Importation of fresh raspberries and blackberries into Canada from all countries for the years 2011 to 2013

Berry type	2011	2012	2013
Fresh raspberries	22,592,542 kg	43,489,086 kg	29,331,774 kg
Fresh blackberries	11,852,525 kg	11,751,929 kg	11,370,160 kg

Table 4. Importation of fresh raspberries and blackberries into Canada in 2013 by country of origin

Country of origin	Raspberries	Blackberries
United States	20,500,315 kg	3,045,360 kg
Mexico	8,779,647 kg	8,240,188 kg
Guatemala	5,553 kg	74,282 kg
Chile	29,853 kg	7,591 kg
Other countries	16,406 kg	2,739 kg

regions, are important factors in the risk of contamination of produce with *C. cayetanensis* oocysts. Following the large foodborne outbreaks of cyclosporiasis in North America in the late 1990s, the U.S. and Canada worked together with Guatemala to investigate production, packaging and exporting practices in that country with respect to fresh raspberries, in an effort to identify the source(s) of contamination. A so-called Model Plan of Excellence (MPE) was developed in an attempt to minimize the likelihood of contamination of raspberries with *C. cayetanensis* oocysts *(26)*. In this HACCP-based approach, stringent control measures were enforced on raspberry farms and packing plants exporting fresh berries to North America. Despite these control measures, further outbreaks associated with Guatemalan berries were reported, suggesting that there may have been compliance problems, or that the control measures were ineffective or not directed against the actual sources of contamination *(42)*. The MPE system, as such, has since been discontinued in Guatemala.

In response to the outbreaks that occurred in the 1990s, Health Canada drafted an import policy entitled “Policy on Fresh Raspberries and Blackberries Imported from Guatemala” in April, 2000. This policy allowed importation of fresh Guatemalan raspberries and blackberries from MPE farms having their own on-farm exporters, or from MPE farms using an off-farm exporter, provided the exporter had procedures in place that adequately addressed the potential for co-mingling between non-MPE and MPE berries. Fresh Guatemalan raspberries and blackberries from medium-risk farms (as a minimum standard) were allowed during the fall season only. Following the drafting of this policy, Health Canada requested that the CFIA also implement an import alert on fresh Guatemalan blackberries such that they were also restricted until the policy was implemented. This import policy was revised in February, 2009, by which time the MPE system was no longer in place in Guatemala. In 2012, following discussions between Health Canada and CFIA, the import restriction on Guatemalan cultivated fresh blackberries was lifted for the period corresponding to March 15 to August 14 of each year. This decision was based on a number of factors, including the information that the current USFDA import alert stipulated an import restriction only for fresh raspberries from Guatemala, and that the continuous importation of fresh blackberries from Guatemala into the U.S. had not resulted in outbreaks or illnesses of cyclosporiasis linked to their consumption. The Canadian import restriction on Guatemalan fresh raspberries remained in effect for the period of March 15 to August 14 of each year.

Little information is available regarding environmental resistance of *C. cayetanensis* oocysts, although they are considered to be more susceptible to adverse environmental conditions than *Cryptosporidium* spp. For example, sporulation may be delayed in oocysts stored at either 4°C or 37°C for 14 days *(104)*. These findings could have important implications regarding risk control during production, storage and transportation.

While extreme hot and cold temperatures are effective at killing *Cryptosporidium,* their effect on *Cyclospora* is not yet well known. It is suspected that commercial freezing kills *Cyclospora (50)*, which is supported by the fact that no outbreaks have been associated with frozen raspberries. However, the amount of frozen Guatemalan raspberries shipped to North America is relatively small compared to peak shipments of fresh raspberries and may not provide absolute evidence that the product is safe. Sathyanarayanan and Ortega *(96)* evaluated the effects of temperature on the sporulation of *C. cayetanensis* in dairy products and basil. The percentage sporulation was used as an indicator of viability. Sporulation occurred after oocysts were resuspended in dairy substrates (i.e., milk, diluted milk, and whipped cream) and stored for up to 24 h at -15°C. On basil, or in water, sporulation still occurred after incubation for up to 2 days at -20°C, and up to 4 days at 37°C. However, few oocysts sporulated after they were incubated for just 1 h at 50°C, and no sporulation was observed after oocysts were incubated at -70°C, 70°C or 100°C. Sporulation was not affected when oocysts were incubated at 4°C and 23°C for up to 1 week.

Gamma irradiation of oocysts has been suggested as a possible way of decontaminating fresh fruits and vegetables. Appropriate irradiation doses to eliminate the infectivity of oocysts of *Toxoplasma gondii* have been published, and it was suggested that similar irradiation procedures would kill *Cyclospora* oocysts attached to fresh raspberries or other fruits and vegetables *(31)*. However, another coccidian, *Eimeria bovis,* was found to be resistant to gamma irradiation *(37)*. Irradiation of raspberries is not currently approved by Health Canada.

The use of ozone has gained approval for use in the U.S. food processing industry as a control measure against bacteria, parasites, viruses and fungi *(3)*. Processors of fresh fruit, vegetables, poultry, and red meat are currently evaluating ozone as a potential disinfectant. The reported effectiveness of ozone in inactivating *Cryptosporidium* in water indicates that the process may also be a useful control measure for *Cyclospora* on raspberries.

While most of the work done on chemical and physical disinfection of protozoans in foods has involved *Cryptosporidium* spp., some studies have demonstrated the effectiveness of commercial freezing, irradiation, UV light, and high-hydrostatic pressure processing (HPP) against *C. cayetanensis,* or *Eimeria acervulina* as a surrogate organism *(54, 76)*. Young broiler chickens inoculated with *E. acervulina* oocysts from UV-treated raspberries had reduced infection rates, while birds inoculated with oocysts from HPP-treated raspberries and basil were asymptomatic and did not shed oocysts *(54)*. As with other protozoan parasites, *C. cayetanensis* oocysts are thought to be very resistant to the chemical disinfectants commonly used in the food industry, as well as in water treatment. Routine chlorination of water is ineffective against coccidian oocysts in general, and some water purification plants relying on chlorination have experienced problems with *Cryptosporidium*. With respect to *Cyclospora*, there is currently only indirect evidence that chlorination is ineffective *(50, 108)*. Ortega et al. *(73)* reported that *Cyclospora* oocyst sporulation was not affected by treatment of inoculated basil and lettuce leaves with gaseous chlorine dioxide.

