

GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

CYCLOSPORA CAYETANENSIS

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Summary

Cyclospora cayetanensis is recognized as an emerging protist that causes diarrheal illness and significantly contributes to the burden of gastroenteritis worldwide. This chapter summarizes the current status of knowledge of the parasite focusing on its public health impact and control strategies. Challenges and limitations for controlling the parasite are discussed.

Cyclospora cayetanensis is an apicomplexan coccidium in the family *Eimeriidae* closely related to *Eimeria* species. The parasite is endemic in tropical areas but reported worldwide. Humans are the only hosts known. *Cyclospora* is responsible for significant morbidity in children and AIDS patients and an important cause of foodborne outbreaks. Young children, older adults, and the immunocompromised are more susceptible to disease.

Globalization of the food supply and increased world travel have contributed to the spread of the parasite to non-endemic areas. Most of the cases have been linked with travelers or foodborne outbreaks. Berries and leafy green vegetables have been implicated as food sources. Consumption of fruits and raw vegetables, drinking untreated water, swimming in rivers, contact with soil or animals, agricultural occupation, and no hand washing have been associated with infection. *Cyclospora* has been isolated from fruits, vegetables, shellfish, drinking water, swimming pools, lakes, rivers, wastewater, sewage water, and soil. The oocysts are highly resistant to environmental conditions, pesticides, and disinfectants.

Cyclospora oocysts can be identified using microscopy, and molecular methods. Techniques for fingerprinting analysis and genotype discrimination are not available. The lack of an animal model and limited DNA sequence data have hampered efforts to develop detection methods. The complete apicoplast and mitochondrial genomes of *C. cayetanensis* were recently obtained which could facilitate the development of genotyping tools.

Prevention and control measures include improvement of personal hygiene, efficient sanitation, and improved water quality management. Food safety training worldwide is necessary. Quantifying risk and controlling *Cyclospora* in the environment are complicated by the low infectious dose, the highly resistant oocysts, and the longer sporulation and pre-patent periods.

Cyclospora cayetanensis

Cyclospora cayetanensis is the only known species of the genus *Cyclospora* to infect humans. Infection results in enteric disease, primarily diarrhea, but asymptomatic infection has been observed. This protozoan parasite is a fecal-oral pathogen in which the oocyst from excreta must mature in the environment (eg sewage, water or soil) to become infectious. About 40% of oocysts sporulate and

become infectious within 14 days at temperatures between 23 and 32°C (Ortega et al., 1994) To date no animal reservoir hosts are known. Transmission appears to be primarily foodborne through fresh fruits and vegetables although contact with human fecally contaminated irrigation water, drinking water, recreational water, sewage, and soil has been documented. Exposure to *C. cayetanensis* in non- endemic locations has increased in parallel with the globalization of the food supply, increased consumption of fresh foods, human migration, and increased world travel. The opening of new markets for fresh fruits and vegetables from endemic areas where crops are grown has transformed food consumption patterns resulting in consumption of raw or undercooked foods potentially exposing consumers to pathogenic contaminants including *C. cayetanensis*. Seasonal variation in the prevalence of cyclosporiasis may be influenced by factors such as rainfall, temperature, and humidity. Climate change is likely to influence the exposure to the parasite.

Water sanitation is essential to control the transmission of cyclosporiasis in both developed and developing countries as well as improved hygiene associated with food safety. A better global understanding of this parasite and the hazard analysis and critical control points (HACCP) arrangements could play a significant role in the control of *Cyclospora* (Buisson et al., 2008). In industrialized countries, surveillance of foodborne diseases has become a fundamental component of food safety systems (Gervelmayer et al., 2008).

1.0 Epidemiology of the Disease and Pathogen

1.1 Global Burden of Disease

1.1.1 Global distribution

Cyclospora cayetanensis infection has been found worldwide, in developed and developing countries and in urban and rural areas but is most common in tropical and subtropical areas (Ortega, 1998). The first documented cases were found in Papua, New Guinea in 1977 and 1978 (Ashford, 1979).

In endemic countries, large-scale surveillance studies of apparently immunocompetent individuals have reported *Cyclospora* infection rates from 0 to 41.6% (Table 1). In immunocompromised patients, mostly HIV/AIDS patients with diarrhea, the percentages of *Cyclospora* infections have ranged from 0 to 36% (Chacin-Bonilla et al., 2001, 2006, 2010). In Colombia, Saudi Arabia, Malaysia, Tanzania, and Cameroon, infection rates of 2.6, 5.9, 4.9, 1.2, and 3.6%, respectively, have been found (Arzuza et al., 2003; Al-Megrin, 2010; Asma et al., 2011; Cegielski et al., 1999; Nsagha et al., 2016). Variations in prevalence of infection may be influenced by study design, geographic area, age, and immunologic status of the population studied, seasonal variability of the parasite, methods of detection used, and expertise of the microscopist.

Table 1. Selected reports of Cyclospora prevalence in immunocompetent individuals from developing countries

Area	Population	Infected Percentage (# of samples)	Reference
Bangladesh	2 to 5 yr	0% (0/289)	Haque et al., 2003
Brazil	All ages	10.8% (9/83)	Días-Borges et al., 2009
China	All ages	5.6% (10/178)	Wang et al., 2002
China	Children	0% (0/252) ^a	Liu et al., 2014
Cuba	0 to 7 yr	4.4% (5/113)	Nuñez et al., 2003
Egypt	All ages	9.2% (12/130)	Nassef et al., 1998
Guatemala	All ages	2.3% (126/5,552)	Bern et al., 1999
Guatemala	Farm families ^b	3.3% (6/182)	Bern et al., 1999
Haiti	All ages	6.0% (24/402)	López et al., 2003
Honduras	All ages	2% (96/4,698)	Kaminsky, 2002
India	All ages	10.6% (33/310)	Gupta, 2011
Indonesia	School children	0.6% (2/348)	Fryauff et al., 1999
Jordan	All ages	6% (12/200)	Nimri, 2003
Lao PDR	All ages	0.1% (1/686)	Kimura et al., 2005
Mexico	Children	3.3% (9/272)	Díaz et al., 2003
Mexico	Children	0.6% (60/8,877)	Orozco-Mosqueda, 2014
Morocco	School children	3.3% (22/673)	El Fatni, 2014
Nepal	All ages	9.2% (128/1,397)	Kimura et al., 2005
Nepal	School children	1.6% (23/1,392)	Tandukar et al., 2013
Nepal	School children	3.9% (20/507)	Bhandari et al., 2015
Nigeria	All ages	1% (11/1,109)	Alakpa et al., 2003
Peru	0 to 2.6 yr	10.9% (41/377)	Ortega et al., 1993
Peru	Children	1.1% (63/5,836)	Madico et al., 1997
Peru	All ages	41.6% (121/291)	Burstein Alva, 2005
Peru	Adults	4.3% (11/256)	Roldán et al., 2009
Saudi Arabia	<5 yr	11.1% (7/63)	Al-Braiken et al., 2003
Thailand	All ages	0.5% (12/2,540) ^a	Thima et al., 2014

Area	Population	Infected Percentage (# of samples)	Reference
Turkey	All ages	0.4% (2/554)	Aksoy et al., 2007
Turkey	All ages	5.7% (129/2,281)	Karaman et al., 2015
Venezuela	Children	5.3% (7/132)	Chacín-Bonilla et al., 2001
Venezuela	All ages	6.1% (13/212)	Chacín-Bonilla et al., 2003
Venezuela	All ages	8.3% (43/515)	Chacín-Bonilla et al., 2007
Venezuela	All ages	24.2% (38/157)	Cazorla et al., 2012
Vietnam	All ages	1% (14/1,425)	Pham-Duc et al., 2013

^aPCR methods; ^b Raspberry farm.

The prevalence of *C. cayetanensis* is unknown in some developing countries. However, the reports of sporadic cases of infections in local residents such as in Argentina (Velasquez et al., 2004), Kuwait (Iqbal et al., 2011), Bangladesh (Albert et al., 1994), New Guinea (Berlin et al., 1994), and South Africa (Markus et al., 1993) or in foreign visitors such as in Puerto Rico (Wurtz et al., 1993), Dominican Republic (Green et al., 2000; Estran et al., 2004; Weitzel et al., 2006), Costa Rica (Cedeño, 2002), Bolivia (Drenaggi et al., 1998), Bulgaria (Ortega et al., 2010), Sri

Lanka, Gabon (Gascon et al., 1995), Lebanon (Lebbad et al., 1993), Cambodia, Solomon Islands (Pollok et al., 1992), Pakistan (Rijpstra et al., 1993), Java, Bali (Deluol et al., 1994), and Madagascar (Bourée et al., 2007) reflect the endemicity of the infection in these nations. In fact, reports of cyclosporiasis from Dominican Republic have been travel-associated cases (Green et al., 2000; Estran et al., 2004; Weitzel et al., 2006). However, of 398 human fecal samples collected from nine different health centers in this country, 9 (2.3%) had *Cyclospora* oocysts (Lalonde et al., 2013). Figure 1 shows the distribution of cyclosporiasis in developing countries.

