An Overview and Assessment of Waterborne Cyclosporiasis in the United States

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Abstract

Foodborne illnesses are an important environmental health concern, with cases often being associated with both food and water contamination. *Cyclospora cayetanensis* is a coccidian parasite that is transmitted through the environment and infects the human intestinal tract, commonly causing diarrheal illness. Cyclosporiasis is most prevalent in tropical and sub-tropical regions of the world, although cases occur all over the world annually. There is strong evidence of seasonality for cyclosporiasis cases with different regions experiencing peaks at different times of year. In the United States, cases have historically been associated with foreign travel and outbreaks associated with imported produce. Recently, domestic cases appear to be rising in the US, and there have been concerns raised that cyclosporiasis has become endemic. The spread of *Cyclospora* through the water supply is of particular environmental health concern to the FDA. To assess this concern, I reviewed cyclosporiasis case reports submitted to the CDC for evidence of trends associated with water. Only two jurisdictions in the United States included questions on water exposures on their case questionnaires, and those questions were not uniform and only included for a limited number of years. There was no evidence of water associated outbreaks or trends, and there does not appear to be a need for comprehensive water exposure questions on case questionnaires at this time.

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1.0 Introduction

Foodborne illnesses comprise a serious, and perhaps under-appreciated, public health concern in the United States. The CDC estimates that there are 48 million cases each year, with the most common pathogens being bacteria and viruses such as *Salmonella*, *Campylobacter*, norovirus, rotavirus, and sapovirus. There are also several foodborne parasites that cause illness within the US, the most prevalent being *Giardia* and *Cryptosporidium*.

In aggregate, foodborne illnesses cause 128,000 hospitalizations a year in the United States costing about $17.6 billion in treatment. Approximately 3000 people die yearly from these infections (CDC; Mead et al., 1999; USDA).

Tracking foodborne illness is an important environmental public health activity that provides insight into the spread, vectors or vehicles, and dangers of foodborne pathogens. These data allow public health officials to help prevent the public from becoming ill, through direct action such as recalls, recommendations, and safety regulations. In particular, identifying sources of infection is important and is an important step in preventing infections in the population (Health.Gov). In this essay we will discuss the growing concern in the United States over a particular foodborne parasite, *Cyclospora cayetanensis*. We will also examine the reporting practices of jurisdictions within the United States, describing and investigating the possibility of waterborne cyclosporiasis as a source of infection. This essay provides a summary of information and assessment of surveillance procedures for public health and environmental health officials.

1.1 Foodborne Illness

A foodborne illness is a disease caused by a pathogen or toxin present in foods or beverages. In the United States, there are an estimated 48 million cases of foodborne illness annually. These infections lead to approximately 128,000 hospitalizations and 3,000 deaths (FDA).

In the United States, surveillance is an important tool used to identify and mitigate the impact of foodborne illness. Public health surveillance is conducted at both the state and local level, with federal support from the Centers for Disease Control and Prevention (CDC), where surveillance data are consolidated and reported at a national level. Certain diseases are mandatory to report to the local or state government. Public health surveillance systems track cases of reportable illnesses, and collect vital information about location, demographics, and risk factors. Doctors, hospitals, and laboratories report instances of potential cases to public health officials who conduct follow-up investigations. Public health officials use this surveillance data to track the spread of illness around the country, identify geographical regions of concern, including outbreaks, identify risk factors and the groups of people most at risk, and track clinical outcomes (CDC; Health.Gov).

Certain risk factors have been linked with acquiring a foodborne illness. For example, not washing fruits and vegetables, undercooking meats, and drinking contaminated water. Foodborne transmission can be prevented with proper food preparation, for example, *Salmonella* can be destroyed with proper cooking of meats. The washing of herbs, berries, and vegetables can effectively remove pathogens from produce, although there is always a risk of infection, especially in uncooked produce (CDC).

Some factors have been associated with worse clinical outcomes in individuals, such as an increased chance of severe symptom presentation, hospitalization, and death. A weakened immune system, age, and pregnancy status are some factors that can affect the body's ability to resist foodborne illness. For people with a compromised or naturally declining immune system it becomes more difficult for the body to fight off infection, including foodborne pathogens. Diseases like cancer, HIV/AIDS, and diabetes can severely weaken the immune system, through both disease progression and treatment side effects (CDC; Lund & O'Brien, 2011).

For people with diabetes, the risk of infection, including foodborne illnesses, is significantly increased. Diabetics have impaired glycemic control, and consequently have impaired immune system function. One study found that salmonellosis was three times more likely, and campylobacteriosis four times more likely, among diabetic patients compared to the general population. Diabetics with listeriosis have been shown to be twenty-five times more likely to develop symptoms than nondiabetics (Lund & O'Brien, 2011).

Pregnant women, and their unborn fetuses, are at particular risk for foodborne illness. Unborn fetuses are just beginning to develop an immune system and are at risk of harmful pathogens crossing the placenta and causing infection. Women have a weakened immune system during pregnancy, and as a result not only have a higher risk of infection for themselves, but for their unborn children as well (CDC; Lund & O'Brien, 2011). Common foodborne illnesses that affect pregnant women include *Listeria monocytogenes* and *Toxoplasma gondii*. While *L. monocytogenes* doesn’t cause life threatening symptoms in mothers, usually mild fever and flu symptoms, it can cause extreme complications with pregnancy. These complications can lead to severe infection at birth for the newborn, or even stillbirth (Lund & O'Brien, 2011).

For young children, under the age of five, their immune systems are not fully developed, and they are unable to fight off infection as well as older people. One of the most common causes of renal failure in children is haemolytic uraemic syndrome (HUS), a disease caused by infectious agents including *E. coli* and the Shiga toxin it produces. A significant number of children with HUS require dialysis therapy in order to treat their symptoms (Scheiring, Rosales, & Zimmerhackl, 2010).

The other age group most at risk for foodborne infection is the elderly, over the age of 65 years. The immune system naturally weakens as people age, and consequently the risk of infection increases. Changes in gastrointestinal physiology and usage of immunosuppressive medication can also contribute to vulnerability. People living in long-term care facilities are at particular risk for foodborne infection, with residents being four times more likely to die from gastroenteritis (Kirk, Veitch, & Hall, 2010). Outbreaks caused by *Campylobacter*, *Clostridium perfringens*, *E. coli*, and *Salmonella* in care facilities provide evidence that the elderly have more serious outcomes from infection of foodborne pathogens, including death (Smith, 1998).