There are also some important control points at the food-preparer or consumer level. As with all enteric pathogens, good personal hygiene, particularly hand washing, is of considerable importance in reducing the risk of transmission. However, as discussed, person-to-person transmission of cyclosporiasis is unlikely, and oocysts contaminating foods are not immediately infective. While rinsing of fresh fruits and vegetables with water is recommended for reducing the risk of transmission of pathogens, it is unlikely that this practice will remove all *C. cayetanensis* oocysts from the foods. Furthermore, delicate fruits such as raspberries will not tolerate anything more than a gentle rinse. Other treatments, such as cooking and freezing, may be used as final barriers against transmission, although most of the produce items implicated in outbreaks of cyclosporiasis are typically consumed raw. Furthermore, few data are available regarding the susceptibility of *C. cayetanensis* to cooking and freezing temperatures. Recent studies suggest that *C. cayetanensis* oocysts are relatively resistant to short exposures to household freezing and to high temperatures *(76)*. *Cyclospora* oocyst sporulation was still observed after up to 45 sec of microwave cooking at 100% power *(72)*.

HAZARD CHARACTERIZATION

Symptoms and pathogenicity

The dose response for cyclosporiasis is generally thought to be relatively low *(32)*. The infectious dose may be similar to that of the closely related animal parasite *Eimeria* spp., for which single oocysts or even individual sporozoites have been found to be sufficient to establish infections in naive hosts.

Cyclosporiasis may result in profuse and prolonged diarrhea and a variety of other symptoms, including abdominal pain, nausea, vomiting, fatigue, fever, and loss of appetite *(63, 76)*. Onset of symptoms has been reported as abrupt in the majority of adult patients *(78)*. These symptoms may be more severe and long-lasting in very young or old people or in immunosuppressed individuals *(76)*. Symptoms will generally appear about a week after becoming infected and, if left untreated, may last anywhere from a few days to several weeks. Relapses have also been reported *(20)*. Infections are often short-lived or asymptomatic in individuals native to endemic areas *(74)*. In the U.S., the hospitalization rate for cyclosporiasis has been reported as 6.5%, and the death rate as zero *(97)*. Mortality associated with *Cyclospora* infections is generally considered to be very rare *(76)*.

The main site of infection is thought to be the small intestine, although infections have been reported in the biliary tract of AIDS patients *(103)*. Altered mucosal architecture with villous atrophy, crypt hyperplasia, inflammatory changes, and other abnormalities have been reported *(21, 67, 74, 77, 108)*.

Susceptibility factors

There is currently insufficient data available regarding susceptibility to *Cyclospora* infection and the likelihood of symptoms. The majority of cluster-related cases in North America associated with the consumption of fresh berries, were adults of relatively high socioeconomic status, which may reflect only food preferences and type of social functions attended. The relatively high cost of imported fresh raspberries and blackberries at the time of the outbreaks could also have been a factor. This population generally has a lower probability of enteric illness because of favorable determinants of health.

Cyclosporiasis has traditionally been considered a traveler's illness, since naive hosts, including travelers and expatriates, generally have a greater probability of developing the illness than the native population. There is also some evidence that young children in endemic regions may be less susceptible to symptomatic infection than older children *(105)*. However, it is not clear whether these differences are due to protective immunity or different exposures. Pape et al. *(81)* reported a higher prevalence of cyclosporiasis in HIV-infected adults with diarrhea than in non-HIV-infected patients with diarrhea.

Symptoms of cyclosporiasis are generally more severe in immunocompromised individuals than in otherwise healthy people. For example, Sifuentes-Osornio et al. *(103)* reported that symptoms were more severe and long-lasting in AIDS patients. Nevertheless, neither Clarke and McIntyre *(19)* nor Petry et al. *(82)* could find any serological evidence for the development of an immune response in individuals infected with *C. cayetanensis*.

Diagnosis and treatment

Diagnosis usually involves a microscopic examination of stool samples for the presence of oocysts. Concentration of oocysts by use of methods such as flotation or sedimentation may improve detection by microscopy *(35, 53, 76, 93)*. Modified acid-fast methods have been widely used in diagnosis; however, *C. cayetanensis* oocysts are variably staining with acid-fast methods, which has led to the use of other, more reliable stains, particularly safranin *(113)*. Although anti-*Cyclospora* antibodies are not commercially available, epifluorescence microscopy can still be used, as *C. cayetanensis* is an autofluorescent organism. Oocysts appear as bright-blue circles when wet mounts are examined under UV-light, using an excitation filter within the range 330–380 nm. Flow cytometry has also been used successfully to detect *C. cayetanensis* in human stool and was reported to be more sensitive than microscopy *(27)*.

PCR-based methods have also been very useful in detecting *C. cayetanensis* and differentiating it from other related parasites. A nested-PCR method, based on the amplification of a portion of the 18S rRNA gene, was developed by Relman et al. *(92)* and has been widely used in the diagnosis of cyclosporiasis and in the detection of *Cyclospora* in environmental samples. Because of cross-amplification, a subsequent Restriction Fragment Length Polymorphism (RFLP) analysis *(51, 52, 102)* may be used to differentiate *Cyclospora* spp. from the closely related *Eimeria* spp. However, because *Eimeria* has not been reported to cause disease in humans, this is of concern only in environmental samples, and possibly in fresh fruits and vegetables. The highly conserved nature of the 18S rRNA gene, however, limits its usefulness in differentiating among isolates of *C. cayetanensis* or other *Cyclospora* species *(76)*. A multiplex PCR assay was subsequently developed to differentiate *Cyclospora* spp. from *Eimeria* spp. without the need for RFLP *(70)*. The ITS region is highly variable and has been used successfully to demonstrate sequence variability among *C. cayetanensis* isolates *(69)*. Unfortunately, the ITS-1 sequence variability was not found to correlate with the geographic origin of the isolates. Piche et al. *(83)* concluded that because of the high degree of ITS-1 sequence variation within samples, and the lack of geographic association among isolates, ITS-1 may be of little use in molecular epidemiology. Real-time quantitative PCR assays have also been used in the detection of *C. cayetanensis (49, 112)*. Lalonde and Gajadhar *(57)* have recently developed a real-time PCR and melting curve analysis for the detection and differentiation of coccidian oocysts.