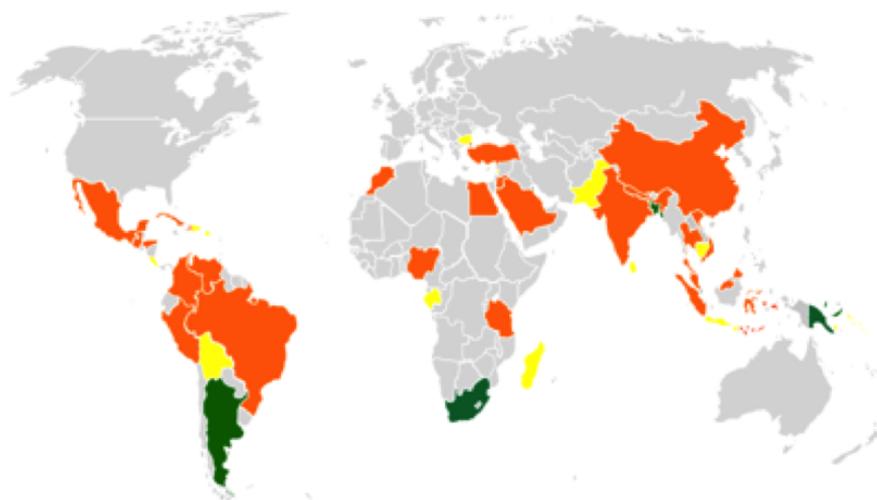


Figure 1. Distribution of cyclosporiasis in developing regions: countries that have reported infection-endemic areas (orange), infection cases without travel history (green), or have been visited by travelers that acquired infection (yellow)

Cyclosporiasis has been found to be common in children in endemic areas. They are often asymptomatic or have relatively mild illness (Madico et al., 1997; Ortega et al., 1997a; Eberhard et al., 1999b; Chacín-Bonilla et al., 2003, 2007). High percentages of asymptomatic carriers (68.2–98.7%, average 87.1%) have been noted in community-based surveys (Chacín-Bonilla, 2010; Bhandari et al., 2015); in some studies, up to 100% of infected children were asymptomatic (Thima et al., 2014), suggesting a development of immune protection from disease but not infection. Thus, in endemic settings, *C. cayetanensis* may not play a consistently pathogenic role. It appears that in these areas, the situation at the general population level is quite different than that observed in children that attended health centers in whom a strong association of the parasite with diarrhea has been recognized (Zerpa et al., 1995; Fryauff et al., 1999; Al-Braiken et al., 2003; Nuñez et al., 2003; Mansfield and Gajadha, 2004). This may be related to the risk of exposure from shared foods and water. This finding suggests that cyclosporiasis is common in impoverished areas where water and food sanitation are poor or nonexistent. It may be that very early and persistent exposure may be

associated with immunity to illness and asymptomatic excretion (Madico et al., 1997; Ortega et al., 1997a; Bern et al., 2002). In fact, after an initial episode of cyclosporiasis, the likelihood of diarrhea and duration of symptoms decreases significantly with each subsequent infection (Bern et al., 2002).

Outbreaks of cyclosporiasis have also been reported among local populations and foreign residents or visitors in the developing world (Shlim et al., 1991; Hoge et al., 1993; Rabold et al., 1994). Table 2 shows epidemics that occurred in the 2000s. The explanation for the epidemics in local adult populations is that acquired immunity in these areas is not long lasting and fades over time (Torres-Slimming et al., 2006) or that geographic distribution, prevalence, and spread of the parasite in one region may vary from one place to another leaving some populations unprotected, particularly those from the upper social class (Mundaca et al., 2008). The limited outbreaks of cyclosporiasis reported in endemic areas could be the indiscriminate use of antibiotics effective against *C. cayetanensis* and the lack of adequate diagnostic capability (Torres-Slimming et al., 2006).

Table 2. Worldwide Cyclospora outbreaks: 2000-2015

Area	Date	No. of Cases ^a	Vehicle	Origin	Reference
Australian cruise ship: Fremantle departure	2010 May-Jun	266 ^b	Lettuce ^c	Malaysia ^c	Gibbs et al., 2013
Canada (BC) ^d	2001 May	17	Thai basil	USA	Hoang et al., 2005
Canada (BC)	2003 Jul	11	Cilantro ^c	UD ^e	Kozak et al., 2013
Canada (BC)	2004	17	Mango, basil ^c	UD	Kozak et al., 2013
Canada (BC)	2004 May-Jun	8	Cilantro ^c	UD	PHAC ^f , 2006
Canada (BC)	2006 Jun-Jul	28	Basil or garlic	UD	Kozak et al., 2013
Canada (BC)	2007 May-Aug	29	Basil	Mexico	Shah et al., 2009
Canada (Ontario)	2005 Apr	44	Basil ^c	UD	Kozak et al., 2013
Canada (Quebec)	2005 Jul	200	Basil	Mexico	PHAC, 2007
Canada ^g	2015 May-Aug	97	UD	UD	PHAC, 2015
Colombia (Medellin)	2002 Apr	31	Salads, juice	UD	Botero-Garcés et al., 2006
Cruise ship (Several countries)	2009 Apr	160	UD	UD	CDC, 2009
Germany	2000 Dec	34	Salads, herbs	France, Italy, Germany	Doller et al., 2002
Indonesia (Bangor)	2001 Sep	14	UD	UD	Blans et al., 2005

Area	Date	No. of Cases ^a	Vehicle	Origin	Reference
Mexico (Monterrey)	2001 Apr	97	Watercress	UD	Ayala-Gaytán et al., 2004
Peru (Lima)	2004 Nov	127	UD	UD	Torres-Slimming et al., 2006
Peru (Lima)	2005 Mar	37	UD	UD	Mundaca et al., 2008
Poland	2013 Nov	3 ^h	Drinking water	Indonesia	Bednarska et al., 2015
Spain (Madrid)	2003 May	11 ^h	Raspberry juice	Guatemala	Puente et al., 2006
Sweden (Stockholm)	2009 May-Jun	18	Snaps peas	Guatemala	Insulander et al., 2010
Turkey (Izmir)	2005 Sep	19	Drinking water	UD	Aksoy et al., 2007
Turkey (Istanbul)	2007 Jul-Aug	286	UD	UD	Ozdamar et al., 2008
United Kingdom ^g	2015 Jun-Sep	79 ^h	UD	Mexico	Nichols et al., 2015
USA (Pennsylvania)	2000 Jun	54	Raspberry cake	Guatemala	Ho et al., 2002
USA (Texas, Illinois)	2004 Feb	95	UD	UD	Ortega et al., 2010
USA (Pennsylvania)	2004 Jun-Jul	96	Snow peas	Guatemala	CDC, 2004
USA (Florida)	2005 Apr	592	Basil	UD	Hammond, 2005
USA ^g : Texas	2013 Jun-Aug	270	Cilantro	Mexico	Abanyie et al., 2013
USA ^g : Iowa, Nebraska	2013 Jun-Aug	227	Lettuce	Mexico	Buss et al., 2016
USA ^g	2014 Jun-Aug	304	Cilantro	Mexico	CDC, 2014
USA ^g	2015 May-Aug	546	Cilantro ^c	UD	CDC, 2015

^a Both laboratory-confirmed and clinically defined cases are included; ^b 34 and 232 cases in two consecutive voyages; ^c Suspect; ^d British Columbia; ^e Undetermined; ^f Public Health Agency of Canada; ^g Multistate outbreak; ^h Travelers.

1.1.1.1 Age and sex distribution

In endemic areas, most of the studies on prevalence of the infection and association with disease have been conducted in children that have attended clinics, hospitals or laboratories and have been skewed towards those with clinical manifestations. The highest risk of infection and diarrhea occur in the first five years of life (Hoge et al., 1995; Madico et al., 1997; Ortega et al., 1998; Chacín-Bonilla et al., 2001; Bern et al., 2002). In children less than 18 months of age, *Cyclospora* infections were detected in Nepal (Sherchand et al., 1999, 2001) but undetected in an outpatient primarycare clinic (Hoge et al., 1995), uncommon in Guatemala (Bern et al., 1999) and Venezuela (Chacín-Bonilla et al., 2001) and present but asymptomatic in Peru (Ortega et al., 1993). It is not known if it is due to weaning maternal antibodies or to limited environmental exposure in this age group.

The community-based studies of *Cyclospora* age distribution are scarce. In a 2-year cross sectional study in Peru, the prevalence of *C. cayetanensis* was highest among children 2-4 years of age and was not observed among individuals older than 18 years of age (Madico et al., 1997). In another study from the same region, the infection was not detected in persons older than 11 years of age (Ortega et al., 1998). In Guatemala (Bern et al., 1999), Honduras (Kaminsky, 2002), Haiti (López et al., 2003), Cuba (Nuñez et al., 2003), Venezuela (Chacín-Bonilla et al., 2007), Nepal (Kimura et al., 2005; Tandukar et al., 2013), Turkey (Turgay et al., 2007), and Thailand (Thima et al., 2014) the infection was more frequent in school children less than 15 years of age. In Henan, China, children 7-17 years of age had the highest detection rate (Zhou et al., 2011). The causes for this age distribution pattern are not clear but may be related to predominant modes of exposure. *C. cayetanensis* is usually transmitted by exposure to contaminated environmental sources from

which young children are relatively protected (Bern et al., 2002). Significant differences of *Cyclospora* infection rate by gender have not been reported. In Haiti and Venezuela, the overall male: female risk ratios were 1.04 and 1.3, respectively (Eberhard et al., 1999b; Chacin-Bonilla et al., 2007).

1.1.1.2 Seasonal distribution

In addition to geographic variability, a marked seasonality of the prevalence of *Cyclospora* infection has been described in several endemic countries. However, it is not uniform among different regions and defies easy explanation (Herwaldt, 2000). The seasonal trend of increased prevalence of cyclosporiasis described in various nations often coincides with warm periods of maximal rainfall as reported in Guatemala (Bern et al., 1999), Honduras (Kaminsky, 2002), Mexico (Orozco-Mosqueda et al., 2014), Jordan (Nimri, 2003), Nepal (Hoge et al., 1993, 1995; Sherchand et al., 2001; Kimura et al., 2005; Bhandari et al., 2015), Indonesia (Fryauff et al., 1999), and China (Zhou et al., 2011). In contrast, infection has been more prevalent in the absence of rain during the drier and hotter months of the year in Lima, Peru (Madico et al., 1997; Bern et al., 2002) and Turkey (Turgay et al., 2007) and in cooler time in Haiti, where temperature fluctuations appear to be the moderator of the infection seasonality (Eberhard et al., 1999b). The seasonal variation of *C. cayetanensis* suggests that environmental factors are important in the life cycle of this parasite and that it is likely to be influenced by several of them such as rainfall, temperature, and humidity.