Every year approximately 1 in 6 Americans become sick with a foodborne illness. These illnesses are caused by a variety of pathogens, including bacteria, viruses, and parasites. These illnesses vary greatly by their source, symptoms, severity, and duration. Illness duration can range from as little as 12-60 hours for norovirus to 2 weeks-3 months for hepatitis A. While many of the symptoms are similar, such as diarrhea, vomiting, and cramps, they differ in severity, the symptoms of botulism can be so severe as to cause respiratory failure and even death. The FDA provides information on common foodborne pathogens and their characteristics (FDA).

Of the 31 most common domestic foodborne illnesses in the United States, norovirus is by far the most common with over an estimated 5.5 million cases per year, followed by Salmonella at an estimated 1 million, and C. perfringens with 965 thousand cases. The CDC provides information on the most commonly reported foodborne illnesses along with data annual on hospitalizations and deaths (CDC, 2018).

1.2 Parasites

Parasites are organisms that infect host animals and receive nutrients at the host's expense. There are three major groups of eukaryotic parasites that infect humans, protozoa, helminths, and ectoparasites. Protozoa are single celled organisms that can thrive in human hosts. They can commonly be transmitted through the fecal oral route or through external vectors, such as mosquitos. Helminths, or parasitic worms, are much larger multicellular organisms. Both helminths and protozoa can be free living or parasitic in their nature. Ectoparasites are organisms that reside on the skin of their hosts, where they suck blood for sustenance. They are commonly arthropods such as mosquitos, lice, ticks, and mites (CDC; Cox, 2002).

There is evidence that over the course of our evolution humans have been infected with approximately 300 species of helminth worms and over 70 species of protozoa. Many of these parasites rarely infect humans today, but some are much more common than others with around 90 species frequently infecting human hosts. An even smaller proportion of these species are responsible for the majority of parasitic diseases, such as malaria, giardiasis, and toxoplasmosis (Cox, 2002).

Infectious protozoa are found all over the world and can be further classified into four major groups. These groups are defined by the organism's form of movement, and include Sarcodina, Mastigophora, Ciliophora, and Sporozoa. For example, Sarcodina are characterized by the extension and retraction of pseudopods, movement that is often defined as amoeboid. A common Sarcodina affecting humans worldwide is *Acanthamoeba* spp., often associated with contact lens usage and contaminated water. *Acanthamoeba* spp. That enters the eye can cause keratitis, or severe inflammation of the cornea. *Naegleria fowleri* is another Sarcodina that can infect humans that come into contact with contaminated water. These "Brain-eating amoebas" can travel through the nose to the brain and cause amebic meningoencephalitis. While rare, amebic meningoencephalitis has a fatality rate over 97%, with only four of the known 154 infected persons surviving infection in the United States (CDC; CDC; CDC).

Helminths are common parasites in humans, and in their adult form some can be visible to the naked eye. Unlike protozoa, helminths cannot multiply in humans and instead rely on their eggs being passed out of the host, usually through feces, and transferred to others. Helminths can be categorized into three main groups: flatworms, thorny-headed worms, and roundworms. Roundworms in particular infect a substantial amount of people, with over one billion cases estimated worldwide. Roundworm infection of the intestines is called ascariasis, and the condition is prolific in areas with poor sanitation. *Ascaris* worms are soil-transmitted helminths and contamination is often caused by exposure to human feces which contain roundworm eggs. Ascariasis is much more common in children for whom it can cause growth problems, along with other symptoms including abdominal pain, nausea, vomiting, malnutrition(CDC; Clinic, 2021).

Ectoparasites largely refer to blood-sucking arthropods that rely on their hosts for survival. These parasites usually remain on or in the skin of their hosts for long periods of time, and often act as transmitters for other pathogens. While ectoparasites are a common source of disease themselves, their ability to act as vectors for other pathogens is what makes them the most dangerous to humans. Mosquito refers to approximately 3,600 species of small flies, which are responsible for a huge burden of disease in humans. Mosquitos transferring malaria is perhaps the most notable example of ectoparasite disease transmission, with malaria causing over 400,000 deaths annually in human populations. Along with malaria, yellow fever, Zika fever, dengue, and filariasis are all dangerous diseases transmitted through mosquito bites (Britannica). The World Health Organization (WHO) estimates that over 17% of all infectious diseases are vector-borne, causing over 700,000 deaths annually. Malaria and dengue in particular account for a large portion of these infections and deaths (CDC; WHO).

1.3 Environmental Health

The environment plays an important role in human health, in particular water quality is associated with the risk of infectious disease. The WHO reports that at least 2 billion people worldwide have drinking water contaminated with feces. Infectious diarrheal diseases such as cholera, dysentery, typhoid, and polio can be acquired through the ingestion of water contaminated with pathogens released from an infected human or animal's feces (WHO). Worldwide, waterborne communicable disease accounted for approximately 3.4 million deaths in 2004 (Pruss-Ustun et al., 2019). In 2016 around eight hundred thousand diarrheal deaths were associated with water, sanitation, and hygiene (WASH) exposures to infection. These WASH exposures contributed to 60% of diarrheal deaths in 2016. Children under the age of 5 are particularly at risk for WASH related infections, with 5.3% of all deaths being attributed to these infections. Waterborne infectious diseases also lead to significant morbidity in those infected, with a combined 49.8 million Disability-Adjusted Life Years lost (Pruss-Ustun et al., 2019; WHO).

While the health burden of WASH related diseases is high, there are effective prevention measures that can be taken. Primarily, the proper construction and maintenance of water and sanitation services helps significantly reduce the danger of infection. Individuals can also contribute to the prevention of disease spread through proper hand-hygiene and personal sanitation practices. Water storage and irrigation systems are critical infrastructure that facilitate the distribution of safe water. However, if contaminated by wastewater or feces, they can become reservoirs of disease potentially leading to large-scale pathogen transmission (Boelee, Geerling, van der Zaan, Blauw, & Vethaak, 2019).

While approximately 74% of people globally have access to clean and reliable drinking water, 2 billion live without adequate safe water sources. Of those people, half do not have access to the most basic safety measures for their water sources. The majority of people, 80%, without clean water access live in rural areas, with half of those living in least developed countries. Lack of clean water access impacts several regions significantly more than the rest of the world, in particular Northern Africa and Southern Asia have problems with water safety and access (Nations).