For most people, an infection with *C. cayetanensis* is treatable and does not result in life-threatening illness. Cyclosporiasis may be effectively treated with the antibiotic trimethoprim-sulfamethoxazole *(63, 76)*.

RISK CHARACTERIZATION

Introduction

Cyclospora oocysts are shed into the environment with the feces of infected individuals. The transmission route is indirect vehicle-borne (foodborne and waterborne). It is very unlikely that the infection would be transmitted person-to-person, or through insects, mammals or birds. Infection with *Cyclospora* often results in profuse and prolonged diarrhea, but is usually self-limiting. Immunocompromised individuals may develop more severe and chronic symptoms.

Imported fresh raspberries, and possibly blackberries, were implicated in a number of outbreaks of cyclosporiasis in North America between 1995 and 2000. The spring harvest appears to present the highest probability for transmission of this parasite. It has been suggested that this could be related to an accumulation of water during the rainy season in the exporting countries, causing potential flooding of crops and wells with fecally-contaminated water. However, this relationship does not always hold, as exemplified by the unusually dry spring seasons in Guatemala in 1997 and 1998, with initial outbreaks in North America preceding any heavy rains. The significance of rainfall alone on the seasonality of infection is also questionable based on prevalence data from both Peru and Haiti.

Contamination of berries by direct contact with fecally-contaminated hands or surfaces is also of concern. The proportion of contaminating *Cyclospora* oocysts that are viable (able to sporulate and cause infection) is unknown. There is insufficient information to determine whether the time frame and the physical conditions imposed on raspberries and blackberries during production, harvest, sorting, storage and transportation would support viability of *Cyclospora* oocysts. Coccidian oocysts, however, are known to be extremely durable and are quite resistant to environmental extremes and chemical control measures.

Control measures that can be monitored at all levels (i.e., production, harvesting, and transportation) are essential to ensure that the risk to consumers is minimized *(99)*. Since most raspberries and blackberries are consumed fresh, there is little that can be done at the consumer level to reduce the probability of infection. Assuming a low dose response, washing of the berries will not remove sufficient oocysts to prevent transmission. Based on current knowledge regarding the susceptibility of related coccidian parasites to freezing and cooking, *Cyclospora*-contaminated raspberries or blackberries that are frozen or cooked (e.g., pie filling, jams) would likely present a lower risk to consumers.

Epidemiology

Cyclosporiasis was originally thought to be largely a travel-associated illness, but from 1995 to 2000 it was associated with the consumption in North America of imported Guatemalan raspberries. Associations with blackberries imported from Guatemala were also apparent following Canadian outbreaks in 1996, 1997, and 1999.

In the mid-to-late 1990s, the greatest risk to Canadian consumers appeared to be associated with the consumption of fresh Guatemalan raspberries during the months of April to June. It is not clear how infectivity patterns may have been biased by the demographics of raspberry consumers. It is also unclear why Canadian illness clusters associated with fresh Guatemalan raspberries and blackberries were primarily reported in Ontario, even though these berries were shipped to several provinces and further distributed inter-provincially. It is possible, however, that there may have been some bias related to physician awareness and/or clinical expertise.

Product identification

Large-scale control measures should also include procedures for the identification of shipments potentially involved in outbreaks, to ensure an effective traceback to the implicated farms. At the time of these outbreaks, the identification system made it very difficult to link illnesses or outbreaks to specific lots of produce. More precise information on implicated farms would have helped define more accurately the potential vehicles of transmission.

CONCLUSIONS

Considerable epidemiological evidence from the mid-to-late 1990s showed that the probability of exposure to infectious doses of *Cyclospora* in imported fresh raspberries during the spring and early summer was unacceptably high. This was supported by the observation of numerous outbreaks in Canada and the U.S. between 1995 and 2000, most of which were strongly associated with the consumption of imported fresh raspberries. The evidence supporting the implication of imported fresh blackberries in illness outbreaks in Canada in the late 1990s was considerably weaker than that for raspberries. While fresh berries have only rarely been implicated in cases of cyclosporiasis in Canada in recent years, a study done at Health Canada, in collaboration with FoodNet Canada (Public Health Agency of Canada) *(14)*, demonstrated three *Cyclospora* PCR-positive samples of imported raspberries, and a single *Cyclospora* PCR-positive sample of imported blackberries, out of a total of 599 fresh berry samples tested. It was not determined if any of the oocysts detected were actually viable and/or infectious.

As the dose response for *Cyclospora* may be very low, an assumption could be made that the consumption of any amount of oocyst-contaminated raspberries or blackberries could result in infection. Berries implicated in outbreaks are usually consumed without any further treatment, i.e., not heat treated, and it is not possible to remove all oocysts from foods by washing; therefore, little can be done at the consumer level to reduce the probability of infection. The risk of transmission can be reduced only by using import restrictions during specific times of the year, or rigorously enforced controls in the country of origin with respect to the production, harvesting, packaging and transporting of berries.

Without fully understanding the modes of contamination of berries, and without knowing precisely the prevalence of cyclosporiasis among farm workers and exporters, the seasonal trends of infection, or the levels of contamination in water sources, it is difficult to conclude what the level of risk is to Canadians from the consumption of imported fresh raspberries or blackberries. Risk management decisions for *Cyclospora* oocysts on these berries must be made with a clear understanding of the many gaps that still exist in our knowledge of the biology and control of this organism. It is also important to note that recommendations and decisions made regarding the importation of fresh raspberries and blackberries will not eliminate the risk of infection with *Cyclospora* to Canadian consumers, because other sources are likely to exist. In recent years, numerous other fresh produce items from a variety of countries have been implicated in outbreaks of cyclosporiasis in North America. These other routes will also need to be assessed to determine what, if any, specific risk management practices may need to be implemented.