In non-endemic industrialized nations, individual cases of cyclosporiasis as well as outbreaks are linked mostly to international travel and consumption of contaminated imported produce, usually from endemic regions. The parasite is a common cause of traveler's diarrhea. The first documented US cases occurred in the mid-1980s in travelers returning from Haiti and Mexico (Soave et al., 1986). Between 1997 and 2008, 33.5% of laboratory confirmed cases of infection in the US were travel related (Hall, 2011), whereas in Canada, 71% of reported cyclosporiasis cases in 2006 were in travelers (Thomas, 2013). The coccidium was only documented as a significant human pathogen in the mid-1990s when it was recognized as the causative agent of multistate outbreaks of diarrheal illness in the US and Canada, mostly associated with fresh

food produce such as soft fruits (berries) and leafy vegetables imported from Mexico and Central America (Chambers et al., 1996; Anonymous, 1997; Herwaldt et al., 1997, 1999, 2000; Dawson, 2005). Since 1990, nearly all reported outbreaks in the US and Canada have been associated with food and almost all the cases have been mostly related to Guatemalan raspberries. These outbreaks occurred during the spring and early summer, a warm and rainy season (Herwaldt, 2000; Shields et al., 2003a). The outbreak that brought cyclosporiasis to importance in North America and established the link to foodborne transmission of infection occurred in the spring of 1996 and was transmitted by fresh raspberries imported from Guatemala. A total of 1,465 cases were reported by 20 states and the District of Columbia in the US, and two Canadian provinces (Herwaldt et al., 1997). In the 1990s, at least 19 outbreaks of cyclosporiasis were documented worldwide, most of them (16) were reported regularly in North America including high-profile, multistate outbreaks in the US and Canada; three were in Nepal (Shlim et al., 1991; Hoge et al., 1993; Rabold et al., 1994; Herwaldt, 2000; Ortega et al., 2010; Kozak et al., 2013). Since 2000, clusters of cases have been documented in the US and Canada; at least 31 epidemics have been reported worldwide, 18 in North America (Table 2). The 2013 multistate outbreaks in the US affected 25 states (primarily Texas, Iowa, and Nebraska) with 631 laboratory confirmed cases of disease. These outbreaks contributed to the largest annual number of reported US cases of cyclosporiasis since 1997 (Abanyie et al., 2013). The 2014 and 2015 multistate epidemics in this country involved 304 and 546 confirmed cases in 19 and 31 states, respectively; most of the cases were reported among Texas residents (CDC, 2014, 2015). The 2015 outbreak in Canada involved Ontario and Quebec provinces (PHAC, 2015). *C. cayetanensis* outbreaks have been mostly reported in North America, probably due to better detection methods and disease surveillance that have helped in tracking outbreaks. Figures 2 and 3 present the distribution by country and continent, of at least 49 outbreaks reported since 1991, excluding one on a cruise ship that involved people from several countries (Ortega et al., 2010).

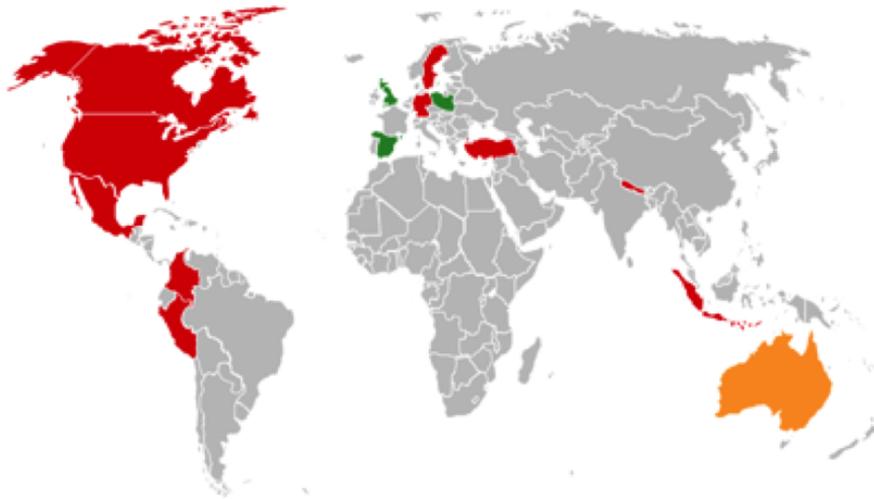


Figure 2. Distribution of worldwide cyclosporiasis outbreaks: countries that have reported epidemics in residents (red), in cruise ship (orange), or in travelers (green)

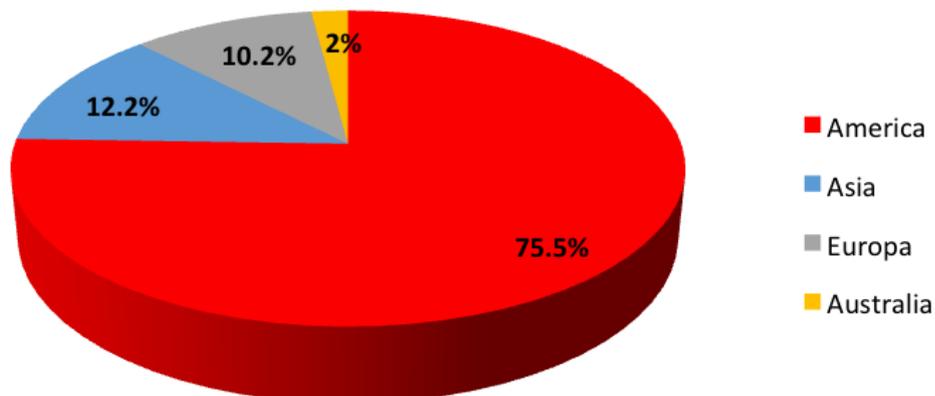


Figure 3. Percentage distribution, by continent, of all reported worldwide cyclosporiasis outbreaks

In North America, outbreaks of waterborne disease have been identified (Huang et al., 1995; Dawson, 2005; Karanis et al., 2007; Baldursson et al., 2011). Exposure to contaminated drinking water, recreational water, or to sewage have spread infection to a lesser extent (Wurtz et al., 1993; Hale et al., 1994; Ooi et al., 1995; Dawson, 2005). Unlike the US and Canada, most cases of cyclosporiasis in

Europe and Australia have been linked with international travel to endemic areas. Cyclosporiasis has been reported in Spain, France, Belgium, Italy, Germany, Greece, United Kingdom, Ireland, Sweden, Switzerland, The Netherlands, New Zealand, and Australia (Shlim et al., 1991; Clarke et al., 1996; Drenaggi et al., 1998; Puente et al., 2006; Bourée et al., 2006, 2007; Ortega et al., 2010). Outbreaks of

cyclosporiasis have also been reported in travelers from Spain (Puente et al., 2006), Poland (Bednarska et al., 2015) and the United Kingdom (Nichols et al., 2015) (Table 2).

Risk factors for cyclosporiasis in industrialized countries include international travel to cyclosporiasis-endemic areas and domestic consumption of contaminated fresh produce imported from these regions. In the US, most of the cases of cyclosporiasis have been linked to imported foods (Herwaldt, 2000). However, there have been sporadic reports of infection where no food source or history of international travel was implicated (Hale et al., 1994; Ooi et al., 1995; Wurtz, 1993). The rate of *Cyclospora* endemic infection in the general population of North America and United Kingdom was less than 0.5% between 1992 and 1995 during non-outbreak periods (Herwaldt, 2000; Ribes et al., 2004). Of a total of 370 laboratory-confirmed cases of *Cyclospora* infection reported during 1997-2009 via the Foodborne Diseases Active Surveillance Network in the USA, 70.3% of them were concentrated in Georgia and Connecticut. During the period of 2004-2009, 37.8% (70/185) of the cases were classified as domestically acquired (Hall et al., 2012). In the USA, cyclosporiasis is not thought to be endemic although the possibility of foci with low-level endemicity has been considered (Herwaldt, 2000, 2006). The sources of a foodborne outbreak in Germany were epidemiologically traced to lettuce and herbs from Germany, France, and Italy; the contamination of food crops could have occurred by seasonal agricultural workers without access to adequate sanitary facilities (Doller et al., 2002). In Europe, *C. cayetanensis* oocysts were detected in 9% of samples tested of drinking water, wastewater, and recreational water in Madrid, Spain (Galvan et al., 2013) and in 15.5% of several environmental matrices including treated wastewater, soil, and vegetables in Apulia, southern Italy with a high prevalence of infection in humans (27.5%, 11/40) (Giangaspero et al., 2015b). In southern Arizona in the US, 19% of wastewater samples were positive for the parasite (Kitajima et al., 2014). The German *Cyclospora* outbreak and the finding of the parasite in different biological matrices in the former countries suggest that irrigation water, soil, and vegetables may represent a source of cyclosporiasis in these areas and illustrates the potential for *C. cayetanensis* to become endemic in industrialized nations. The study on the occurrence of *C. cayetanensis* in treated wastewater from Arizona showed that infection is prevalent in this area. It is conceivable there is some level of waterborne cyclosporiasis in developed nations since several outbreaks of cyclosporiasis have been attributable to water (Rabold et al., 1994; Huang et al., 1995; Aksoy et al., 2007).

1.1.2 Symptomatology

Cyclospora cayetanensis is an important emerging cause of diarrhea worldwide that can lead to significant morbidity in children and AIDS patients. It is also a major cause of foodborne diarrheal illness in industrialized countries (Mansfield and Gajadha, 2004). Asymptomatic carriage of *Cyclospora* occurs. For others, the clinical course and severity of infection can vary considerably from patient to patient, depending in large part on the immune

status of the person. The disease is characterized by watery diarrhea, anorexia, fatigue, body aches, mild to severe nausea, abdominal cramping, flatulence, low-grade fever, and weight loss (Herwaldt, 2000). Severe dehydration can occur (Gajadhar et al., 2015). The infection is usually self-limiting, but symptoms can relapse for several weeks or months. In immunocompetent hosts, mild-to-moderate, self-limiting diarrhea is common. In immunocompromised hosts, severe intestinal injury and prolonged diarrhea is observed (Shields et al., 2003a).

In endemic areas, younger children have more severe symptomatology but frequent exposure may result in a gradual reduction in the severity of illness as they age to asymptomatic infections, and in the absence of symptomatic infections in adults (Madico, 1997; Ortega et al., 1997a; Bern et al., 2002; Chacin-Bonilla, 2010; Thima et al., 2014). In the developed world, travelers and expatriates infections are almost always symptomatic.

Infection causes significant morbidity in immunocompromised individuals, in particular those with HIV infection. The risk of infection and severity of illness are related to the state of immunosuppression of the patients. They tend to present severe, chronic or intermittent diarrhea that may last for weeks with significant weight loss. The average duration of diarrhea for HIV patients is longer than that for HIV negative patients (199 days vs 57.2 days) (Sifuentes-Osorio et al., 1995). There is a high recurrence rate of cyclosporiasis in HIV patients (Pape et al., 1994; Soave, 1996). Acalculous cholecystitis has been reported in these patients (Sifuentes-Osorio et al., 1995; Zar et al., 2001).

Cyclosporiasis has been associated with various sequelae including biliary disease (Sifuentes-Osorio et al., 1995; de Gorgolas et al., 2001), acalculous cholecystitis (Sifuentes-Osorio et al., 1995; Zar et al., 2001), Guillain-Barre syndrome (Richardson et al., 1998), and Reiter syndrome (Connor et al., 2001).

Histopathological alterations of the small intestine include diffuse edema and infiltration by inflammatory cells with villous atrophy and crypt hyperplasia, characterized by shortened blunted villi and increased crypt length (Ortega, 1997a). Loss of villar surface in the intestine can occur (Gajadhar et al., 2015). An accumulation of an electron-dense phospholipid membrane/myelin-like material of the enterocytes has been described (Connor et al., 1999).