2.0 Cyclosporiasis

2.1 History

In the early 1990's *C. cayetanensis* was often referred to as a cyanobacteria-like parasite with researchers describing the novel organism present in the stool of patients experiencing diarrhea. In 1993 a report by Ortega et al. was published describing a novel coccidian parasite causing gastro-intestinal illness in humans. They conducted two large scale prospective cohort studies with children in Lima, Peru. The studies collected biological samples from children living in shantytowns, with the intent of better understanding the epidemiology of the parasite *Cryptosporidium parvum*. While conducting this research, investigators identified undefined cyanobacterium-like bodies among the stool samples. Unknown cysts, distinguishable from that of *C. parvum*, were identified in the feces of children gathered, 18% containing cysts in the first study and 6% from the second. These cysts were both visually distinguishable from those of *C. parvum* and relatively unreactive to *C. parvum* antibodies. Researchers were able to place the new species within the genus *Cyclospora* after observing the organism during sporulation, and excystation (Ortega, Sterling, Gilman, Cama, & Diaz, 1993).

While this was the first time that *C. cayetanensis* had been classified by researchers, the novel organism had first been described in 1979. Three cases in Papua New Guinea were documented by Ashford, who isolated cysts from patients but was unable to classify the genus of parasite. For over a decade, reports of gastro-intestinal infections caused by an unknown parasite were described. These reports came from Haiti, Mexico, Nepal, and Peru, where researchers described the organisms as a larger version of *Cryptosporidium*. In 1994 Ortega et al. proposed the name *Cyclospora cayetanensis* (Ashford, 1979; Herwaldt, 2000; Ortega, Gilman, & Sterling, 1994).

Throughout its short, documented history, cyclosporiasis has often been misidentified due to its similarity to cryptosporidiosis. Like cryptosporidiosis, cyclosporiasis was described as affecting children and the immunocompromised, and appeared to be endemic to tropical countries where untreated drinking water was common (Bern et al., 1999; Herwaldt, 2000; Sherchand, Cross, Jimba, Sherchand, & Shrestha, 1999). There was also early evidence of seasonality to cyclosporiasis infections, with notable rises during the early rainy season (Bern et al., 1999; Sherchand et al., 1999). In 1998 Sturbaum et al. were the first to identify contaminated water as a source of infection after finding *C. cayetanensis* oocysts in 64% of wastewater samples taken in Lima, Peru (Sturbaum et al., 1998).

2.2 Biology

The parasite *C. cayetanensis* is an obligate intracellular parasite, one which requires an animal cell to reproduce. As a coccidian protozoan *C. cayetanensis* is a single celled spore forming organism. To date only humans have been identified as hosts for *C. cayetanensis*, with no documentation of an animal reservoir. The life cycle of *C. cayetanensis* has seven stages, starting with the excretion of unsporulated oocysts in human stool. Oocysts are a resilient and infective stage of many sporozoa life cycles, characterized by thick cell walls that are able to survive in the environment for extended periods of time. Once excreted, the oocysts sporulate in the environment, where it then contaminates fresh produce or water. Sporulation describes the process of dormant coccidian oocysts maturing into their infective form (CDC, 2019; Mathison & Pritt, 2021).

There is evidence that temperature, humidity, soil chemistry, and light exposure affect the sporulation process, which lasts approximately one to two weeks. Humans become infected upon the consumption of sporulated oocysts from a contaminated source. Notably, it is not possible for direct contamination between human hosts without the intermediary step of environmental contamination. The invasive oocysts excyst within the human gastrointestinal tract, releasing sporozoites (Figure 1), a mobile spore-like cell stage, which infect nearby epithelial cells. The *C. cayetanensis* parasites then undergo both sexual and asexual reproduction before fertilized oocysts are excreted in the stool, restarting the life cycle (Figure 2) (CDC, 2019; Mathison & Pritt, 2021).

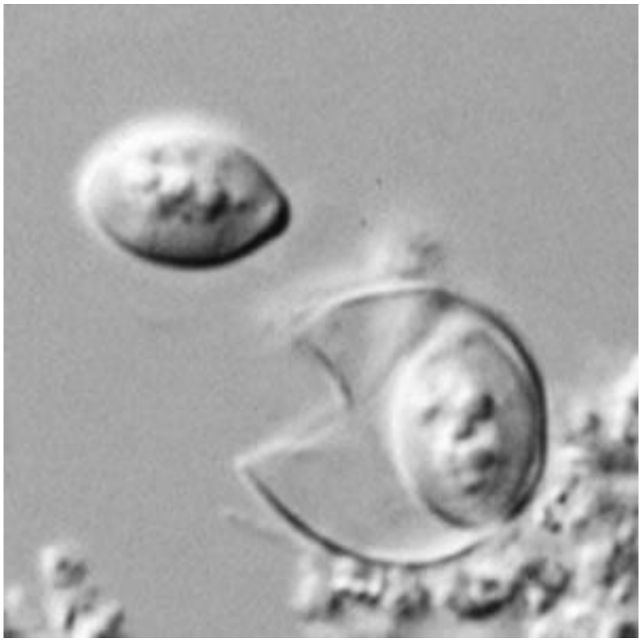

Figure 1 C. cayetanensis oocysts viewed under differential interference contrast (DIC) microscopy. Rupturing oocyst of C. cayetanensis viewed under DIC microscopy. One sporocyst has been released from the mature oocyst; the second sporocyst is still contained within the oocyst wall. CDC DPDx, accessed 7/30/2023, https://www.cdc.gov/dpdx/cyclosporiasis/index.html