RESEARCH NEEDS/DATA GAPS

Many gaps still exist in our knowledge of *Cyclospora* as an important foodborne pathogen. Since *C. cayetanensis* is found only in humans, and effective cell culture methods are not yet available, oocysts and other life stages are not readily available to researchers, and this has resulted in a lag of research behind that of other important foodborne protozoan parasites such as *Cryptosporidium* and *Giardia*. Specifically, work is required in development of improved methods for the detection of *Cyclospora* oocysts on berries and other foods in surveillance studies and outbreak investigations, in order to better understand the risks to consumers and to monitor the effectiveness of control methods. Effective methods for the molecular characterization of *C. cayetanensis*, down to the genotype or subgenotype level, are also urgently needed. Combined with epidemiological investigations and traceback studies, the molecular characterization of *C. cayetanensis* isolates will also be of great value in identifying the possible origin of contaminated foods and the potential sources of food contamination (e.g., water used in irrigation or processing, food handlers, etc.). Furthermore, laboratory research is also needed on the effectiveness of various chemical and physical disinfectants and on-farm control measures, and the sensitivity of oocysts to cooking and freezing.

ACKNOWLEDGMENTS

The authors thank Irina Frenkel, Fresh Produce Safety, Canadian Food Inspection Agency, for providing the Canadian import data on imported fresh raspberries and blackberries, and Vanessa Morton, Regan Murray, Elizabeth Hillyer, Meghan Hamel, and Rachel McCormick, Centre for Foodborne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada, for providing data on reported cases and rates of cyclosporiasis in Canada, as well as Canadian outbreak summaries.

REFERENCES

1. Abou el Naga, I. F. 1999. Studies on a newly emerging protozoal pathogen: *Cyclospora cayetanensis. J. Egypt. Soc. Parasitol.* 29:575–586.
2. Anh, V. T., N. T. Tram, L. T. Klank, P. D. Cam and A. Dalsgaard. 2007. Faecal and protozoan parasite contamination of water spinach *(Ipomoea aquatica)* cultivated in urban wastewater in Phnom Penh, Cambodia. *Trop. Med. Int. Health* 12(Suppl. 2):73–81.
3. Anon. 1998. Food safety technology: a potential role for ozone? Agricultural Outlook, Economic Research Service/USDA, June-July, 1998:13–15.
4. Bern, C., B. Hernandez, M. B. Lopez, M. J. Arrowood, M. A. de Mejia, A. M. de Merida, A. W. Hightower, L. Venczel, B. L. Herwaldt, and R. E. Klein. 1999. Epidemiologic studies of *Cyclospora cayetanensis* in Guatemala. *Emerg. Infect. Dis.* 5:766–774.
5. Bern, C., B. Hernandez, M. B. Lopez, M. J. Arrowood, A. M. de Merida, and R. E. Klein. 2000. The contrasting epidemiology of *Cyclospora* and *Cryptosporidium* among outpatients in Guatemala. *Am. J. Trop. Med. Hyg.* 63:231–235.
6. Brennan, M. K. et al. 1996. Cyclosporiasis: a new cause of diarrhea. *Can. Med. Assoc. J.* 155:1293–1296.
7. British Columbia Centre for Disease Control. 2002. Unpublished data.
8. British Columbia Centre for Disease Control. 2003. Unpublished data.
9. Cáceres, V. M., R. T. Ball, S. A. Somerfeldt, R. L. Mackey, S. E. Nichols, W. R. MacKenzie, and B. L. Herwaldt. 1998. A foodborne outbreak of cyclosporiasis caused by imported raspberries. *J. Family Practice* 47:231–234.
10. Calvo, M., M. Carazo, M. L. Arias, C. Chaves, R. Monge, and M. Chinchilla. 2004. Prevalence of *Cyclospora* sp., *Cryptosporidium* sp., microsporidia and fecal coliform determination in fresh fruit and vegetables consumed in Costa Rica. *Arch. Latinoam. Nutr.* 54:428–432.
11. Cama, V. 2006. Coccidian parasites, p. 33–55. *In* Y. R. Ortega (ed.), Foodborne Parasites. Springer, New York, NY.
12. Canadian Notifiable Disease Surveillance System. http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/index-eng.php (accessed Oct. 3, 2014).
13. Cann, K. J., R. M. Chalmers, G. Nichols, and S. J. O'Brien. 2000. *Cyclospora* infections in England and Wales: 1993 to 1998. *Comm. Dis. Publ. Health* 3:46–49.
14. C-EnterNet. 2012. C-EnterNet 2011 Short Report. Public Health Agency of Canada. http://publications.gc.ca/collections/collection_2012/aspc-phac/HP37-8-1-2011-eng.pdf (accessed Oct. 29, 2014).
15. Centers for Disease Control and Prevention. 2013. Cyclosporiasis Outbreak Investigations — United States, 2013 (Final Update). Available at: http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/investigation-2013.html. Accessed 24 November 2014.
16. Centers for Disease Control and Prevention. 2015. U.S. Foodborne Outbreaks of Cyclosporiasis—2000–2014. Available at: http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/ foodborneoutbreaks.html. Accessed 19 August 2015.
17. Centers for Disease Control and Prevention. 2015. Cyclosporiasis Outbreak Investigations — United States, 2014. Available at: http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2014/. Accessed 2 July 2015.
18. Chu, D.-M.T., J. B. Sherchand, J. H. Cross, and P. A. Orlandi. 2004. Detection of *Cyclospora cayetanensis* in animal fecal isolates from Nepal using an FTA filter-base polymerase chain reaction method. *Am. J. Trop. Med.* 71:373–379.
19. Clarke, S. C., and M. McIntyre. 1997. An attempt to demonstrate a serological immune response in patients infected with *Cyclospora cayetanensis* (Letter to Editor). *Br. J. Biomed. Sci.* 54:73–74.
20. Colley, D. G. 1996. Widespread foodborne cyclosporiasis outbreaks present major challenges (Letter to Editor). *Emerg. Infect. Dis.* 2:354–356.
21. Connor, B. A., J. Reidy, and R. Soave. 1999. Cyclosporiasis: clinical and histopathologic correlates. *Clin. Infect. Dis.* 28:1216–1222.
22. Connor, B. A., and D. R. Shlim. 1995. Foodborne transmission of *Cyclospora* (Letter to Editor). *The Lancet* 346:1634.