1.1.3 Economic impact

Cyclosporiasis has serious implications for young children, travelers to endemic areas, immunocompromised patients, and naive populations. Although the global incidence and prevalence of morbidity, disability, and mortality associated with acute and chronic cyclosporiasis have not been estimated, diarrheal disease disproportionately affects developing countries, but gastroenteritis also is a significant problem in industrialized nations.

About 99% of the USA cyclosporiasis cases are estimated to be foodborne (Mead et al., 1999), resulting in an estimated 11,407 foodborne incident cases and 11 hospitalizations per year (Scallan et al., 2011). From these estimates, the annual cost of infection in the US has been estimated to be \$11 million using a basic Cost of Illness (COI) model and \$17 million when pain and suffering were also considered in the COI model (Scharff, 2012). In 1996 and 1997, the US and Canadian health officials reported 2,944 cases (132 clusters) of cyclosporiasis (Shields et al., 2003a).

A study was conducted to estimate the disease burden of 14 pathogens in food sources in the US, using attribution data from outbreak investigation and expert elicitation, from 1999 through 2008. The health burden associated with each pathogen was measured using new estimates of the cost of illness and loss of quality-adjusted life year (QALY) from acute and chronic illness and mortality. For *Cyclospora*, annual number of illnesses, hospitalizations, and QALY losses were 11,407 (137-37,673), 11 (0-109), and 10 (0-33), respectively. Annual burden of disease was \$2 million and ranged from \$0 to \$8. Based on exposure to this pathogen, produce was responsible for 96% of illness burden (Batz et al., 2012).

Sporadic outbreaks of cyclosporiasis, including the multistate outbreaks in the USA in 2013-2015 (Abanyie et al., 2013; CDC, 2014, 2015) and Canada in 2015 (PHAC, 2015), underscore the continued burden of illness this protist presents in developed countries. Cyclosporiasis might have long-term negative consequences since early childhood diarrheal illness could have serious impacts on children's growth and cognitive development and may predispose them to chronic metabolic disease in later life (Guerrant et al., 2013).

1.2 Taxonomic Classification of the Agent

1.2.1 Physical description of the agent

The environmental stage excreted in the feces is the oocyst. Oocysts are microscopic with a diameter of 7.7-9.9 µm, and spheroidal in shape. With the modified acid-fast stain, some stain dark red and have a variable number of dark inclusion bodies, whereas others do not stain at all and appear as transparent spheres. Viewed with epifluorescence ultraviolet microscopy using a 365 nm or a 490 nm dichromatic filter, oocysts autofluoresce blue or green (Ortega et al., 1993). Viewed with transmission electron microscopy, a freshly excreted oocyst contains a granular undifferentiated cytoplasm surrounded by a two-layered oocyst wall (63 and 50 nm thick, respectively); the cytoplasm has no unique distinguishing structures. Upon exposure to air the oocyst undergoes sporulation. This process takes 7-15 days. The oocysts are not infectious upon excretion. About 40% of oocysts sporulate and become infectious within 14 days, at temperatures between 23 and 32°C (Ortega et al., 1994). A sporulated oocyst contains two sporocysts, each with 62-nm-thick walls surrounding a plasma membrane. Each sporocyst has a Stieda and substiedal bodies at one end and a residuum

consisting of spherical globules. Each sporocyst has two sporozoites. The presence of two sporozoites in each of the two sporocysts is the defining diagnostic criterion for the genus *Cyclospora* (Ortega et al., 1993, 1994).

1.2.2 Taxonomy

Members of the genus *Cyclospora* are protozoan parasites in the subphylum *Apicomplexa*, subclass *Coccidiasina*, order *Eucoccidiorida*, family *Eimeriidae* (Shields et al., 2003a). Nineteen species of *Cyclospora* have been described, based mainly on conventional microscopic analysis of oocysts in feces from reptiles (mostly snakes), insectivores, rodents, primates, and humans (Lainson, 2005). *Cyclospora cayetanensis* is the only species in the genus known to infect humans. *Cyclospora colobi*, *C. papionis*, and *C. cercopitheci* were identified on the basis of 18S rRNA gene sequence analysis from primates in Ethiopia and Kenya. These three species are host specific although they are closely related to *C. cayetanensis* based on morphologic and molecular studies (Eberhard et al., 1999a). *C. colobi*-like organisms were identified in snub-nosed golden colobus monkeys in northwestern China (Zhao et al., 2013). Three additional species have been reported to infect dairy cattle in China (Li et al., 2007), drills on Bioko Island, western Africa (Eberhard et al., 2014) and rhesus monkeys; the latter was named *Cyclospora macacae* (Li et al., 2015).

Beginning in 1979, before *C. cayetanensis* was identified and named, there were reports describing an *Isospora*-like organism, a coccidian-like body, large *Cryptosporidium*, or *Cyanobacterium*-like body, associated with diarrhea in humans (Ashford, 1979; Long et al., 1991; Shlim et al., 1991; Gascon et al., 1993). Subsequently, following successful sporulation and excystation of the oocysts isolated from Peruvians with persistent diarrhea, *C. cayetanensis* was described and named (Ortega et al., 1993, 1994). Molecular analysis of nuclear *ssrDNA* sequences suggested that *C. cayetanensis* is phylogenetically closely related to other coccidia, especially members of the genus *Eimeria* (Relman et al., 1996; Ogedengbe et al., 2015; Cinar et al., 2015). Phylogenetic analysis grouped the *Cyclospora* species infecting primates, including *C. cayetanensis* in humans, forming a group closely related to avian *Eimeria* species (Li et al., 2015). Because of limited molecular testing of specimens from humans it is not yet known whether all human *Cyclospora* isolates belong to the same species and whether the closely related *Cyclospora* species described from lower primates infect humans.

Different genes have been assessed for elucidating evolutionary relationships between *C. cayetanensis* strains to aid in molecular epidemiology. Analysis of heat shock protein and 18 ribosomal RNA (18S rRNA) genes of *C. cayetanensis* from humans in Mexico, Peru, and Nepal showed existence of genetically homogeneous population for the *C. cayetanensis* parasites at both genes (Sulaiman et al., 2013, 2014). Analysis of the 18S rRNA gene of *C. cayetanensis* isolates and among members of *C. colobi*, *C. papionis*, and *C. cercopitheci* showed a significant distinct

genetic variation among species and a minor genetic diversity within the species (Sulaiman et al., 2014). Examination of 18S rRNA gene sequences of isolates from China also revealed only minor sequence polymorphisms (Zhou et al., 2011). The intervening transcribed spacer 1 (ITS-1) is highly variable even within individual oocysts and is therefore not reliable for inferring relationships between strains (Olivier et al., 2001). Efforts are underway to characterize a few more genetic loci to better understand the population genetic structure and transmission dynamics of *Cyclospora*.

Recently, the full-length mitochondrial and apicoplast genomes of *C. cayetanensis* have been reported (Tang et al., 2015; Qvarnstrom et al., 2015; Cinar et al., 2015). Both genomes are highly similar to those of cecum-infecting avian *Eimeria* spp. Sequence variations in the mitochondrial genome between two Chinese isolates and one US *C. cayetanensis* isolate have been identified (Tang et al., 2015). Another study found the mitochondrial genome to have a close phylogenetic relationship with *Eimeria magna*, a coccidian infecting rabbits (Cinar et al., 2015). Through a greater availability of whole genome sequencing and comparative genomic analysis, it was shown that sequences would improve our understanding of the biology of *C. cayetanensis* which probably possesses a classical coccidian metabolism and has a host cell invasion system very similar to *Eimeria* spp. and *Toxoplasma gondii*. The dominant surface antigens observed in other coccidian are not present or significantly diminished (Liu et al., 2016). Nevertheless, these results need to be validated. Further characterization of the genomes of additional *C. cayetanensis* isolates and other *Cyclospora* species is needed to improve our comprehension of the taxonomic position and biology of *Cyclospora*.

1.2.3 Life cycle

Humans are the only known host for *C. cayetanensis*. It is an obligate intracellular parasite that requires a single host to complete the entire life cycle. Asexual and sexual

stages have been observed within the epithelium of the gastrointestinal tract of the host (Sun et al., 1996; Ortega et al., 1997a; Connor et al., 1999).

The life cycle (Figure 4) starts with the ingestion of the sporulated oocyst, which excysts in the gut releasing infective sporozoites that invade the epithelial cells of the duodenum and jejunum (Sun et al., 1996; Ortega et al., 1997a). The sporozoites transform into trophozoites which undergo merogony and form two types of meronts. Type I meronts contain 8–12 merozoites which penetrate host cells and each merozoite develops into a type II meront that develops to contain four merozoites. Once liberated, these merozoites enter other host cells and begin the gametogony cycle by differentiating into either male (microgametocyte) or female (macrogametocyte) stages. The male stage forms flagellated microgametes. The fertilized macrogametocyte develops into a zygote. A resistant wall is then formed around it and develops into an oocyst which contains the sporont (Ortega et al., 1997a; Connor et al., 1999). The unsporulated, noninfective oocysts are passed in the stool and sporulation occurs yielding infective oocysts containing two sporocysts, each one with two banana-shaped sporozoites (Ortega et al., 1993, 1994). The environmental conditions for sporulation are not yet completely understood although for other genera of coccidia, exposure to air is required. Most coccidians pathogenic to humans require short periods of time to sporulate. However, *Cyclospora* oocysts require prolonged time outside the host, depending on climatic factors, for sporulation to take place in the environment (Ortega et al., 1998). Experimentally, sporulation has been carried out by suspending oocysts in 2.5% potassium dichromate in water often with constant or intermittent stirring (Ortega et al., 1993). About 40% of oocysts sporulate within 14 days at temperatures between 23 and 32°C (Ortega et al., 1994). It is not known why *C. cayetanensis* requires a much longer time to sporulate than other coccidia.

1.3.2 Waterborne transmission

In countries where *C. cayetanensis* is endemic and water and sewage treatment systems insufficient or lacking, waterborne oocysts are a likely source of infection because they are environmentally robust (Mansfield and Gajadha, 2004), sufficiently small to penetrate the physical barriers of water treatment, and insensitive to many disinfectants used in the water industry (Rabold et al., 1994; Soave et al., 1998). Furthermore, the infectious dose is low, although it has not been fully described (Sterling et al., 1999; Dixon et al., 2005), probably between 10 and 100 oocysts (Adam et al., 1999).

