Cyclospora cayetanensis

Cyclospora cayetanensis
Protozoa, class of Sporozoea

Parasite

Characteristics and sources of *Cyclospora cayetanensis*

Main microbial characteristics

Cyclospora cayetanensis is the causative agent of cyclosporiasis. It is a unicellular parasite belonging to the Coccidia (Coccidiasina) subclass of the phylum *Apicomplexa*.

C. cayetanensis is an intracellular parasite whose monoxenous[1] biological cycle has not yet been fully characterised. The cycle begins with the ingestion of mature sporulated oocysts, each one containing two sporocysts which each contain in their turn two infecting sporozoites. When exposed to gastric acid, the sporozoites are liberated into the intestinal lumen and penetrate the enterocytes of the small intestine. Type I and II meronts are then produced through asexual reproduction. The meronts can then evolve to a sexual stage, with the formation of male gametes (microgametogony) or female gametes (macrogametogony). Fusion of the gametes produces an immature oocyst (8 to 10 µm) which is excreted in the faeces when the enterocyte dies. The parasite only becomes infectious after maturing in the external environment, depending upon the temperature (one to two weeks if the temperature is between 23 and 27°C), once the oocyst reaches its sporulated stage.

The biological cycle of the parasite is summed up in Figure 1.

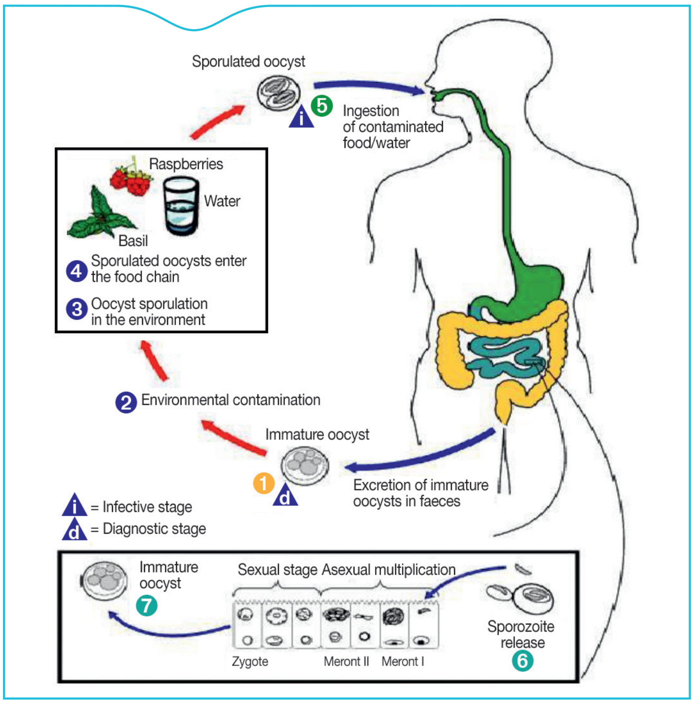


Figure 1: **Biological life cycle (based on a diagram presented by the CDC) of *Cyclospora cayetanensis***

Sources of the hazard

There are currently no known animal reservoirs for this parasite. The only reservoir appears to be humans. The main environmental sources of the hazard, *via* the faecal elimination of oocysts, are water and plants in contact with soil or irrigated through spraying.

The role of free-living nematodes or other mechanical vectors (filter-feeding shellfish, insects, rotifers) in the contamination of food crops is also worth exploring.

Transmission routes

Transmission of the parasite is by the faecal-oral route, through food or water contaminated by oocysts from human faecal matter. The disease is therefore most often contracted through ingesting contaminated water or raw vegetables or plants.

Cyclospora is not directly infectious when it is excreted in the faeces of an infected individual, since the oocysts are excreted in their immature form. Direct transmission from one individual to another is therefore impossible.

Recommendations for primary production

- **Compliance with good hygiene practices during growing and harvesting, especially in endemic countries which produce plant products and export them to Europe.**
- **Strict adherence to good farming practices in order to limit the risk of contaminating raw materials (ready-to-eat raw fruits and vegetables) through spray irrigation water.**
- **Treatment of wastewater and banning human fertiliser.**

(1) Parasite life cycle involving a single host.

Data sheet on foodborne biological hazards
February 2014

Human foodborne disease

Nature of the disease (Table 1)

Table 1. **Characteristics of the disease**

Mean incubation period	Target population	Main symptoms (according to surveys)	Duration of symptoms	Duration of infectious period (shedding)	Complications	Asymptomatic forms
2 to 6 days	All people exposed to ingestion of viable sporulated *C. cayetanensis* oocysts*	• Watery diarrhoea (96- 99%), • Abdominal pain (75 to 85%). • Nausea (70-88%) • Fatigue (75-92%) • Weight loss (72%) • Anorexia (65%) • Fever (43-54 %) • Vomiting (27-32 %) • Headache (45-47%)	17.5 days (10-30 days)	a few weeks	VIH/AIDS patients: prolonged diarrhoea	Yes: Higher frequency in endemic regions

* Especially people travelling in endemic regions (essentially Latin America, the Indian sub-continent and South-East Asia), consumers of raw products imported from inter-tropical regions (berries, fruits and vegetables, spices) stored and transported in a humid environment.