23. County of Lambton Community Health Services Department. 2010. *Cyclospora* Outbreak Investigation Closed Locally. Available at: http://www.lambtonhealth.on.ca/News-Releases/669. Accessed 24 November 2014.

24. Crist, A., et al. 2004. Outbreak of cyclosporiasis associated with snow peas — Pennsylvania, 2004. *Morb. Mortal. Wkly. Rep.* 53:876–878.

25. DeGraw, E., S. Heber, and A. Rowan. 1997. Update: outbreaks of cyclosporiasis — United States and Canada, 1997. *Morb. Mort. Weekly Rep.* 46:521–523.

26. Dixon, B. R. 2003. The prevalence and control of foodborne protozoan parasites, p. 31–76. *In* B. W. Blais (ed.), CurrentChall-enges in Food Microbiology. Research Signpost, Kerala, India.

27. Dixon, B., J. Bussey, L. Parrington, and M. Parenteau. 2005. Detection of *Cyclospora cayetanensis* oocysts in human faecal specimens using flow cytometry. *J. Clin. Microbiol.* 43:2375–2379.

28. Dixon, B. R., R. Fayer, M. Santin, D. E. Hill, and J. P. Dubey. 2011. Protozoan Parasites: *Cryptosporidium, Giardia, Cyclospora,* and *Toxoplasma,* Chapter 24, p. 349–70. *In* J. Hoorfar (ed.), Rapid Detection, Characterization and Enumeration of Foodborne Pathogens. ASM Press, Washington, USA.

29. Dixon, B, L. Landry, and F. Milord. 2012. Investigations of foodborne outbreaks caused by a parasite in Canada, Chapter 9, p. 80–91. *In* Hoorfar, J. (ed.), Case Studies in Food Safety and Authenticity: Lessons from Real-Life Situations. Woodhead Publishing. Cambridge, UK.

30. Dixon, B., L. Parrington, A. Cook, F. Pollari, and J. Farber. 2013. Detection of *Cyclospora, Cryptosporidium* and *Giardia* in ready-to-eat packaged leafy greens in Ontario, Canada. *J. Food Protect.* 76:307–313.

31. Dubey, J. P., D. W. Thayer, C. A. Speer, and S. K. Shen. 1998. Effect of gamma irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts. *Int. J. Parasitol.* 28:369–375.

32. Eberhard, M. L., and M. J. Arrowood.2002. *Cyclospora* spp. *Curr. Opin. Infect. Dis.* 15:519–522.

33. Eberhard, M. L., A. J. da Silva, B. G. Lilley, and N. J. Pieniazek. 1999. Morphologic and molecular characterization of new *Cyclospora* species from Ethiopian monkeys: *C. cercopitheci* sp.n., *C. colobi* sp.n., and *C. papionis* sp.n. *Emerg. Infect. Dis.* 5:651–658.

34. Eberhard, M. L., E. K. Nace, and A. R. Freeman. 1999. Survey for *Cyclospora cayetanensis* in domestic animals in an endemic area in Haiti. *J. Parasitol.* 85:562–563.

35. Eberhard, M. L., N. J. Pieniazek, M. J. Arrowood. 1997. Laboratory diagnosis of *Cyclospora* infections. *Arch. Pathol. Lab. Med.* 121:792–797.

36. El Said Said, D. 2012. Detection of parasites in commonly consumed raw vegetables. *Alexandria J. Med*. 48:345–352.

37. Fitzgerald, P. R. 1968. Effects of ionizing radiation from cobalt-60 on oocysts of *Eimeria bovis. J. Parasitol.* 54:233–240.

38. Fleming, C. A., D. Caron, J. E. Gunn, and A. Barry. 1998. A foodborne outbreak of *Cyclospora cayetanensis* at a wedding. *Arch. Intern. Med.* 158:1121–1125.

39. Ghimire, T. R., P. N. Mishra, and J. B. Sherchand. 2005. The seasonal outbreaks of *Cyclospora* and *Cryptosporidium* in Kathmandu, Nepal. *J. Nepal Health Res. Council* 3:39–48.

40. Guzewich, J. 2002. US Food and Drug Administration. Personal communication.

41. Hall, R. L., J. L. Jones, S. Hurd, G. Smith, B. E. Mahon, and B. L. Herwaldt. 2012. Population-based active surveillance for *Cyclospora* infection — United States, Foodborne Diseases Active Surveillance Network (FoodNet), 1997–2009. *Clin. Infect. Dis.* 54:S411–417.

42. Herwaldt, B. L. 2000. *Cyclospora cayetanensis*: a review, focusing on the outbreaks of cyclosporiasis in the 1990s. *Clin. Infect. Dis.* 31:1040–1057.

43. Herwaldt, B. L., M.-L. Ackers, and the *Cyclospora* Working Group. 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *New Engl. J. Med.* 336:1548–1556.

44. Herwaldt, B. L., M. J. Beach, and the *Cyclospora* Working Group. 1999. The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. *Ann. Int. Med.* 130:210–220.

45. Ho, A.Y., A. S. Lopez, M. G. Eberhart, R. Levenson, B. S. Finkel, A. J. da Silva, J. M. Roberts, P. A. Orlandi, C. C. Johnson, and B. L. Herwaldt. 2002. An outbreak of cyclosporiasis associated with imported raspberries in Pennsylvania in 2000. *Emerg. Infect. Dis.* 8:783–788.