In Nepal, infection was associated with untreated water in several studies (Hoge et al., 1993; Tandukar et al., 2013; Bhandari et al., 2015) and three other outbreaks were related to drinking water (Shlim et al., 1991; Hoge et al., 1993; Rabold et al., 1994). An outbreak affecting foreign soldiers and dependents was linked with drinking water containing *Cyclospora* oocysts. This water was a mixture of river and municipal water that was chlorinated and filtered but the organisms were not completely removed (Rabold et al., 1994).

The first reported outbreak of cyclosporiasis in the US was in Chicago, where 23 cases were linked to a hospital water supply. Epidemiologic studies implicated tap water in the physician's dormitory as the most likely source of the outbreak. Stagnant water in a storage tank may have contaminated the water supply after a pump failure. Examination of water samples did not reveal *Cyclospora* oocysts (Huang et al., 1995). A follow-up study of this epidemic revealed that drinking tap water and attendance at a house staff party were significant risk factors. For this reason, the possibility of a food-borne outbreak associated with the food served at the house staff party has been pointed out (Ortega et al., 2010).

In Peru, cyclosporiasis was associated with consumption of unchlorinated water (Zerpa et al., 1995). In a case-control study from Guatemala, several variables related to water were associated with risk for *Cyclospora* infection including drinking untreated water, and

swimming in rivers or springs (Bern et al., 1999). In Haiti, the only factor associated with infection was drinking water from an artesian well (López et al., 2003). In another study in Turkey, a cyclosporiasis outbreak was linked to drinking water (Aksoy et al., 2007).

Sewage water was also identified as a possible source of cyclosporiasis in Nepal (Sherchand et al., 1999, 2001). In an Egyptian village, *Cyclospora* oocysts, possibly from sewage contamination, were detected in several water sources suggesting water was an important source of infection (el-Karamany et al., 2005). Of 524 water-associated outbreaks of protozoan disease reported worldwide, *C. cayetanensis* was the causative agent in nine (1.7%) (Karanis et al., 2007; Balduresson et al., 2011).

It has been demonstrated that shellfish Identification of *C. cayetanensis* in shellfish in Alexandria, Egypt (Negm, 2003) and Izmir, Turkey (Aksoy et al., 2014) suggests that freshwater run-off from land can carry oocysts into the marine ecosystem, a further concern for waterborne oocysts in the spread of infection where seafood consumed raw and recreation in marine water could potentially increase the risk of infection.

These findings of *C. cayetanensis* in several types of water (Table 3) suggest the potential spread of the parasite by drinking and recreational water, including chlorinated water, and wastewater in endemic areas and potentially in non-endemic areas as a single event. It has been hypothesized that contamination of Guatemalan raspberries could have occurred during the preparation of insecticides and fungicides using contaminated river water or by cross-contamination from hands of pickers or handlers of crops (Sterling et al., 1999; Sathyanarayanan et al., 2004). However, even when *C. cayetanensis* has been detected in water and food related to an outbreak, the source of contamination has not been established (Huang et al., 1995; Colley, 1996; Herwaldt et al., 1997). It remains a matter of speculation. *C. cayetanensis* can contaminate crops via different pathways including black water used for irrigation or spraying of crops, contact with contaminated soil, or contact with infected food handlers with hands that have been in contact with contaminated soil (Dawson, 2005).

Table 3. Isolation and prevalence of Cyclospora in environmental matrices from several countries

Area	Matrices Analyzed	Contaminated Percentage (# of Samples)	Reference
Cambodia	Water spinach	8.3% (3/36)	Vuong et al., 2007
Canada	Pre-cut salads, leafy greens	1.6% (9/544)	Dixon et al., 2013
Costa Rica	Lettuce	4% (2/50)	Calvo et al., 2004
Egypt	Drinking water and rivers	0.2% (2/840)	el-Karamany et al., 2005
Egypt	Potable water	21.3% (64/300)	Elshazly et al., 2007

Area	Matrices Analyzed	Contaminated Percentage (# of Samples)	Reference
Ghana	Sachet drinking water	59.3% (16/27)	Kwakye-Nuako et al., 2007
Ghana	Vegetables	11.9% (20/168)	Duedu et al., 2014
Guatemala	Rivers	6.7% (2/30)	Bern et al., 1999
Guatemala	Drinking water sources	41.7% (5/12) ^a	Dowd et al., 2003
Italy	Tap water	30% (3/10) ^a	Gianguaspero et al., 2015a
Italy	Vegetables and fruits	12.2% (6/49) ^a	Gianguaspero et al., 2015b
Italy	Treated wastewater	21.3% (20/94) ^a	Gianguaspero et al., 2015b
Italy	Well water	6.2% (1/16) ^a	Gianguaspero et al., 2015b
Italy	Soil	11.8% (6/51) ^a	Gianguaspero et al., 2015b
Peru	Vegetables	1.7% (3/172)	Ortega et al., 1997b
Peru	Wastewater	72.7% (8/11)	Sturbaum et al., 1998
Spain	DWTP ^b , WWTP ^c , rivers	9% (20/223)	Galván et al., 2013
Turkey	Shellfish	26.4% (14/53) ^a	Aksoy et al., 2014
Tunisia	Wastewater	0.4% (1/232) ^a	Ben-Ayed et al., 2012
USA	WWTP influent	25% (6/24) ^a	Kitajima et al., 2014
USA	WWTP effluent	12.5% (3/24) ^a	Kitajima et al., 2014
Venezuela	Lettuce	5.9% (6/102)	Devera et al., 2006
Vietnam	Lakes and rivers	63.6% (84/132) ^a	Miegeville et al., 2003
Vietnam	Herbs and water	10.1% (58/575)	Tram et al., 2008

^a PCR methods; ^b Drinking water treatment plants; ^c Wastewater treatment plants

1.3.3 Soil transmission

In developing countries, contact with soil is considered a risk factor for cyclosporiasis (Chacin-Bonilla, 2008a). Studies from Peru (Madico et al., 1997), Guatemala (Bern et al., 1999), Venezuela (Chacin-Bonilla et al., 2007), and Egypt (el-Karamany et al., 2005) found soil to be a potential source of infection. In a study from Nepal, the *C. cayetanensis* was more prevalent where agriculture work and lack of hand washing were risk factors for infection (Tandukar et al., 2013). Several studies indicated that a variety of parasites were present in leafy vegetables probably resulting from exposure of the edible parts to the

soil surface (Uga et al., 2009).

Also in developed regions, contact with soil appears to play a role in the spread of infection. In an outbreak of cyclosporiasis in Florida, US, soil was a risk factor for infection (Koumans et al., 1998). In Germany, an outbreak was associated with lettuce from farms in Germany, France, and Italy; contamination of food crops could have occurred by seasonal agricultural workers from endemic areas without access to adequate sanitary facilities (Doller et al., 2002).

Variables associated with low socioeconomic status

could predispose persons to infection. In Venezuela, the majority of cases of cyclosporiasis were clustered in the areas of extreme poverty where living in a hut, not having a toilet, and having contact with fecal-contaminated soil were strongly associated with infection (Chacin-Bonilla et al., 2007). The main finding of this study was the strong correlation of stool positivity for *Cyclospora* with environments conducive to human fecal contamination, which suggests that anthroponotic transmission is possible through contact with contaminated soil in this area. Indeed, this factor was strongly linked to infection. The findings indicated an inverse relationship between socioeconomic status and infection and showed that cyclosporiasis, as well as other communicable infections, affects families living in substandard housing developments. In Haiti and China, higher rates of infection have been noted in areas, where deficient sanitary facilities and personal hygiene and soil frequently contaminated with feces were present (Lopez et al., 2003; Wang et al., 2002; Zhou et al., 2011).

The reasons for a higher prevalence of infection in older children (Chacín-Bonilla, 2010; Zhou et al., 2011; Tandukar et al., 2013; Thima et al., 2014) could be explained by other exposure and behavioral sub-factors strongly correlated with low socioeconomic status rather than age alone. Contamination of soils by inadequate defecation practices might be significant determinants for infection. Since outdoor defecation is frequent, non-supervised children may be more exposed to infection.

These results highlight the potential links between social marginalization and *Cyclospora* infection. Individuals of all socioeconomic strata can acquire cyclosporiasis. However, social inequality could mediate patterns of human exposure and infection. Impaired social environments could also influence patterns of human exposure, as persons within these areas may lack resources necessary for proper sanitation or educational avoidance of transmission routes. Living in physically impaired environments, where access to clean water and food is limited or where contact with soil is frequent, can increase exposure to *Cyclospora* oocysts. The effects of family wealth on cryptosporidiosis risk have also been demonstrated in several countries including the US (Chacin-Bonilla et al., 2008b; Becker et al., 2015).

Infections linked to contact with soil provide reasons to believe that this route of spread could be a mayor source of infection in areas of poor environmental sanitation, and poverty a predisposing factor. Large studies in endemic countries are required to elucidate soil transmission in vulnerable populations.

1.3.4 Reservoirs: The role of animals in transmission

Humans are the only known hosts of *C. cayetanensis*. However, the mechanical spread of the parasite through domestic animals was suggested in early studies in developing regions. Contact with animals is considered a risk factor for infection in Guatemala (Bern et al., 1999), Peru (Bern et al., 2002), Jordan (Nimri, 2003), Nepal (Sherchand et al., 1999, 2001; Bhandari et al., 2015) and Egypt (el-Karamany et al., 2005). Oocysts resembling those

of *C. cayetanensis* have been identified, using conventional methods, in the feces of several animals including ducks (Zerpa et al., 1995), chickens (García-López et al., 1996; Sherchand et al., 1999, 2001), mice and rats (Sherchand et al., 2001), dogs (Yai et al., 1997; Sherchand et al., 2001), and birds (Perez Cordon et al., 2009). *Cyclospora*-like oocysts were observed in feces of animals (carnivores, artiodactyla, and nonhuman primates) from a Spanish zoological garden (Perez Cordon et al., 2008). The presence of *C. cayetanensis* has also been demonstrated by PCR in feces of one chicken, two dogs and one monkey (Chu et al., 2004) and in one rhesus monkey (Li et al., 2015). No histological evidence of *Cyclospora* infecting tissues were presented in the prior studies. In contrast to these findings, the parasite was not detected in Haiti from 327 domestic animals, including pigeons, chickens, ducks, turkeys, guinea pigs, cats, dogs, goats, pigs, horses, and cattle (Eberhard et al., 1999c) and in Brazil from 140 stray dogs (Carollo et al., 2001), and Lima, Peru (Ortega et al., 1997b). Attempts to infect several animals with *C. cayetanensis* have been unsuccessful, suggesting host specificity (Eberhard et al., 2000). Although *C. cayetanensis* was reported to be propagated in albino mice (Sadaka et al., 2001) and guinea pigs (Wang et al., 2002) the findings could not be confirmed (Ortega et al., 2010). The parasite has been detected in shellfish (Negm, 2003; Aksoy et al., 2014). Free living nematodes, insects, and rotifers could play a role in the spread of *Cyclospora* (Ortega et al., 2010).