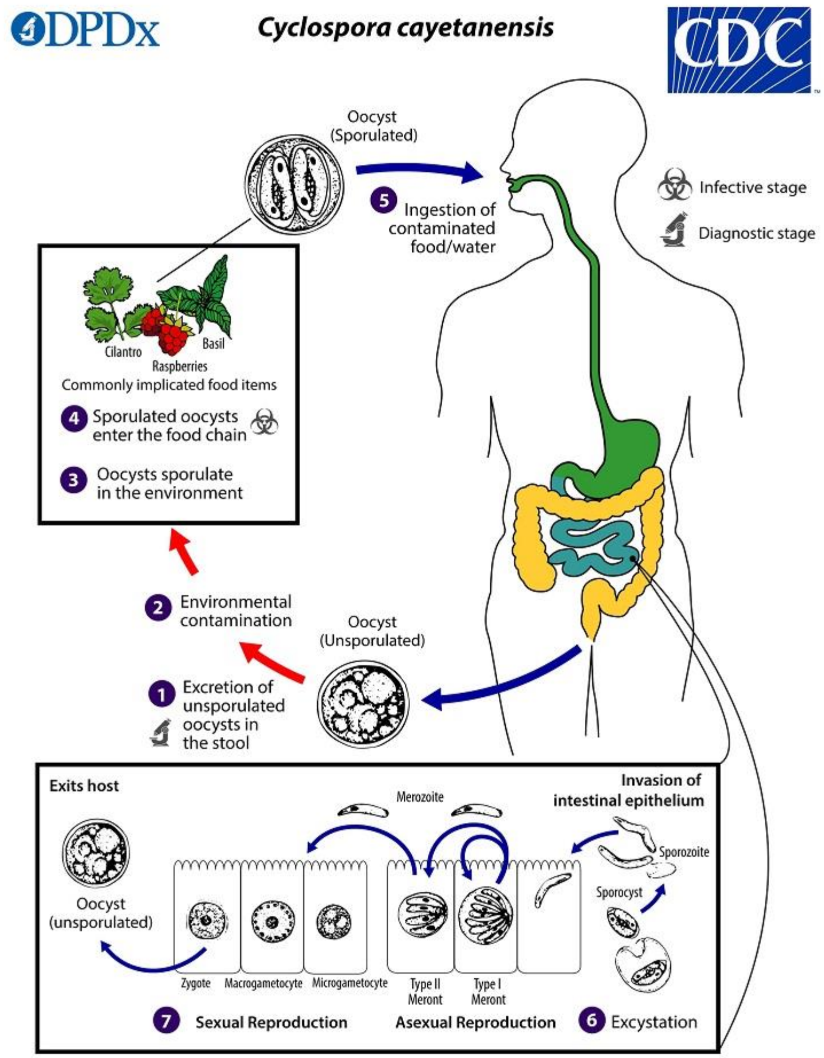

Figure 2 Life Cycle of Cyclospora cayetanensis. CDC DPDx, accessed 7/30/2023,

https://www.cdc.gov/dpdx/cyclosporiasis/index.html

2.3 Transmission

C. cayetanensis spreads to human hosts via the ingestion of sporulated oocysts. Infections have been associated with the consumption of contaminated drinking water, and fresh produce that has come in contact with contaminated water or soil.

In areas where *C. cayetanensis* is endemic, there is strong evidence that contaminated water sources facilitate the spread of the parasite between human hosts. In Guatemala, Bern et al. found that almost all the variables associated with cyclosporiasis risk involved contaminated water consumption or exposure (Bern et al., 1999). In Peru 7/11 (64%) of wastewater samples tested contained *C. cayetanensis* oocysts (Herwaldt, 2000). In a study in Nepal drinking water, sewage water, and vegetables at market, likely washed in contaminated water, all tested positive *for C. cayetanensis* (Sherchand et al., 1999). Furthermore, hand washing, and water boiling were significant mitigation factors associated with school children infection (Tandukar et al., 2013). In a study in Vietnam 12% of market produce samples were contaminated, compared to 8% from farm samples (Tram et al., 2008). Many of these studies also support the theory that *C. cayetanensis* infection is strongly dependent on seasonality (Bern et al., 1999; Giangaspero & Gasser, 2019; Sherchand et al., 1999) and that children and immunocompromised individuals are more likely to suffer from cyclosporiasis (Bern et al., 1999; Sherchand et al., 1999; Wurtz et al., 1993)

Food, fresh produce in particular, has been commonly associated with the spread of cyclosporiasis amongst communities. In the United States, outbreaks of cyclosporiasis have been thought to be caused by the import of contaminated fresh produce. Imported raspberries, lettuce, cilantro, and basil have all been linked to outbreaks in the country. Outside of the United States, there is also substantial evidence of contaminated food being a transmission route for *Cyclospora*. Produce that is consumed raw provides a particular risk of infection, for example, herbs (CDC).

2.4 Clinical Presentation

The clinical presentation of cyclosporiasis ranges greatly, from asymptomatic to severe symptoms and rarely death. Symptom presentation begins between 2 days and 2 weeks, (average 7 days) after infection, as the newly released sporozoites infect epithelial cells in the gastrointestinal tract. Without treatment, symptoms can last for several weeks, and in some cases over a month. The most common symptom for those infected is watery diarrhea, allowing for the proliferation of oocysts into the environment. Other common symptoms include bloating, cramping, fatigue, nausea, and loss of appetite. Less commonly, vomiting and fever may occur (Almeria, Cinar, & Dubey, 2019; CDC; Ortega & Sanchez, 2010).

Similar to other foodborne illnesses, symptoms are more likely to last longer and be more severe among vulnerable groups who are less able to fight off infection. Children, along with the elderly and the immunocompromised are at risk for developing more severe symptoms. Individuals living in areas where cyclosporiasis is endemic have shown heightened levels of immunity, and adults are more likely to be asymptomatic despite infection (Almeria et al., 2019; CDC; Ortega & Sanchez, 2010).

For those suffering from severe cases of cyclosporiasis, prolonged watery diarrhea can lead to dangerous levels of dehydration and malnutrition. This prolonged strain on the body can lead to death. Rarely, other complications can result from cyclosporiasis including: acalculous cholecystitis, Guillain-Barré syndrome, sterile urethritis, ocular inflammation, and reactive arthritis. A report of cardiac arrest resulting in death has also been documented while the patient suffered from a prolonged fever (Ortega & Sanchez, 2010).

2.5 Diagnosis

Most individuals infected with cyclosporiasis experience mild or no symptoms. For these people, testing is not necessary, and symptoms that do present can resolve on their own after a short period of time. If healthcare providers suspect cyclosporiasis in their patients with symptoms, such as prolonged diarrhea, there are methods to test for the parasite. In the United States, testing for evidence of *Cyclospora* is recommended for patients with compatible symptoms and symptom onset during peak cyclosporiasis seasonality, late spring and summer. Additional risk factors for clinicians to consider are foreign travel to areas where cyclosporiasis is endemic, and the consumption of potentially contaminated produce. (Almeria et al., 2019; CDC; Mathison & Pritt, 2021; Ortega & Sanchez, 2010)