46. Hoang, L. M. N., M. Fyfe, C. Ong, J. Harb, S. Champagne, B. Dixon, and J. Isaac-Renton. 2005. Outbreak of cyclosporiasis in British Columbia associated with imported Thai basil. *Epidemiol. Infect.* 133:23–27.

47. Hoge, C. W., D. R. Shlim, R. Rajah, J. Triplett, M. Shear, J. G. Rabold, and P. Echeverria. 1993. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travelers and foreign residents in Nepal. *Lancet* 341:1175–1179.

48. Huang, P., J. T. Weber, D. M. Sosin, P. M. Griffin, E. G. Long, J. J. Murphy, F. Kocka, C. Peters, and C. Kallick. 1995. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. *Ann. Intern. Med.* 123:409–414.

49. Hussein, E. M., A. A. el-Moamly, H. A. Dawoud, H. Fahmy, H. E. el-Shal and N. A. Sabek. 2007. Real-time PCR and flow cytometry in detection of *Cyclospora* oocysts in fecal samples of symptomatic and asymptomatic pediatrics patients. *J. Egypt. Soc. Parasitol.* 37:151–170.

50. Jackson, G. J., J. E. Leclerc, J. W. Bier, and J. M. Madden. 1997. *Cyclospora* — still another new foodborne pathogen. *Food Technol.* 51:120.

51. Jinneman, K. C., J. H. Wetherington, A. M. Adams, J. M. Johnson, B. J. Tenge, N.-L. Dang, and W. E. Hill. 1996. Differentiation of *Cyclospora* sp. and *Eimeria* spp. by using the polymerase chain reaction amplification products and restriction fragment length polymorphisms. FDA Laboratory Information Bulletin, Number 4044.

52. Jinneman, K. C., J. H. Wetherington, W. E. Hill, A. M. Adams, J. M. Johnson, B. J. Tenge, N.-L. Dang, R. L. Manger, and M. M. Wekell. 1998. Template preparation for PCR and RFLP of amplification products for the detection and identification of *Cyclospora* sp. and *Eimeria* spp. oocysts directly from raspberries. *J. Food Prot.* 61:1497–1503.

53. Kimura, K., S. K. Rai, K. Takemasa, Y. Ishibashi, M. Kawabata, M. Belosevic, and S. Uga. 2004. Comparison of three microscopic techniques for diagnosis of *Cyclospora cayetanensis. FEMS Microbiol. Letters* 238:263–266.

54. Kniel, K. E., A. E. H. Shearer, J. L. Cascarino, G. C. Wilkins, and M. C. Jenkins. 2007. High hydrostatic pressure and UV light treatment of produce contaminated with *Eimeria acervulina* as a *Cyclospora cayetanensis* surrogate. *J. Food Prot.* 70:2837–2842.

55. Koumans, E. H. A., D. J. Katz, J. M. Malecki, S. Kumar, S. P. Wahlquist, M. J. Arrowood, A. W. Hightower, and B. L. Herwaldt. 1998. An outbreak of cyclosporiasis in Florida in 1995: A harbinger of multistate outbreaks in 1996 and 1997. *Am. J. Trop. Med. Hyg.* 59:235–242.

56. Lalonde, L. F., and A. A. Gajadhar. 2008. Highly sensitive and specific PCR assay for reliable detection of *Cyclospora cayetanensis* oocysts. *Appl. Environ. Microbiol.* 74:4354–4358.

57. Lalonde, L. F., and A. A. Gajadhar. 2011. Detection and differentiation of coccidian oocysts by real-time PCR and melting curve analysis. *J. Parasitol.* 97:725–730.

58. LeBer, C. 1998. Ontario Ministry of Health. Unpublished data.

59. Lopez, A. S., J. M. Bendik, J. Y. Alliance, J. M. Roberts, A. J. da Silva, I. N. Moura, M. J. Arrowood, M. L. Eberhard, and B. L. Herwaldt. 2003. Epidemiology of *Cyclospora cayetanensis* and other intestinal parasites in a community in Haiti. *J. Clin. Microbiol.* 41:2047–2054.

60. Lopez, A. S., D. R. Dodson, M. J. Arrowood, P. A. Orlandi, A. J. da Silva, J. W. Bier, S. D. Hanauer, R. L. Kuster, S. Oltman, M. S. Baldwin, K. Y. Won, E. M. Nace, M. L. Eberhard, and B. L. Herwaldt. 2001. Outbreak of cyclosporiasis associated with basil in Missouri in 1999. *Clin. Infect. Dis.* 32:1010–1017.

61. Lopez, F. A., J. Manglicmot, T. M. Schmidt, C. Yeh, H. V. Smith, and D. A. Relman. 1999. Molecular characterization of *Cyclospora*-like organisms from baboons. *J. Infect. Dis.* 179:670–676.

62. Madico, G., J. McDonald, R. H. Gilman, L. Cabrera, and C. R. Sterling. 1997. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. *Clin. Infect. Dis.* 24:977–981.

63. Mansfield, L. S., and A. A. Gajadhar. 2004. *Cyclospora cayetanensis,* a food- and waterborne coccidian parasite. *Vet. Parasitol.* 126:73–90.

64. Manuel, D. G., S. Neamatullah, R. Shahin, D. Reymond, J. Keystone, J. Carlson, C. Le Ber, B. L. Herwaldt and D. H. Werker. 2000. An outbreak of cyclosporiasis in 1996 associated with consumption of fresh berries — Ontario. *Can. J. Infect. Dis.* 11:86–92.

65. Manuel, D. G., R. Shahin, W. Lee, and M. Grmusa. 1999. The first reported cluster of foodborne cyclosporiasis in Canada. *Can. J. Public Health* 90:399–402.

66. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.

67. Miller, M. A., and J. C. Paige. 1998. Other food borne infections. *Vet. Clin. North America: Food Animal Practice* 14:71–89.

68. Milord, F., E. Lampron-Goulet, M. St-Amour, E. Levac, and D. Ramsay. 2012. *Cyclospora cayetanensis*: a description of clinical aspects of an outbreak in Quebec, Canada. *Epidemiol. Infect.* 140:626–632.