Susceptible population groups(2): immunodepressed individuals (especially AIDS patients) can develop serious, prolonged diarrhoea leading to severe dehydration which can also cause cachexia. Extra-intestinal infections (cholangitis) have been described in AIDS patients, but no fatal cases due to cyclosporiasis have been declared.

Dose-effect relationships(3)

The epidemiological data suggests that approximately ten oocysts are enough to bring on the disease.

Epidemiology

Surveillance system

In France, only clustered cases of cyclosporiasis manifesting as foodborne illness outbreaks are subject to mandatory reporting. Sporadic cases are not reported, but since 2006 a survey-based annual report of the number of cases diagnosed by the parasitology laboratories of the Crypto-Anofel network (InVS network) has been issued.

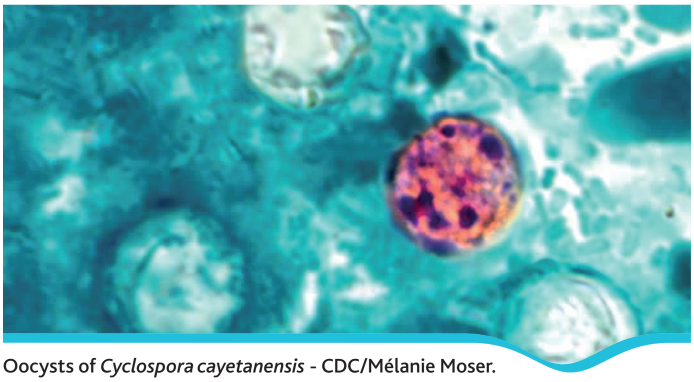
In the USA, confirmed cases have been reported to the Center for Disease Control and Prevention (CDC) since 1997. They have been reported to the Public Health Agency of Canada (PHAC) since 2000.

Prevalence

Although it has a cosmopolitan distribution, cyclosporiasis is essentially found in the inter-tropical geographical area (where the environmental conditions are favourable to the parasite) with a higher prevalence in certain countries such as Nepal, Peru, Guatemala, Mexico and Haiti. In these countries, the overall prevalence is from 2.3% to 9.2% in immunocompetent subjects and 3.8% to 11.3% in AIDS patients. In these endemic areas, cases are frequently distributed seasonally, often in relation to the rainy season. Epidemiological studies have also found that the majority of infected subjects are children, and that asymptomatic carriage is common. The prevalence of *Cyclospora* infection in industrialised countries is only partially known. In the US, from 1997 to 2008, the overall rate of incidence went from 0.01 out of 100,000 people in 1997 to 0.07 in 2002; excluding epidemic cases, the observed cases mainly concerned migrants and travellers (destinations such as Mexico, Guatemala and Peru were often reported).

In France, since 2006 about approximately ten sporadic cases of cyclosporiasis have been reported by the national Crypto-Anofel network each year, mainly imported cases.

(2) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard (in the case of the ANSES data sheets).
(3) Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.



Oocysts of *Cyclospora cayetanensis* - CDC/Mélanie Moser.

Outbreaks

Since 1990, about sixty foodborne cyclosporiasis epidemics (and three waterborne ones) have been reported, most in the US (including one which occurred from mid-June 2013 to August 2013 with 643 cases reported over 25 states) and in Canada (from 3 to 1475 cases). When the source of contamination could be identified through an epidemiological survey, the incriminated foods were essentially fruits and vegetables from the endemic area, in particular raspberries, basil, lettuce and snow peas.

Role of food

Main foods to consider

Water is the main vector of food contamination. Fruits and vegetables can be contaminated by both soil and waterborne oocysts (crop irrigation water). Varieties with a large surface area (leafy vegetables for example) or with a shape and texture promoting the trapping or attachment of oocysts (raspberries for example) are more likely to be contaminated. These foods present a risk if they are not thoroughly washed or cooked.

Retention and inactivation treatments in industrial environments

Retention treatments

The most commonly used retention treatments for treating water are flocculation, decantation and filtration. However, due a lack of *in vivo* and *in vitro* models, no serious studies have been able to be conducted.