The most common method for detection is the examination of stool samples for the presence of oocysts. These ova and parasite exams use traditional microscopy and staining to identify the parasite within a patient's stool (Figure 3). It can be difficult to identify *Cyclospora* oocysts and parasites within stool samples, and the use of ultraviolet autofluorescence helps highlight the parasite against the background (Almeria et al., 2019; CDC; Mathison & Pritt, 2021; Ortega & Sanchez, 2010). Diagnostic laboratories are able to identify the unique morphology of *Cyclospora* oocysts under direct light microscopy. The oocysts are non-refractile spheres 8-10 micrometers in length, which contain cytoplasm and refractile globules. When the stool sample is acid-fast stained, the oocytes typically appear pink, red, or purple. *Cyclospora* oocysts can be easy to miss, with less shedding in stool than the similar *Cryptosporidium parvum*. While *Cyclospora* and *Cryptosporidium* oocysts are very similar in appearance, *Cyclospora* oocysts are notably larger. Multiple samples may need to be taken and observed if oocysts are not identified but cyclosporiasis is still suspected. (Almeria et al., 2019; CDC; Mathison & Pritt, 2021; Ortega & Sanchez, 2010)

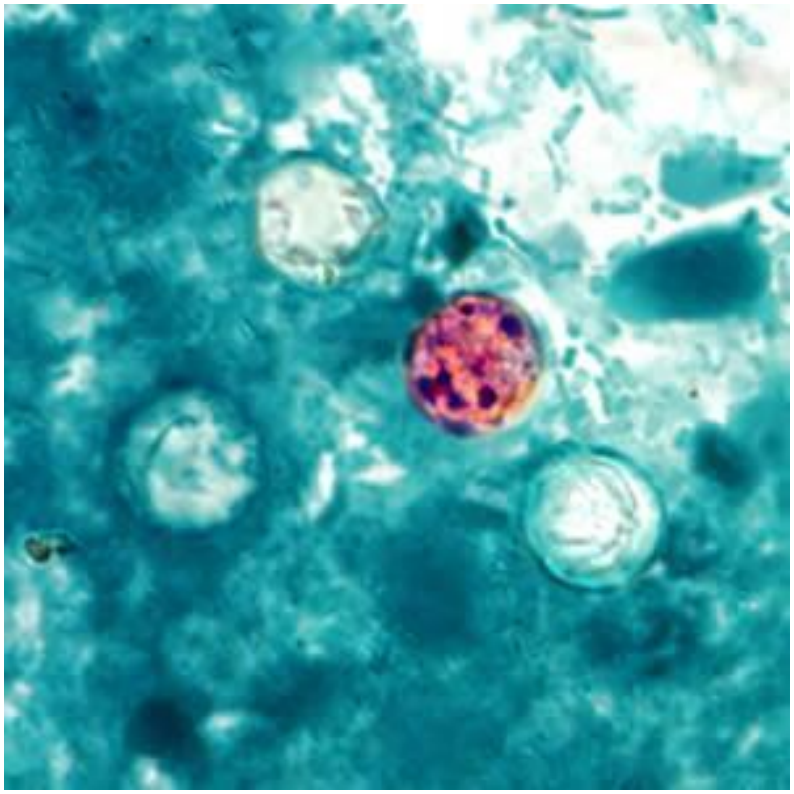

Figure 3 Two oocysts of C. cayetanensis stained with modified acid-fast stain. CDC DPDx, accessed 7/15/2023

https://www.cdc.gov/dpdx/cyclosporiasis/index.html

Molecular diagnostics can also be used to help identify the presence of *Cyclospora* in stool samples. Polymerase chain reaction analysis can be used to identify *Cyclospora* through the presence of DNA markers. While the genome of *Cyclospora cayetanensis* was recently mapped, and diagnostic assays are being developed, light microscopy and ultraviolet autofluorescence are still the preliminary method for the diagnosis of cyclosporiasis (Almeria et al., 2019; CDC; Mathison & Pritt, 2021; Ortega & Sanchez, 2010)

2.6 Treatment

In most immunocompetent patients, especially those living in areas where *C. cayetanensis* is endemic, infections are asymptomatic or mild in symptom presentation. For these people treatment is likely unnecessary, and their immune systems will be able to resolve the infection without intervention. For those who do experience prolonged or severe symptoms, whether immunocompetent or not, the standard treatment is the combination drug trimethoprim-sulfamethoxazole (TMP-SMX) (Almeria et al., 2019; Ortega & Sanchez, 2010).

The use of TMP-SMX has proven effective for both children and adults, with daily treatment regimens successfully eliminating the excretion of oocysts in stool. This reduction in *Cyclospora* in the stool was correlated with an improvement to clinical presentation, with diarrhea subsiding in a matter of days. For patients with sulfonamide allergies, ciprofloxacin and nitazoxanide are the recommended alternative treatments, although they have proven less effective than the standard TMP-SMX regimen. For patients with drastically compromised immune systems, such as transplant recipients and those with AIDS, longer regimens and stronger doses may be required. Due to an increased chance of relapse, TMP-SMX is suggested to be taken as a prophylaxis for several weeks following initial treatment for immunocompromised patients (Almeria et al., 2019; CDC; Mathison & Pritt, 2021; Ortega & Sanchez, 2010)

3.0 Cyclosporiasis Epidemiology

3.1 Risk Factors

There are several risk factors associated with the acquisition of cyclosporiasis. These risk factors include living in, or traveling to, an endemic area, exposure to contaminated water or food, often caused by poor sanitation practices, exposure during peak seasonality in the late spring and early summer and having a compromised or developing immune system (Bern et al., 1999; Bhandari et al., 2015; Ortega & Sanchez, 2010; Tram et al., 2008).

While cyclosporiasis cases occur around the globe, the majority of reported cases come from tropical and subtropical regions of the world where the disease is endemic. Central and South America, the Indian subcontinent, Southeast Asia, North Africa, and the Middle East are areas where *C. cayetanensis* are considered endemic. In areas where cyclosporiasis is prevalent, poor sanitation practices and compromised infrastructure are strongly associated with the spread of the disease. Issues with drinking water infrastructure and poor sanitary practices leading to the fecal contamination of water are strongly associated with outbreaks of cyclosporiasis in communities. Fresh produce can also become contaminated, either in the fields, or when cleaned with water containing *Cyclospora* before consumption (Almeria et al., 2019; Ortega & Sanchez, 2010).