69. Olivier, C., S. van de Pas, P. W. Lepp, K. Yoder, and D. A. Relman. 2001. Sequence variability in the first internal transcribed spacer region within and among *Cyclospora* species is consistent with polyparasitism. *Int. J. Parasitol.* 31:1475–1487.

70. Orlandi, P. A., L. Carter, A. M. Brinker, A. J. da Silva, D.-M.Chu, K. A. Lampel, and S. R. Monday. 2003. Targeting single-nucleotide polymorphisms in the 18S rRNA gene to differentiate *Cyclospora* species from *Eimeria* species by multiplex PCR. *Appl. Environ. Microbiol.* 69:4806–4813.

71. Orlandi, P. A., and K. A. Lampel. 2000. Extraction-free, filter-based template preparation for rapid and sensitive PCR detection of pathogenic parasitic protozoa. *J. Clin. Microbiol.* 38:2271–2277.

72. Ortega, Y. R., and J. Liao. 2006. Microwave inactivation of *Cyclospora cayetanensis* sporulation and viability of *Cryptosporidium parvum* oocysts. *J. Food Protect.* 69:1957–1960.

73. Ortega, Y. R., A. Mann, M. P. Torres, and V. Cama. 2008. Efficacy of gaseous chlorine dioxide as a sanitizer against *Cryptosporidium parvum, Cyclospora cayetanensis,* and *Encephalitozoon intestinalis* on produce. *J. Food Protect.* 71:2410–2414.

74. Ortega, Y. R., R. Nagle, R. H. Gilman, J. Watanabe, J. Miyagui, H. Quispe, P. Kanagusuku, C. Roxas, and C. R. Sterling. 1997. Pathologic and clinical findings in patients with cyclosporiasis and a description of intracellular parasite life-cycle stages. *J. Infect. Dis.* 176:1584–1589.

75. Ortega, Y. R., C. R. Roxas, R. H. Gilman, N. J. Miller, L. Cabrera, C. Taquiri, and C. R. Sterling. 1997. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *Am. J. Trop. Med. Hyg.* 57:683–686.

76. Ortega, Y. R., and R. Sanchez. 2010. Update on *Cyclospora cayetanensis,* a foodborne and waterborne parasite. *Clin. Microbiol. Rev.* 23:218–234.

77. Ortega, Y. R., and C. R. Sterling. 1996. *Cyclospora cayetanensis*: epidemiology and diagnosis. *Clin. Microbiol. Newsletter* 18:169–172.

78. Ortega, Y. R., C. R. Sterling, and R. H. Gilman. 1998. *Cyclospora cayetanensis. Adv. Parasitol.* 40:399–418.

79. Ortega, Y. R., C. R. Sterling, R. H. Gilman, V. A. Cama, and F. Díaz. 1993. *Cyclospora* species — a new protozoan pathogen of humans. *New Engl. J. Med.* 328:1308–1312.

80. Osterholm, M. T. 1999. Lessons learned again: cyclosporiasis and raspberries. *Ann. Int. Med.* 130:233–234.

81. Pape, J. W., R. I. Verdier, M. Boncy, J. Boncy, and W. D. Johnson. 1994. *Cyclospora* infection in adults infected with HIV. Clinical manifestations, treatment, and prophylaxis. *Ann. Intern. Med.* 121:654–657.

82. Petry, F., J. Hofstatter, B. K. Schulz, G. Deitrich, M. Jung, and P. Schirmacher. 1997. *Cyclospora cayetanensis*: first imported infections in Germany. *Infection* 25:167–170.

83. Piche, R., R. Chen, L. Hoang, B. Dixon, J. H. Cross, H. D. A. Lindquist, M. W. Fyfe, S. Champagne, J. L. Isaac-Renton, and C. S. L. Ong. 2002. Molecular epidemiological studies on two cyclosporiasis outbreaks in Vancouver, British Columbia, p. 89–92. *In* Proceedings of the 10th International Congress of Parasitology, Vancouver, B.C. Aug. 4-9, 2002. Monduzzi Editore S.p.A.-Medimond Inc.

84. Pieniazek, N. J., and B. L. Herwaldt. 1997. Reevaluating the molecular taxonomy: is human-associated *Cyclospora* a mammalian *Eimeria* species? *Emerg. Infect. Dis.* 3:381–383.

85. Pratdesaba, R. A., M. González, E. Piedrasanta, C. Mérida, K. Contreras, C. Vela, F. Culajay, L. Flores, and O. Torres. 2001. *Cyclospora cayetanensis* in three populations at risk in Guatemala. *J. Clin. Microbiol.* 39:2951–2953.

86. Pritchett, R., C. Gossman, V. Radke, J. Moore, E. Busenlehner, K. Fischer, K. Doerr, C. Winkler, M. Franklin-Thomsen, J. Fiander, J. Crowley, E. Peoples, L. Bremby, J. Southard, L. Appleton, D. Bowers, L. Lipsman, H. Callaway, D. Lawrence, R. Gardner, B. Cunanan, R. Snaman, J. Rullan, G. Miller, S. Henderson, M. Mismas, T. York, J. Pearson, C. Lacey, J. Purvis, N. Curtis, K. Mallet, R. Thompson, D. Portesi, D. M. Dwyer, M. Fletcher, M. Levy, T. Lawford, M. Sabat, and M. Kahn. 1997. Outbreak of cyclosporiasis — Northern Virginia – Washington, D.C. — Baltimore, Maryland, metropolitan area, 1997. *J. Am. Med. Assoc.* 278:538–539.

87. Public Health Agency of Canada. 2005. Unpublished data.

88. Public Health Agency of Canada. 2014. Centre for Foodborne, Environmental and Zoonotic Infectious Diseases. Outbreak Summaries Database. Data extracted on 19 February 2014.

89. Public Health Agency of Canada. 2015. Unpublished data.

90. Rabold, J. G., C. W. Hoge, D. R. Shlim, C. Kefford, R. Rajah, and P. Echeverria. 1994. *Cyclospora* outbreak associated with chlorinated drinking water (Letter to Editor). *The Lancet* 344:1360–1361.