1.3.5 Incubation period

The median incubation period in most foodborne outbreaks has been 7 days (Herwaldt et al., 1997, 1999; Koumans et al., 1998; CDC, 1998). Among symptomatic individuals in outbreaks, the incubation period averages one week and ranges from approximately 2 to 14 days (Herwaldt, 2000, 2006). In a *Cyclospora* outbreak from Peru in 2004, analysis of the epidemiological curve suggested an incubation period of 2 to 6 days (Torres-Slimming et al., 2006).

1.3.6 Period of communicability

1.3.6.1 Shedding levels

Cyclospora oocysts typically are shed in relatively low numbers, even by non-immune ill persons (Herwaldt, 2000). Oocysts are not shed during the first week of infection, but in heavy infections, numerous oocysts are passed with loose feces (Gajadhar et al., 2015). In fecal material the number of *Cyclospora* oocysts may range from 10^2 to 10^4 oocysts per gram of stool (Shields et al., 2003a).

1.3.6.2 Time of shedding

Disappearance of symptoms and shedding of oocysts usually occur within a few days to 1 or 2 weeks (Soave et al., 1986; Shlim et al., 1991; Hoge et al., 1993). However, intermittent shedding of *Cyclospora* oocysts can continue even when the host is asymptomatic. Some untreated patients excrete oocysts after symptoms resolve (Shlim et al., 1991; Huang et al., 1995; Gajadhar et al., 2015) or have

symptoms longer than oocysts excretion (Hoge et al., 1993; Gajadhar et al. 2015) for several weeks. Untreated young children shed *Cyclospora* for a mean of 22–23 days (Ortega et al., 1993).

1.3.7 Population susceptibility

The susceptible populations to symptomatic illness include the very young, the elderly, immune-compromised persons, and those without previous exposure. In endemic and non-endemic areas, the models of susceptibility are different.

In developing countries, risk categories for cyclosporiasis include children, foreigners, and immunocompromised patients. Young children, in the first five years of age, are more likely to develop clinical symptoms (Hoge et al., 1995; Madico et al., 1997; Ortega et al., 1998; Sherchand et al. 1999, 2001; Chacín-Bonilla et al., 2001; Bern et al., 2002). Among resident foreigners and expatriates, the disease is common (Clarke and McIntyre, 1996; Drenaggi et al., 1998; Shields et al., 2003a; Puente et al., 2006; Bourée et al., 2006, 2007) and outbreaks have been reported (Shlim et al., 1991; Hoge et al., 1993; Rabold et al., 1994; Blans et al., 2005; Puente et al., 2006; Bednarska et al., 2015; Nichols, 2015) (Table 2). Among HIV-infected patients, *Cyclospora* is an important cause of diarrhea (Chacín-Bonilla, 2010).

In the developed world, cyclosporiasis is observed in the general population regardless of age including immunocompetent individuals, HIV-infected individuals, and immunocompromised patients (Kurniawan et al., 2009; Gajadhar et al., 2015).

1.4 Population and Individual Control Measures

1.4.1 Vaccines and drug therapy

No vaccine is available for cyclosporiasis.

Trimethoprim-sulfamethoxazole (TMP-SMX) was first used to treat cyclosporiasis in 1993 (Madico et al., 1993) and since 1995, it has been the drug combination of choice for managing infection (Hoge et al., 1995). It can be treated with the drug at 160–800 mg twice a day for 7 days or the same dose 4 times a day for 10 days in immunocompromised patients with AIDS, often with resolution of symptoms and oocysts shedding in 1–2 days (Madico et al., 1993; Hoge et al., 1995). In Peru, children with *Cyclospora* infection received a 3-day course of TMP-SMX at 5–25 mg/kg of body weight and stopped diarrhea and oocysts shedding (Madico et al., 1993, 1997). In Nepal, adults with cyclosporiasis were treated with TMP-SMX at 160–800 mg twice a day for 7 days; 84% of them were negative for oocysts upon stool examination whereas in the remainder the infection resolved extending therapy for an additional week (Hoge et al., 1995). In Haiti, HIV infected patients with cyclosporiasis were treated with TMP-SMX but 43% had recurrent infection. As a secondary prophylaxis, these patients received the drug three times a week for one month successfully controlling the infection

(Pape et al., 1994). For AIDS patients, the same dosage for 10 days and afterwards three times a week indefinitely is recommended (Guerrant et al., 2001).

As alternative treatments of cyclosporiasis, ciprofloxacin (Verdier et al., 2000) and in few cases nitazoxanide were effective for controlling infection (Diaz et al., 2003; Zimmer et al., 2007). The efficacy of these drugs is controversial. These drugs are usually recommended for treatment in patients that are sulpha allergic.

The close relatedness between *Cyclospora* spp. and *Eimeria* spp. suggests that many of the drugs used in the treatment of poultry coccidiosis may be effective against *C. cayetanensis* infection (Tang et al., 2015). Drugs affecting the mitochondrial and apicoplast metabolism could be developed and evaluated in clinical trials to test their effectiveness for cyclosporiasis (Saremy et al., 2011; Goodman et al., 2013; Stocks et al., 2014).

1.4.2 Hygiene measures

Improving personal and environmental sanitation may reduce exposure to human feces and contamination of the environment. Proper hygiene habits, and food washing and sanitizing may reduce the risk of acquiring infections. However, these practices do not completely remove *Cyclospora* oocysts from contaminated produce. Washing produce does not eliminate the risk of acquiring infection (Herwaldt et al., 1997, 1999). In fact, some oocysts remain on produce after washing (Ortega et al., 1997b). Good agricultural and manufacturing practice, and globally harmonized system are important to prevent introduction of the pathogen in the agricultural crops.

In the developing world, the most important steps to prevent infection are health education, personal hygiene, adequate hand washing, changing eating habits, safe drinking water, proper sanitary infrastructures, and treatment of human sewage. However, these steps are difficult challenges for low income-countries. Preventing geophagia in children is important because of the soilborne transmission of infection. The relationship between social marginalization and cyclosporiasis carries important implications for targeted public health interventions for infection in resource-poor groups. Great awareness of the parasite and increased familiarity with it or with the disease would improve surveillance programs for the coccidium and would increase the likelihood and early detection of future epidemics. It is necessary to implement detection techniques in the laboratories and in the field that would help to control the infection and prevent outbreaks locally and associated to imported contaminated produce in the developed world. Understanding interactions between socioeconomic and environmental conditions along with longitudinal and genotyping approaches will be the key to guiding prevention and control strategies to cyclosporiasis.

For prevention and control of waterborne *Cyclospora* infection, specific instructions and regulations developed by international organizations for controlling waterborne protozoa could be used for *C. cayetanensis*. From a public

health perspective, potential spread of the parasite from water can be avoided only by adequate treatment of household water sources. Studies to assess the quality of stored water and household practices which stimulate post-treatment contamination are highly recommended. Consumers should be aware of risks associated with consumption of raw, unwashed leafy greens and berries. Boiled or filtered water must be used for drinking, food preparation, and washing of any fruits and vegetables that are eaten raw.

The use of wastewater and excreta in agricultural production may facilitate the dissemination of parasites and impact human health (Rimhanen-Finne et al., 2004); most common health risks are diarrheal diseases and soil-transmitted pathogens (Blumenthal et al., 2001). The identification of *C. cayetanensis* in wastewater (Sturbaum et al., 1998; Sherchand et al., 1999, 2001; Ben-Ayed et al., 2012) indicates that development of measures to minimize human exposure to this protist and to improve the safety of discharge and reuse of wastewater and sludge are needed. The use of untreated manure as a fertilizer on farms can lead to produce contamination when it is not treated properly. The quality of the water used for both irrigating produce and washing it after harvest is essential for preserving hygiene in farming operations. Farmers should be educated regarding the risks of using sewage and contaminated water in fertilizing and irrigating crops of fruits and vegetables. Toilet facilities should be provided for food pickers and handlers in place.

For the developed world, consumers should be aware of risks associated with consumption of raw, unwashed leafy greens and berries. Development, implementation and monitoring of on-farm control measures in endemic areas are necessary to diminish or avoid future epidemics locally and in non-endemic areas. Application of disinfection techniques for decontaminating imported produce will improve food quality and safety. However, as they are not available, prevention is the only option. Control methods should be devised for the potential routes used by the coccidium to enter the food production process. To prevent foodborne contamination, establishment of preventive or control measures in the processing and production operation is necessary for raw foods entering a factory or contamination of food products inside the factory (Dawson, 2005; Keller, 2009).

In the 2013-2015 US multistate outbreaks of cyclosporiasis (Table 2), some diseases were linked to fresh cilantro from Puebla, Mexico. As a consequence, the FDA and the government of Mexico enhanced the safety of fresh cilantro with produce safety controls on both sides of the border. The FDA implemented import controls to detain without physical examination shipments of fresh cilantro from the state of Puebla. Shipments of fresh cilantro from other states in Mexico will be allowed to enter into the US if documentation is submitted at entry demonstrating that the cilantro was harvested and packed outside of Puebla. The controls implemented by Mexico incorporate a system for risk reduction, including export controls, for cilantro from the state of Puebla. Mexico's Systems of Risk

Reduction of Contamination ensure that agriculture, aquaculture, seafood, and livestock products are produced and processed in optimal sanitary conditions to reduce the risk of contamination. Cilantro producers in the state of Puebla must comply with 11 minimal requirements on good agricultural and food safety practices (FDA, 2015). This collaborative effort will ensure that fresh fruits and vegetables are being prepared and stored under sanitary conditions.

In developed countries, the efficacy of conventional wastewater treatment processes at removing *Cyclospora* oocysts is limited (Galvan et al., 2013; Kitajima et al., 2014; Giangaspero et al., 2015b). Therefore, more advanced treatments must be used for further reduction of oocysts for reclamation purposes (Kitajima et al., 2014).