Cyclosporiasis infections show clear seasonality, although there is significant variation by region. These variations could be associated with changes in rainfall, temperature, humidity, and human behavior resulting in increased environmental contamination. These factors likely play an important role in determining peak conditions for the sporulation stage of the *Cyclospora* life cycle while in soil, as well as human related conditions allowing for environmental transmission. In some countries like Guatemala, Nepal, Mexico, and China, cyclosporiasis cases are most prevalent during warm rainy seasons. In Peru and Turkey, infections peak during dry warm periods. Some areas tend towards cooler seasons, with Haiti seeing increased infections during cool dry seasons, and Indonesia during cool wet seasons. In the United States infections are most common during the summer months, when travel associated cases and foodborne outbreaks occur most often. Overall seasonality plays an important role in cyclosporiasis cases across the globe, but there is a large amount of variation in the conditions that correspond with peak cases. The underlying causes of seasonal trends require additional research at local levels (Almeria et al., 2019; Bern et al., 1999; Bhandari et al., 2015; Frickmann et al., 2021; Orozco-Mosqueda, Martinez-Loya, & Ortega, 2014; Ortega & Sanchez, 2010; Tram et al., 2008).

Individuals with weak or compromised immune systems are at a higher risk of *Cyclospora* infection and the development of symptoms. In endemic areas children, the elderly, and those with immunodeficiencies are most likely to become infected. Adults are less likely to become ill due to their increased immunities to *Cyclospora* developed over their lifetime, although immune systems deteriorate as individuals reach old age increasing the likelihood of infection. In areas where cyclosporiasis is not endemic, age is not as significant of a factor for infection as the adult population has not had a chance to develop any resistance to *Cyclospora* (Bhandari et al., 2015; Mathison & Pritt, 2021; Ortega & Sanchez, 2010).

Certain pre-existing conditions can leave people at particular risk for *Cyclospora* infection. Some of the earliest cases of cyclosporiasis involved people living with HIV/AIDS, and it remains a common opportunistic disease among them (Pape, Verdier, Boncy, Boncy, & Johnson, 1994; Ramezanzadeh et al., 2022; Wurtz et al., 1993). Currently, cyclosporiasis prevalence estimates range from 2.8% in Asia to 7.8% in South America and it is significantly more likely to affect people living with HIV/AIDS (Ramezanzadeh et al., 2022).

3.2 Cyclosporiasis Outside Of The United States

While cases of cyclosporiasis appear all around the world yearly, the majority of cases, and therefore research, is done in tropical and subtropical regions of the world. Several studies were reviewed that screened for *C. cayetanensis*, and many of them found the parasite in abundance during stool sample screenings. In a study in Nepal almost 30% of children with diarrhea symptoms were positive for the parasite (Sherchand et al., 1999), similarly in a study in Peru assessing children 18% of tested children were infected (Ortega et al., 1993). A study of raspberry farmers in Guatemala found 6.7% had positive test results (Bern et al., 1999). A study conducted in Haiti of household and water supplies found 12% of persons tested were infected as well as one water source (Lopez et al., 2003), and in Vietnam, 11.8% of market water and herb samples contained *Cyclospora* (Tram et al., 2008).

Outside of endemic areas, waterborne transmission of cyclosporiasis has been identified in Europe as well. A 2015 preliminary study of tap water from Italian trains found *Cyclospora* oocysts present in toilet water, this study describes the first documented case of water being contaminated with *C. cayetanensis* in Italy. Ten water samples were collected from different coaches of a busy passenger train from October 2012 to July 2013, with 30% of the samples testing positive for *C. cayetanensis* oocysts. The toilet water was pumped directly from the potable water tanks stored onboard the train, and the water tanks were filled daily at the train's station of departure. There are multiple factors that could have led to the contamination of the water, such as a tainted supply source, contaminated holding tanks, or the entry of coprophagic animals which came into contact with fecal matter (Giangaspero, Marangi, & Arace, 2015).

3.3 Cyclosporiasis In The United States

Cyclosporiasis cases have been reported in the United States since the mid 1990's. From 2016 to 2020 12,642 cases of cyclosporiasis were reported in the country. In the United States, the CDC, along with state and local officials, monitor cases of cyclosporiasis year-round. Health agencies work together in order to detect and contain outbreaks linked to a common source of contamination. Sick individuals, who meet the surveillance case definition, are given a questionnaire focusing on risk factors such as food and water exposures and foreign travel in order to identify common sources of contamination (CDC; CDC).

Surveillance case definitions utilize a set of criteria in order to provide consistent identification of cyclosporiasis cases across jurisdictions within the United States. These case definitions are not the same as a diagnosis from a medical professional. Cases are classified as "Probable" when individuals meet the clinical description while also being epidemiologically linked to a confirmed case. Cases are classified as "Confirmed" when they both meet the clinical description and have laboratory confirmed evidence of *Cyclospora* and/or *Cyclospora* DNA in a stool or biopsy sample. For the case definition, the clinical criteria include an individual experiencing watery diarrhea, appetite loss, weight loss, cramps, bloating, nausea, aching, fatigue, vomiting, and/or fever. Since 1999, cyclosporiasis has been a nationally notifiable disease, with jurisdictions across the USA requiring by law that laboratories and healthcare facilities report cyclosporiasis data to their public health agencies (CDC).

In order to gather additional information on cyclosporiasis cases, local jurisdictions administer the Cyclosporiasis National Hypothesis Generating Questionnaire (CNHGQ). The questionnaire contains sections on demographics, clinical information such as symptoms and illness onset date, domestic and international travel, information on the consumption of fresh produce, herbs, berries, fruits, leafy greens, vegetables, visiting restaurants and eating outside the home (Appendix A). These case reports allow health officials to recognize outbreaks and conduct traceback investigations in order to identify potential sources of contamination and work to prevent further spread of the disease (CDC).

While the task of collecting case questionnaires falls to state and local jurisdictions, the data are submitted to the CDC which is responsible for monitoring the 120 notifiable diseases and conditions at the national level. This system is called the *National Notifiable Diseases Surveillance System* (NNDSS), which includes the compilation, analysis, and sharing of information. The CDC uses this and other information to help organize efforts to prevent the occurrence of disease (CDC; Health.Gov).

The number of reported cases has been trending upwards since 2016 with a minimum of 537 cases, up to a maximum of 4,703 in 2019. Each year, cyclosporiasis cases are reported for individuals that have reported no known international travel within two weeks of symptom onset; these cases are referred to as domestically acquired cases. From 2016 to 2020, 6679 domestically acquired cases were reported in the United States, 8828 from 2016 to 2022. The peak of domestically acquired cases was 2408 in 2019, and the lowest was 134 cases in 2016. Texas had the most reported total cases of cyclosporiasis for 2016, 2017, 2019, and 2020, with Illinois having a higher case count in 2018 (Table 1). Regionally, cases were more spread out over the five-year sample, with four different regions topping the annual case counts (CDC; CDC).