91. Raccurt, C. P., B. Fouche, P. Agnamey, J. Menotti, T. Chouaki, A. Totet, and J. W. Pape. 2008. Presence of *Enterocytozoon bieneusi* associated with intestinal coccidia in patients with chronic diarrhea visiting an HIV center in Haiti. *Am. J. Trop. Med. Hyg.* 79:579–580.

92. Relman, D. A., T. M. Schmidt, A. Gajadhar, M. Sogin, J. Cross, K. I. Yoder, O. Sethabutr, and P. Echeverria. 1996. Molecular phylogenetic analysis of *Cyclospora,* in the human intestinal pathogen, suggests that it is closely related to *Eimeria* species. *J. Infect. Dis.* 173:440–445.

93. Riner, D. K., A. S. Mullin, S. Y. Lucas, J. H. Cross, and H. D. A. Lindquist. 2007. Enhanced concentration and isolation of *Cyclospora cayetanensis* oocysts from human fecal samples. *J. Microbiol. Methods* 71:75–77.

94. Robbins, J. A., T. M. Sjulin, and H. P. Rasmussen. 1988. Scanning electron microscope analysis of drupelet morphology of red raspberry and related *Rubus* genotypes. *J. Amer. Soc. Hort. Sci.* 113:474–480.

95. Robertson, L. J., B. Gjerde, and A. T. Campbell. 2000. Isolation of *Cyclospora* oocysts from fruits and vegetables using lectin-coated paramagnetic beads. *J. Food Prot.* 63:1410–1414.

96. Sathyanarayanan, L., and Y. Ortega. 2006. Effects of temperature and different food matrices on *Cyclospora cayetanensis* oocyst sporulation. *J. Parasitol.* 92:218–222.

97. Scallan, E., R. M., Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States — major pathogens. *Emerg. Infect. Dis.* 17:7–15.

98. Shah, L., L. MacDougall, A. Ellis, C. Ong, S. Shyng, L. LeBlanc, and the British Columbia Cyclospora Investigation Team. 2009. Challenges of investigating community outbreaks of cyclosporiasis, British Columbia, Canada. *Emerg. Infect. Dis.* 15:1286–1288.

99. Shellabear, C. K., and A. J. Shah. 1999. *Cyclospora cayetanensis:* an emerging food pathogen. *Food Austral.* 51:30–32.

100. Sherchand, J. B., K. Sherpa, S. Tandukar, J. H. Cross, A. Gajadhar, and J. B. Sherchand. 2010. Infection of *Cyclospora cayetanensis* in diarrhoeal children of Nepal. *J. Nepal Paediatr. Soc.* 30:23-30.

101. Sherchand, J. B., J. Cross, M. Jimba, S. Sherchand, and M. P. Shrestha. 1999. Study of *Cyclospora cayetanensis* in health care facilities, sewage water and green leafy vegetables in Nepal. *S. E. Asian J. Trop. Med. Publ. Health* 30:58–63.

102. Shields, J. M., and B. H. Olson. 2003. PCR-Restriction fragment length polymorphism method for detection of *Cyclospora cayetanensis* in environmental waters without microscopic confirmation. Appl. Environ. Microbiol. 69:4662–4669.

103. Sifuentes-Osornio, J., G. Porras-Cortés, R. P. Bendall, F. Morales-Villarreal, G. Reyes-Terán, and G. M. Ruiz-Palacios. 1995. *Cyclospora cayetanensis* infection in patients with and without AIDS: biliary disease as another clinical manifestation. *Clin. Infect. Dis.* 21:1092–1097.

104. Smith, H. V., C. A. Paton, M. M. A. Mtambo, and R. W. A. Girdwood. 1997. Sporulation of *Cyclospora* sp. oocysts. *Appl. Environ. Microbiol.* 63:1631–1632.

105. Soave, R. 1996. *Cyclospora*: an overview. *Clin. Infect. Dis.* 23:429–437.

106. Steele, M., S. Unger, and J. Odumeru. 2003. Sensitivity of PCR detection of *Cyclospora cayetanensis* in raspberries, basil, and mesclun lettuce. *J. Microbiol. Methods* 54:277–280.

107. Sturbaum, G. D., Y. R. Ortega, R. H. Gilman, C. R. Sterling, L. Cabrera, and D. A. Klein. 1998. Detection of *Cyclospora cayetanensis* in wastewater. *Appl. Environ. Microbiol.* 64:2284–2286.

108. Taylor, A. P., L. J. Davis, and R. Soave. 1997. *Cyclospora. Curr. Clin. Topics Infect. Dis.* 17:256–268.

109. Thomas, M. K., R. Murray, L. Flockhart, K. Pintar, F. Pollari, A. Fazil, A. Nesbitt, and B. Marshall. 2013. Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. *Foodborne Pathog. Dis.* 10: 639–648.

110. Toronto Public Health et al. 1998. Outbreak of cyclosporiasis — Ontario, Canada, May 1998. *Morb. Mortal. Wkly. Rep.* 47:806–809.

111. Tram, N. T., L. M. N. Hoang, P. D. Cam, P. T. Chung, M. W. Fyfe, J. L. Isaac-Renton, and C. S. L. Ong. 2008. *Cyclospora* spp. in herbs and water samples collected from markets and farms in Hanoi, Vietnam. *Trop. Med. Int. Health* 13:1415–1420.

112. Varma, M., J. D. Hester, F. W. Schaefer, M. W. Ware, and H. D. A. Lindquist. 2003. Detection of *Cyclospora cayetanensis* using a quantitative real-time PCR assay. *J. Microbiol.* Methods 53:27–36.

113. Visvesvara, G. S., H. Moura, E. Kovacsnace, S. Wallace, and M. L. Eberhard. 1997. Uniform staining of *Cyclospora* oocysts in fecal smears by a modified safranin technique with microwave heating. *J. Clin. Microbiol.* 35:730–733.

114. Wurtz, R. 1994. *Cyclospora*: a newly identified intestinal pathogen of humans. *Clin. Infect. Dis.* 18:620–623.