2.0 Environmental Occurrence and Persistence

2.1 Detection Methods

Cyclospora cayetanensis oocysts can be identified in clinical and environmental samples using microscopy and sporulation studies by trained technicians and parasitologists. Molecular techniques can also be used. Samples can be stored in 2.5% aqueous potassium dichromate for molecular detection or sporulation and in 10% formalin for direct microscopy, concentration techniques, and staining. *Cyclospora* can be identified by bright-field or phase contrast microscopy in wet-mount preparations of fecal smears, but they are not easily distinguished from other particles (Mansfield and Gajadha, 2004). The oocysts stain variably with acid-fast techniques (Ortega et al., 1993) but stain uniformly with the safranin procedure modified by microwave treatment (Visvesvara et al., 1997) or with safranin at 85°C for 5 min using a water bath instead of microwave heating (Maratim et al., 2002).

Ultraviolet fluorescence microscopy is a useful technique for screening wet mounts of stool for *Cyclospora* oocysts which autofluoresce white-blue or green under epifluorescence microscopy using a 330-380 DM or 450-490 DM excitation filter, respectively (Ortega et al., 1993; Sterling et al., 1999). Concentration of the oocysts using ethyl acetate-formalin sedimentation, sucrose gradients, cesium chloride or discontinuous density Percoll gradients may be useful to maximize sensitivity and specificity of detection solely by microscopy (Kimura, 2004; Ortega et al., 2010).

The diagnosis of *Cyclospora* infection can also be confirmed by demonstrating sporulation of oocysts. If the sample is stored at 23 to 30°C for 1 to 2 weeks, the oocysts will differentiate into sporulated oocysts that contain two sporocysts (Ortega et al., 1994).

Limitations of traditional microscopy and morphological methods are the intermittent shedding of oocysts and the need to examine several fecal samples, variable staining of the parasite, and the time required for oocysts to sporulate for taxonomic classification; additionally, they require

skilled microscopists, and does not allow for species identification. Currently, commercial immunofluorescent antibody kits are not available for *Cyclospora*.

Molecular biological tools have been developed to detect and differentiate *Cyclospora* at the species levels but they are not in widespread use for routine testing. These methods have greater sensitivity and specificity than microscopy for detection and diagnosis but they must be carefully designed and validated to avoid misidentifying closely related *Eimeria* species and robust enough for use in clinical and environmental matrices containing polymerase chain reaction (PCR) inhibitors and high levels of background DNA. Conventional PCR, PCR-fragment length polymorphism, and real-time quantitative PCR with melting curve analysis have been developed for detection of the parasite (Relman et al., 1996; Jinneman et al., 1998; Lalonde et al., 2008, 2011, 2013; Shields et al., 2003b; Varma et al., 2003). Application of a bead-based multiplex eukaryotic enteropathogens assay has also been developed. This multiplex PCR protocol provides a sensitive and specific assay for *Cyclospora* (Taniuchi, 2011; Buss et al., 2015).

Methodologies that could be used for fingerprinting analysis and genotype discrimination had not been available. The conserved sequence nature of rRNA and HSP70 genes and intra-isolate variations among different copies of ITS-1 and ITS-2 had made the development of genotyping tools for the parasite difficult (Adam et al., 2000; Olivier et al., 2001; Riner et al., 2010; Zhou et al., 2011; Sulaiman et al., 2013, 2014). The recent availability of whole mitochondrial and apicoplast genome sequences (Tang et al., 2015; Qvarnstrom et al., 2015; Cinar et al., 2015) and whole genome sequencing (Liu et al., 2016) beyond rRNA and heat shock protein genes could facilitate development of genotyping tools for investigations of *Cyclospora* outbreaks. Recently, whole-genome sequence data from *C. cayetanensis* protozoa enabled the development of a MLST genotyping tool for characterizing isolates. In this study, 2 to 10 geographically segregated sequence types at each of 5 selected loci were observed. There was clear geographic clustering of MLST types. Most specimens from China clustered together in 1 major group, whereas specimens from epidemics in the US formed 2 other groups with specimens from Peru. A sample from Spain appeared to be different. The apparent existence of geographic clusters and the high resolution of the typing tool could be useful for infection/contamination source tracking (Guo et al., 2016).

Environmental samples are more difficult to examine than stool samples. The detection of any protozoan from any substrate follows a three-step process: concentration, purification using methods as immune-magnetic separation or density gradient centrifugation, and detection. The target pathogen has to be efficiently concentrated or the following procedures might not reveal the parasite. The third step is detection by several methods such as microscopy, flow cytometry, and nucleic acid amplification.

Methods to detect *Cyclospora* oocysts in environmental

samples are limited. In water, the low frequency of the target requires large amounts of this matrix to be screened. Filtration using cartridge, hollow-fibre ultra-filters or capsule filters is performed to capture oocysts. High turbidity causes filters to clog. An alternative method of collection and concentration not affected by turbidity is flocculation (Vesey et al., 1993). *Cyclospora* can be isolated from water samples by filtration using Hannifin polypropylene cartridge filters or Envirocheck® capsules. Particles trapped in the filters are released using an elution buffer, and centrifuged. Pellets are stored in 2.5% potassium dichromate and examined for the presence of the parasite (Sturbaum et al., 1998).

Limited availability of suspected food products and spotty distribution of oocysts present sampling difficulties; given the long incubation period of cyclosporiasis, little or no product may be available for testing (Shields et al., 2003a). A good elution method is necessary to retrieve oocysts from the suspected product. Due to the low infectious dose of *C. cayetanensis* and the unavailability of an enrichment procedure for this parasite, it is important to develop methods to maximize its detection. To recover the oocysts from food products, de-ionized water, saline solution, elution buffers, glycine buffer pH 5.5, 0.1% Alconox, 3% levulinic acid and 1% HCL-pepsin, and lectin coated paramagnetic beads have been used (Lalonde et al., 2008; Shields et al., 2012; Chandra et al., 2014).

Recovery rates for certain products such as leafy vegetables and herbs, tend to be low, ranging from 12 to 14% (Ortega et al., 1997b; Robertson et al., 2000). Detection limit can be as low as 0.3 oocysts per gram of raspberries (Orlandi et al., 2000) recoveries can be improved with better washing and detection techniques (Ortega et al., 1997b; Orlandi et al., 2000).

Molecular assays are a useful diagnostic tool in combination with oocyst extraction from water and foods. Nuclei acid amplification has been used for detecting *C. cayetanensis* in water (Shields et al., 2003b; Lalonde et al., 2008). Continuous separation channel centrifugation appears to be an efficient method for recovering *Cyclospora* oocysts but its main limitation is the availability of centrifuges (Borchardt et al., 2009).

To assess the potential risk of matrices contaminated with the parasite, the viability and sporulation stage of *Cyclospora* oocysts have to be determined. Due to a lack of vital dyes, tissue culture methods or animal models, viability assessments of *C. cayetanensis* oocysts in foods or water samples are often overlooked. Oocysts can be induced to sporulate in vitro between 8–14 days in distilled water or potassium dichromate at 22 to 30°C (Smith et al., 1997). The sporulated oocysts are treated with bile salts, sodium taurocholate and subjected to mechanical pressure to release sporozoites through excystation (Ortega et al., 1994; Smith et al., 1997). The viability and sporulation of *Cyclospora* oocysts have also been determined by the electron rotation method (Dalton et al., 2001). These methods work. However, when using environmental and food samples the number of parasites present are

extremely low, making these methods hard if not impractical to use.

2.2 Data on Occurrence

In areas of endemicity where *C. cayetanensis* is common and water and sewage treatment systems, sanitary facilities, and standard housing developments are insufficient or lacking, oocysts can spread readily through water supplies and distribution systems, foods, and soil. The parasite has been isolated in developing and developed countries from several environmental matrices such as fresh produce, shellfish, drinking and recreational water, wastewater, and soil (Table 3).

2.2.1 Sewage and wastewater

In Perú, 72.7% (8/11) of water samples from a primary oxidation lagoon contained *Cyclospora* oocysts (Sturbaum et al., 1998). Oocysts also were detected in sewage water in Nepal (Sherchand et al., 1999, 2001), and Tunisia (Ben-Ayed et al., 2012). In Spain, oocysts were isolated in wastewater treatment plants with an annual prevalence of 16.1% (9/56) in raw water and 10.7% (6/56) in finished water. The highest prevalence was noted in spring (Galvan et al., 2013). In Italy, oocysts were detected in 21.3% (20/94) of wastewater samples, mainly in autumn (Giangaspero et al., 2015b). In the US (Arizona), oocysts were found in two wastewater treatment plants in raw and treated water (Kitajima et al., 2014).

2.2.2 Sludge

No data are available.

2.2.3 Surface waters

Water from rivers and lakes in Guatemala, Vietnam, Egypt, and Spain were positive for *Cyclospora* (Bern et al., 1999; Miegeville et al., 2003; el-Karamany et al., 2005; Galvan et al., 2013). In surface waters, oocyst occurrence may be highly variable with low frequency. The estimated concentration of the parasite in rivers from Guatemala was 15,000 or more oocysts per 10-liter specimen (Bern et al., 1999). In Egypt, the coccidium was isolated in five residential areas, from a drain, an irrigation canal, underground water and piped water, reflecting the high environmental contamination of the area. In the irrigation canal, the water contamination was 1900 oocysts / liter (el-Karamany et al., 2005). In rivers and lakes samples from Vietnam, the level of positivity reached 63.6% (Miegeville et al., 2003). In four river basins in Spain, the annual prevalence of the parasite was 2% (Galvan et al., 2013).

2.2.4 Ground waters

Limited information of *Cyclospora* in ground water is available. In Egypt, the densities of contamination by oocysts / liter in underground water and piped water at shallow depth and underground water > 35 m deep were respectively 700 and zero (el-Karamany et al., 2005). In Italy, oocysts were identified in 6.2% (1/16) of well water

samples (Giangaspero et al., 2015b).

2.2.5 Drinking waters

Oocysts have been detected in municipal drinking water that was associated with an outbreak in Nepal. The drinking water consisted of a mixture of municipal and river water. Coliform bacteria were not detected suggesting, perhaps like other coccidia (eg. *Cryptosporidium*) that water chlorination is not sufficient to inactivate the coccidium (Rabold et al., 1994). In rural areas of Guatemala, *C. cayetanensis* was detected in 3 of 5 water samples used for public consumption by amplification of *Cyclospora* 18S-rDNA (Dowd et al., 2003). In Vietnam, oocysts were identified in drinking water (Miegeville et al., 2003). In Egypt, oocysts were isolated from drinking water in five residential areas (el-Karamany et al., 2005) and 0.24% (2/840) of surveyed drinking water samples from seven districts contained oocysts (Elshazly et al., 2007). In Ghana, Accra, 59.2% (16/27) of sachets containing drinking water had oocysts (Kwakyenuako et al., 2007). In Italy, 30% (3/10) of tap water samples collected in a train were *C. cayetanensis* positive and contained copies of DNA corresponding to 4–11 oocysts per liter (Giangaspero et al., 2015a). This high concentration is a cause of concern for the possibility of presence of sporulated oocysts due to the high viability of *Cyclospora* oocysts (Smith et al., 1996) and the low infectious dose (Dixon et al., 2005).