In the USA, a mean of two outbreaks were reported a year between 2000 and 2017, with a total of 39 occurring in the time period. The vast majority of USA cyclosporiasis outbreaks occurred between May and July, demonstrating the seasonality of the disease. From 2000 to 2017 the most common foods associated with outbreaks were raspberries, basil, and cilantro. While it has proven difficult to identify a particular source of an outbreak, some of the outbreaks were associated with imported produce from countries such as Peru and Guatemala (CDC).

Since 2019, cases have been linked to contaminated produce from both foreign and domestic sources. In 2019 a multistate outbreak of *Cyclospora* was traced to fresh basil imported from Mexico. The outbreak affected 241 people across 11 states and resulted in 6 hospitalizations. The distributor of the basil recalled potentially contaminated basil approximately two months after cases began to appear. In 2020, 701 people across 14 different states had laboratory confirmed cases of *Cyclospora* infection, with exposure being confirmed in 13 of those 14 states. After traceback investigation and interviews, a major USA produce brand of bagged salad mix containing iceberg lettuce and other produce that had been processed at a plant in Illinois was identified as the likely source of infection. The outbreak resulted in 38 (5%) hospitalizations and no deaths; the distributor voluntarily recalled the identified produce item. The years 2021 and 2022 each had two large, multistate, outbreaks of *Cyclospora* linked to the consumption of leafy greens. None of the four outbreaks could be traced back to a specific source of contamination (CDC).

The first reported outbreak of cyclosporiasis in the United States, and the only one linked to contaminated drinking water, occurred in Illinois in July 1990. An investigation was conducted after several physicians at a Chicago area hospital developed diarrhea over the course of a weekend. Other hospital workers experiencing diarrhea were interviewed, stool samples were collected, and the City of Chicago's water service authorities inspected the plumbing and collected water samples from the hospital facilities. After investigation it was found that 21 individuals met the clinical definition for cyclosporiasis, with 11 laboratory confirmed cases. The investigators identified tap water from the physicians' dormitory as a risk factor and assumed that water was contaminated with *Cyclospora*. Upon environmental investigation the water tank was found to have several issues, including poor maintenance and stagnant water (Huang et al., 1995).

Table 1 Total and domestically acquired probable and confirmed cases reported to the US CDC by, 2016-2022*

Year	Total cases	Domestically acquired
2016	537	134
2017	1,194	597
2018	3,519	2299
2019	4,703	2408
2020	2,689	1241
2021	-	1020
2022	-	1129

* Data from Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System (NNDSS) Annual Summary Data for years 2016-2019, United States, CDC WONDER online database, https://wonder.cdc.gov/nndss/nndss_annual_tables_menu.asp, accessed 6/23/2023

4.0 Is Cyclosporiasis Endemic To The USA?

4.1 Introduction

Given the number of annual cases of cyclosporiasis in the United States, there is concern as to whether *C. cayetanensis* has become endemic to the USA. The Food and Drug Administration (FDA) is an agency within the Department of Health and Human Services which is responsible for the safety of the USA food supply, drugs, medical devices, and other consumer goods. Recalling food products that have been identified as sources of infection with food and waterborne pathogens falls under the FDA's purview, and therefore the possible increase of domestic cyclosporiasis cases is of interest to the agency along with the CDC. In particular, there is concern that cyclosporiasis is being spread through water, which has been shown to be a transmission route outside of the USA.

This section describes my work at the CDC reviewing cases of cyclosporiasis reported within the United States between 2017 and 2019 with the aim of evaluating to what degree waterborne cyclosporiasis is a risk. Specific research goals were:

1. *Complete a systematic review of cyclosporiasis case report forms used to collect public health surveillance data in jurisdictions within the United States, with the goal of informing national recommendations around environmental risk for cyclosporiasis in water.*

2. *Conduct an evaluation activity for cyclosporiasis case reporting. The evaluation assessed data collection tools and efficacy. Case data was formatted and cleaned, and a random sampling was conducted. The efficacy of data collection elements utilized by the jurisdictions was assessed for relevancy and practicality.*

3. *To analyze United States public health surveillance data in order to quantify the reported environmental health risk factors for cyclosporiasis. This analysis was used to determine the efficacy of data collection techniques, and to make recommendations to the Food and Drug Administration regarding the environmental health risk pertaining to water.*

In order to understand the possible role of contaminated water as infection sources in the United States, data from jurisdictions reporting cyclosporiasis to the CDC from 2017-2019 were compiled and reviewed.

4.2 Methodology

Copies of questionnaires submitted by jurisdictions to CDC were reviewed. Jurisdiction A and jurisdiction B were the only jurisdictions that included water related questions. Data from both jurisdictions were reviewed and summarized. Due to the large number of cases reported by jurisdiction A over the three-year period, every tenth case questionnaire was systematically sampled for review. All cases reported by jurisdiction B were included in the review. Notably, the CDC received scanned PDF files of printed questionnaires. This meant that some longer open-ended answers were only partially legible. Of a total of 373 cases, 37 jurisdiction A case questionnaires were reviewed, 10 of 102 in 2017, 9 of 87 in 2018, and 18 of 184 in 2019. All 37 jurisdiction B case questionnaires from 2019 were reviewed.

4.3 Results

4.3.1 Water Exposure Questions

In 2017 and 2018, jurisdiction A collected data on potential water exposures to cyclosporiasis, the same water exposure questions were asked in both 2017 and 2018. In 2019 the water exposure questions were not asked. Jurisdiction A's water exposure questions focused on swimming-based exposures, asking about the case patient's recent swimming and use of water parks, hot tubs, jacuzzis, spas, and hydrotherapy pools. No drinking water questions were included.

In 2019 jurisdiction B asked case patients about any recent swimming in the 14 days prior to symptom onset and about their drinking water sources, both at home and at work. jurisdiction B's questions about swimming were open-ended, while the drinking water questions were multiple choice with an open-ended "other" option.

Both jurisdictions asked open-ended follow-up questions concerning the location and date of any swimming and jurisdiction A also asked about water parks, hot tubs, jacuzzis, spas, and hydrotherapy pools.