Table 2. **Physical and chemical treatments**

Inactivation treatment		Matrix	Effectiveness of treatment
Chemical treatments			
Chlorine dioxide gas (4.1 mg/L) for 20 min		lettuce leaves and basil	Ineffective
Chlorine (concentrations commonly used for the public water supply)		water	Ineffective
Physical treatments			
Refrigeration/ ambient temperature		/	Ineffective
Freezing	-15°C, 24 h	milk	Ineffective
	-15°C, 48 h	milk	Effective
	-20°C, 48h	basil and water	Ineffective
	-20°C, 96 h	basil and water	Effective
	-70°C, 1 h	basil and water	Effective
Cooking/reheating	50°C, 1 h	basil and water	Ineffective
	70°C, 15 min	basil and water	Effective
	Microwaves – 45 sec (temperature reached in centre: 96°C)	Aqueous solution	Partial destruction
High pressure*	550 MP, 40°C, 2 min	basil and raspberries	6 $\log_{10}$ reduction
Ionisation*	1 kGy	raspberries	2.8 $\log_{10}$ reduction
UV*	261 mW/cm^2 Continual treatment at 254 nm	basil	4 $\log_{10}$ reduction

* model used: *Eimeria acervulina*, a *Coccidium* close to *Cyclospora cayentanensis*

Inactivation treatments

For the same reasons, the resistance/susceptibility properties of *Cyclospora* oocysts to the physical and/or chemical treatments commonly used as inactivation processes for micro-organisms are poorly understood.

The few existing studies use the sporulation ability of oocysts (sporulation test) as a test of viability for *Cyclospora cayetanensis*. Treatments that do not affect the ability of oocysts to sporulate are considered to be ineffective.

Monitoring in food

In Europe and in the rest of the world, there are no regulatory criteria regarding *Cyclospora cayetanensis* in food.

Screening in water

There is no standardised method for detecting *Cyclospora cayetanensis* oocysts in water. However, due to the size of oocysts, certain authors use the filtration-based concentration methods described for *Giardia* cysts and *Cryptosporidium* oocysts (EPA method 1623 (2005), Drinking Water Inspectorate (2005), ISO 15553 (2006) et NF T90-455 (2001)). Identification is based on microscope observations and molecular assays (nested PCR and sequencing or real-time PCR).

Screening in foods

While the FDA has described methods for isolating *Cyclospora* oocysts from leafy vegetables (lettuce, aromatic herbs), berries (raspberries), and beverages (juice, cider, milk, water), their effectiveness is not known.

Recommendations for operators

- **Good hygiene practices should be adhered to during the transformation of basic ingredients and potable water used in processing.**
- **Kitchen personnel and all other individuals who may be called on to handle foodstuffs should be made aware of the faecal-oral route risks and of the importance of following strict hygiene measures (thorough hand washing). This is especially important if the food is intended to be eaten raw or lightly cooked.**
- ***Cyclospora* should be taken into account in the hazard analysis by operators concerned with plant-based foods which are immersed in, or spray-irrigated with, potentially contaminated water (especially foods coming from endemic regions). Appropriate measures should be taken accordingly.**

Domestic hygiene

Recommendations to consumers

- **Basic rules of hygiene should be followed, hands washed thoroughly, especially after using the lavatory, and kitchen utensils and countertops washed, especially before handling food.**
- **Foods that may be contaminated by *Cyclospora* oocysts (fruits and vegetables intended to be eaten raw) should be washed thoroughly with potable water.**
- **Foods should be cooked if proper washing conditions cannot be applied (no potable water available, or water from an unsure source), especially in endemic regions.**
- **In endemic regions, it is recommended to drink only bottled spring or mineral water from sealed bottles. If this is not possible, boil water for 10 minutes or treat it using a microfiltration system before drinking.**

References and links

General references

- Chacín-Bonilla L. Epidemiology of *Cyclospora cayetanensis*: A review focusing in endemic areas. Acta Trop. 2010 Sep;115(3):181-93. Review.
- Hall RL, Jones JL, Herwaldt BL. Surveillance for laboratory-confirmed sporadic cases of *cyclosporiasis*-United States, 1997-2008. MMWR Surveill Summ. 2011 Apr 8;60(2):1-11.
- Milord F, Lampron-Goulet E, St Amour M, Levac E, Ramsay D. *Cyclospora cayetanensis*: a description of clinical aspects of an outbreak in Quebec, Canada. Epidemiol. Infect. (2012), 140, 626-632.
- Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. Clin Microbiol Rev. 2010 Jan;23(1):218-34.

Useful links

- ANSES: www.anses.fr
- CDC: http://www.cdc.gov/parasites/cyclosporiasis/
- CDC-DPDx: http://www.dpd.cdc.gov/dpdx/HTML/Cyclosporiasis.htm
- FDA:http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm073638.htm
- National Reference Laboratory (NRL) for foodborne parasites, excluding *Echinococcus* sp.: ANSES Laboratory for Animal Health – Maisons-Alfort – France
- European Union Reference Laboratory for parasites: Istituto Superiore di Sanità (ISS) I-00161, Rome – Italy (http://www.iss.it/crlp/index.php)