Some have reported the presence of *C. cayetanensis* throughout the year in treated potable water from tanks (el-Karamany et al., 2005) and treated piped water (Elshazly et al., 2007). Others have detected *Cyclospora* in drinking water, wastewater, and river water in Spain (Galvan et al., 2013), and in train tap water in Italy (Giangaspero et al., 2015a) being higher in spring months, even though differences in prevalence between the seasons were not statistically significant. In another study from Italy, the highest prevalence was in autumn in vegetables, wastewater, and soil (Giangaspero et al., 2015b).

2.2.6 Seawater

No data are available for the presence of *C. cayetanensis* oocysts in the marine environment. Detection of oocysts in shellfish in Alexandria, Egypt (Negm, 2003), and Izmir, Turkey (Aksoy et al., 2014) suggests contamination of coastal waters of these areas.

2.2.7 Soil

In a study from Apulia, Italy, 11.8% (6/51) of soil samples were found positive (Giangaspero et al., 2015b).

2.2.8 Irrigation water and on crops

Water used either for irrigation or processing of vegetables contained *Cyclospora* oocysts in Guatemala (Bern et al., 1999), Vietnam (Tram et al., 2008), and Italy (Giangaspero et al., 2015b). In Guatemalan raspberry fields, river water used for irrigation and application of pesticides contained oocysts and could have been the

source of contamination of berries involved in several outbreaks in North America (Bern et al., 1999).

In Vietnam, 11.8% (34/288) of market water and herb samples and 8% (24/287) of farm samples were positive for *Cyclospora* including Vietnamese mint, marjoram, basil, lettuce, and coriander. Contamination was observed before the rainy season but not during this time (Tram et al., 2008).

Among fresh produce from markets in Peru, *C. cayetanensis* was detected on basil, cabbage, celery, cilantro, green onions, green chili, herbs, leeks, lettuce, and parsley (Ortega et al., 1997b). In Peru, of 110 vegetables examined, 1.8% (2) contained *Cyclospora* in one survey, and of 62 vegetables sampled in a second survey, 1.6% (1) contained oocysts (Ortega et al., 1997b). In Canada (Dixon et al., 2013), the US (Lopez et al. 2001), Costa Rica (Calvo et al., 2004), Venezuela (Devera et al., 2006), Nepal (Sherchand et al., 1999, 2001), Vietnam (Tram et al., 2008), Cambodia (Vuong et al., 2007), and Egypt (Abou el Naga, 1999; el Said, 2012), oocysts were detected on green leafy vegetables. In the US, the parasite was found in the raspberry filling of a cake (Ho et al., 2002). The report from Canada represents the first large-scale surveillance study examining packaged ready-to-eat leafy greens in North America for the presence of protozoan parasites. A total of 544 samples were purchased from a variety of retail grocery stores in Ontario, Canada between April 2009 and March 2010; most of these products were grown in the US, with some from Canada and Mexico. A relatively high prevalence (1.7%, 9/544) of *Cyclospora* spp. were identified by PCR-restriction fragment length (Dixon et al., 2013). This result established a baseline for further studies and suggested a need for more research in relation to the possible sources of contamination of these foods, the assessment of parasite viability and means to reduce foodborne transmission to humans.

In Costa Rica and Venezuela, the parasite was identified on lettuce. In Costa Rica, *Cyclospora* was detected during the dry season (Calvo et al., 2004; Devera et al., 2006). In Ghana, *Cyclospora* was isolated from cabbage, pepper, carrot, onion, tomato, and lettuce in 5% of the samples studied (Duedu et al., 2014). The relationship between numbers of organisms found on fresh produce and numbers in the environment in which crops were grown is unclear (Dawson, 2005).

Produce can become contaminated in the field, during harvesting, storage or transportation. Because of changes in processing, more precutting and coring of produce may occur in the field during harvest, increasing the probability for contamination (Sewell et al., 2001). *Cyclospora* oocysts present on produce also can originate in the soil where the food is grown and can be present in irrigation water or fertilizer. To prevent foodborne contamination establishment of preventive or control measures in the processing and production operation is necessary for raw foods entering a factory or contamination of food products inside the factory (Dawson, 2005; Keller, 2009). Enforcement of international food trade and

implementation of methods using HACCP may help control cyclosporiasis (Buisson et al., 2008).

2.2.9 Fish and shellfish

No data are available for fish. Oocysts were detected in marketed shellfish in Alexandria, Egypt (Negm, 2003) and in farmed and wild mussels from Izmir Province, west coast of Turkey (Aksoy et al., 2014). Shellfish concentrate *Cyclospora* oocysts from contaminated waters. Controlled laboratory studies with freshwater clams (*Corbicula fluminea*) showed that 48 to 100% of the clams retained *Cyclospora* oocysts for up to 13 days (Graczyk et al., 1998). The parasite has been detected in shellfish in Egypt and Turkey (Negm, 2003; Aksoy et al., 2014).

2.3 Persistence

Cyclospora oocysts require several days to sporulate in the environment prior to being ingested by a susceptible host. This suggests that oocysts are quite hardy and environmentally resistant (Herwaldt, 2000). The oocysts require time, moisture, and moderate temperature (optimal 20–25°C) to become infective (sporulate), thus following 7 to 15 days in a warm humid environment, sporulation occurs yielding infective oocysts and become infective. It has been suggested that oocyst suspension in water facilitates both the development and transmission of coccidian oocysts (Mansfield and Gajadha, 2004).

The percent sporulation of *C. cayetanensis* oocysts, as an indicator of viability, has been determined under a variety of conditions to examine persistence.

Whether *Cyclospora* oocysts are as resilient as other coccidian parasites is unknown. However, naturally occurring *Cyclospora* oocysts may survive for extended periods in the environment, given the marked seasonality of infection in endemic regions (Herwaldt et al., 1999). Little is known about the effects of environmental conditions on the rate of sporulation and on the viability of oocysts (Ortega et al., 1993; Smith et al., 1997; Herwaldt, 2000). *Cyclospora* oocysts may survive for extended periods, 7 days to 2 months, in water depending on the temperature (Ortega et al., 1998). No oocysts sporulated after storage at 4°C for 2 months and 1.3 log₁₀ reduction was seen (only 5% of the oocysts sporulated) at the highest temperature after 7 days (37°C).

In other studies the equivalent of a 0.92 log₁₀ reduction was seen (very close to a T90) where by up to only 12% of the oocysts sporulated after being stored at 4°C for 1 to 2 months and this same level of reduction was seen at 30°C after 6–7 days (Smith et al., 1997).

Many more experiments were run with a yes or no result without quantification. For example viability of unsporulated oocysts were subjected to freezing and heating conditions in dairy and basil substrates and then placed in 2.5% potassium dichromate. *Cyclospora* sporulation was then observed to occur when Oocysts incubated at 23°C or stored at 4°C and then

brought to 23°C. (Note oocysts incubated at 30 or 37°C, did not sporulate after various exposures (Sathyanarayanan et al. 2006).

The results of these studies showed that sporulation occurred for oocysts re-suspended in dairy substrates stored at -15°C within 24 h; in water or basil at 20°C for up to two days and at 37°C for up to 4 days. *C. cayetanensis* sporulation was also not affected after microwave heating for up to 45 s (Ortega et al., 2006). Few oocysts sporulated at 50°C for 1 h and sporulation did not occur at -70, 70, and 100°C in water or basil leaves (Sathyanarayanan et al., 2006). These results were corroborated a number of times showing that oocysts could not be induced to sporulate after freezing at -18°C or -20°C for 24 h or after heating at 60°C for 1 h (Sterling et al., 1999; Smith et al., 1997; Ortega et al., 1998).

Oocysts are very sensitive to desiccation and the oocyst wall ruptures after 15 min (Long et al., 1991).

3.0 Reductions by Sanitation Management

Very little is known about reductions of *C. cayetanensis* oocysts by sanitation management.

3.1 Wastewater Treatment

In a few studies the occurrence of oocysts before and after conventional wastewater treatment has been examined to evaluate processes for removing *Cyclospora* oocysts. However, no quantitative studies have reported log reductions.

In Spain, oocysts were isolated in conventional wastewater treatment plants with an annual prevalence of 16.1% (9/56) in raw sewage and 10.7% (6/56) in effluents (Galvan et al., 2013).

In Italy, significant differences were noted between the prevalence of contamination in treated wastewater samples (13% positive) from a treatment plant with advanced technologies (i.e. membrane ultrafiltration, GDF plus UV

radiation) compared to one with traditional water treatment techniques (55% of the samples positive $p < 0.003$) (Giangaspero et al., 2015b).

In the US, Arizona, oocysts were detected using qPCR methods in the influent with the highest concentration of 1.2×10^4 copies/L. No concentrations in the effluent were noted in this work. The oocysts were detected in 3/12 samples (25%) in the influent in two plants and 1/12 and 2/12 (8% and 17%) of the effluent samples. Also, the prevalence of *Cyclospora* in soil irrigated with effluents was higher than that at other sites in Italy (Giangaspero et al., 2015b).

The possibility of parasites such as *Cyclospora* surviving various biosolids treatments is low if temperatures reach above the 37°C in anaerobic digestion and achieve high desiccation (See persistence above) as oocysts should not survive long under low-moisture conditions (Gerba et al., 2002). Yet studies validating this are not available.

3.2 Disinfection

Cyclospora oocysts seem to be resistant to many disinfectants, including chlorination at levels used in water treatment (Rabold et al., 1994; Soave et al., 1998).

Gaseous chlorine dioxide at 4.1 mg/L for 20 min (Ortega et al., 2008) did not affect sporulation of the oocysts (inoculated onto lettuce and basil).

High-hydrostatic-pressure processing and UV light radiation have been suggested to reduce the risk of cyclosporiasis associated with produce as observed using *Eimeria acervulina* as a surrogate for *Cyclospora* (Kniel et al., 2007).

Oocysts are not killed when exposed to pesticides such as captan 50% wettable powder (W.P.), benomyl 50% W.P., diazinon 47.5%, malathion 25% W.P., and zineb 75% W.P., at lower and higher than recommended doses, were not effective in inactivating oocyst sporulation (Sathyanarayanan et al., 2004).

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