4.4 Review of Data

Table 2 Summary of recreational water exposure data for reviewed cyclosporiasis cases

	Jurisdiction A 2017	Jurisdiction A 2018	Jurisdiction B 2019
Use of whirlpool spa, jacuzzi, hot tub, hydrotherapy pool	0/10 (0%)	1/9 (11%)*	-
Visited recreational water park	0/10 (0%)	0.9 (0%)	-**
Swimming in a pool or other body of water	0/10 (0%)	2/9 (22%)***	10/37 (27%)****

* 1/9 (11%) respondents reported the location(s) where they swam. 0/9 (0%) respondents reported the date(s) when they swam.

** One Respondent from jurisdiction B reported swimming at a recreational water park in the open-ended swimming section.

*** 2/9 (22%) respondents reported the location(s) where they swam. 1/9 (11%) respondents reported the date(s) when they swam.

**** 10/37 (27%) respondents listed the location(s) where they swam. 8/37 (22%) respondents listed the date(s) when they swam.

Table 3 Summary of drinking water consumption for Jurisdiction B 2019 cyclosporiasis cases

	Drinking Water at Home	Drinking Water at Work
Response Rate	34/37 (92%)	17/37 (50%)
Municipal Water	19/37 (51%)	7/37 (19%)
Well Water	10/37 (27%)	1/37 (3%)
Bottled Water	9/37 (24%)	7/37 (19%)
Unknown	0/37 (0%)	1/37 (3%)
Other	4/37 (11%)	2/37 (5%)

4.5 Discussion

Both jurisdiction A and jurisdiction B included water related exposure questions in their case investigations for cyclosporiasis. Jurisdiction B included questions related to swimming as well as water consumption whereas jurisdiction A included only swimming questions. Though water is a known transmission source for *Cyclospora* in other countries (Naganathan et al., 2021) it appears unlikely that either jurisdiction's water exposure questions contributed significantly to their respective case investigations. Despite both jurisdiction A and jurisdiction B asking about swimming prior to symptom onset, neither jurisdiction attributed any cases of *Cyclospora* infection to swimming.

Recreational water contamination can cause illnesses in individuals who come in contact with contaminated water while swimming recreationally in any body of water. Parasites such as *Cyclospora*, *Giardia*, and *Cryptosporidium* can survive for extended periods of time in the environment, even in chlorinated water; for *Giardia* and *Cryptosporidium*, recreational swimming has been documented as a common transmission route (CDC).

While a large portion of sampled cases, in both jurisdiction A and jurisdiction B, reported swimming in the two-week period before symptom onset, none of the cases reported swimming in the same locations. The two jurisdiction A cases that reported swimming did so abroad, in the Philippines and Mexico. In contrast, all of the sampled jurisdiction B cases that reported swimming did so in the U.S. Cases reported swimming at local lakes, at pools, and one case reported going to a jurisdiction B waterpark. There does not appear to be any geographic clustering around swimming locations contaminated with *Cyclospora*.

Only one of the sampled jurisdiction A cases reported using a hot tub in the weeks preceding symptom onset, and none of the sampled cases reported going to a waterpark.

Only jurisdiction B gathered data about drinking water, and while most cases reported their source(s) of drinking water at home, there does not appear to be any correlation between water source and cyclosporiasis cases. The majority of respondents reported drinking municipal water at home and bottled water at work.

It is difficult to assess the danger of bottled water contamination given our limited data. The only jurisdiction in the country to ask about bottled water was jurisdiction B. In their data collection, they asked no follow-up questions about brand, bottle type, or purchase location which could assist with traceback investigations. If standardized water exposure questions are added to the CNHGQ it would be beneficial to gather more details, similar to the food exposure sections, in order to effectively trace any common exposures.

It is unlikely that any municipal water supplies in jurisdiction B were contaminated with *Cyclospora*. Any such contamination would likely result in a large number of cases in the geographic area that utilized the contaminated municipal water source. There was no evidence of any such outbreak in our sampled data set.

5.0 Conclusion

Cyclospora cayetanensis is a relatively common parasite found across the world, thriving in areas where sanitation is poor. While most cases of infection, especially in areas of endemicity, are asymptomatic or mild, large outbreaks are not uncommon and are an environmental health concern to communities and public health agencies. Both food and water can act as avenues of transmission for cyclosporiasis, in the United States foodborne outbreaks are the most common, while waterborne outbreaks have been historically rare. Due to the increasing number of cyclosporiasis cases, and the precedent of waterborne outbreaks in other countries, there is concern that waterborne cyclosporiasis infections may be a rising, under-monitored, threat.

Cyclosporiasis has affected people living in different geographic areas in the United States. Historically, Texas has been the most affected state, but other states have seen increased numbers of cases recently. Although most U.S. cyclosporiasis outbreaks have been linked to imported produce, the public health response to future outbreaks must consider the potential for domestic soil and water contamination. Awareness of environmental health patterns is important for planning public health interventions. Changes in human behavior, land use, food production and distribution, and local climate could have impacts on the incidence of cyclosporiasis in the country. Public health surveillance is an important tool in understanding these changing dynamics.

While data are routinely gathered on *Cyclospora* infection in the United States, only three years of water exposure data were collected between two jurisdictions. From this limited data set there is no evidence of *Cyclospora* infection associated with water exposures. Given this lack of evidence, there is no strong indication that these questions asked as their own discrete, detailed, section are necessary at this time. It is important for public health agencies to strive for parsimony when developing questionnaires so as to not create an undue burden on the officials conducting the interviews or the individuals reporting their symptoms.

However, without any questions dedicated to water exposure it will be difficult to identify changing trends. In order to maintain baseline surveillance of water exposures it could be beneficial to include a small number of general questions. Alternatively, open ended questions could prove sufficient at capturing baseline information on water exposures while maintaining efficient questionnaire length.

An additional environmental surveillance tool that could be used to help track C*yclospora* in the U.S. is the National Wastewater Surveillance System (NWSS). The NWSS was launched in 2020 to help track the presence of SARS-CoV-2 in community level wastewater samples. There are over 1,200 NWSS testing sites across the US, covering wastewater systems that serve almost 150 million Americans. While the NWSS only tests for SARS-CoV-2, and more recently mpox, the CDC is exploring ways to test for additional pathogens. This surveillance could provide an important early warning for potential C*yclospora* outbreaks (CDC).

If the number of cases of cyclosporiasis continues to increase in the United States, especially if there is evidence of waterborne transmission, the case questionnaires should be augmented with detailed water consumption and exposure sections. Given the national nature of cyclosporiasis surveillance, these questionnaires should be standardized, and reporting should be consistent across jurisdictions.

Appendix A

Cyclosporiasis National Hypothesis Generating Questionnaire

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