

MICHELLE DANYLUK UF – MODERATOR

YNES ORTEGA, UGA

MAURICIO DURIGAN, FDA

NICOLE CLAUSEN, FDA

RICHARD DE LOS SANTOS, TX



Cyclospora Interactive Panel

Instructions

Go to

www.menti.com

Enter the code

7319 3359



Or use QR code

Cyclospora is a:

0
Virus

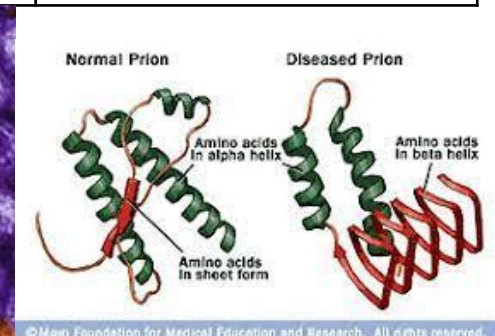
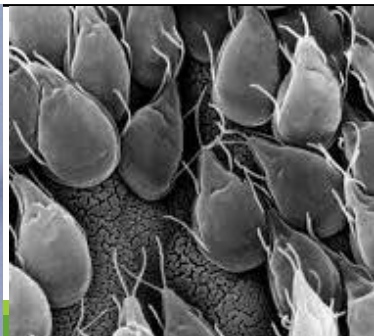
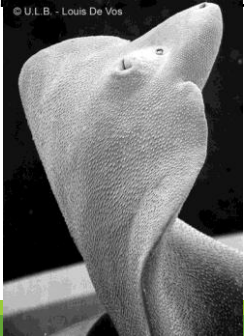
0
Parasite

0
Protozoa

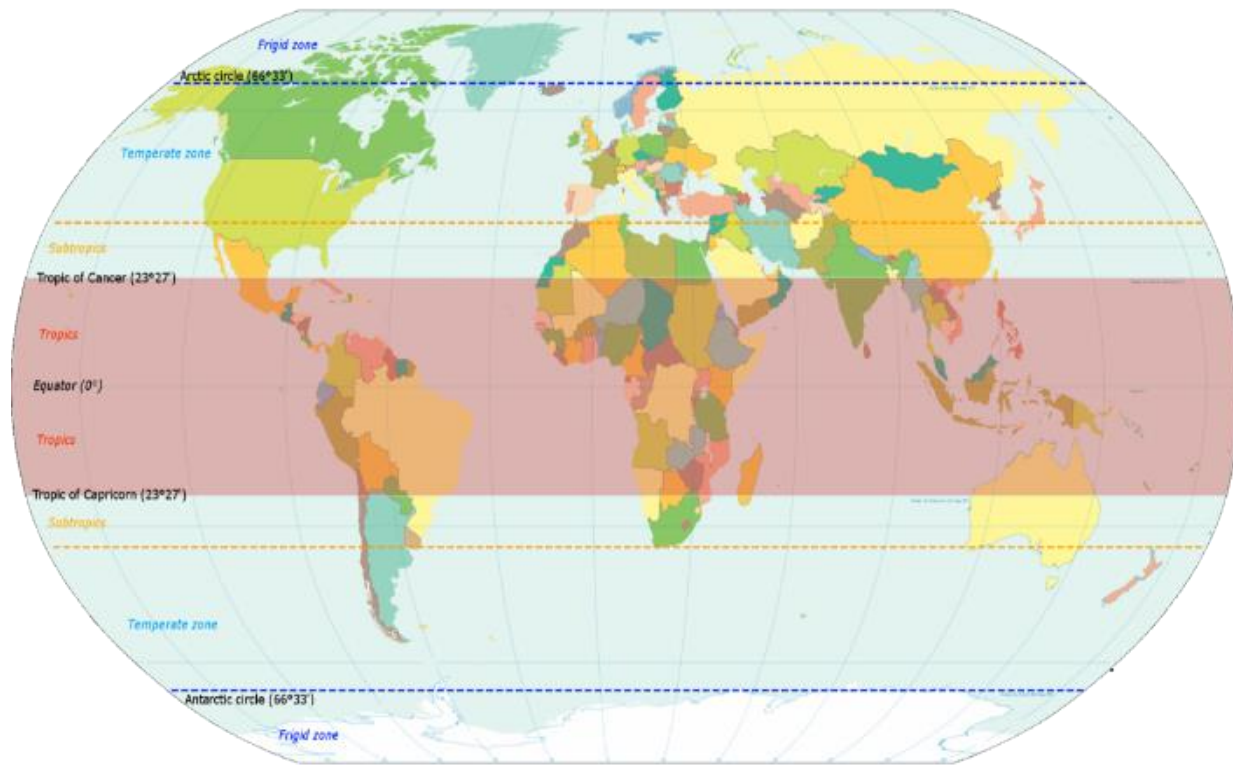
0
Bacteria

0
Prion

Parasite	Protozoa	bacteria	virus	prion
Organism that lives on or in a host and feeds at the expense of the host. Multicellular or unicellular Eukaryote	Single celled microorganism. Larger than bacteria Can be Amoeba, flagellates, ciliates, sporozoans, etc Eukaryote	Small single celled organism. Ubiquitous Contain a single loop of DNA. May contain a plasmid Prokaryote	Microscopic organism consisting of a segment of nucleic acid surrounded by a protein coat	Protein that can induce abnormal folding of specific proteins
<i>Taenia</i>	<i>Giardia</i>	<i>Salmonella</i>	Hepatitis A	Creutzfeldt-Jakob Disease
<i>Giardia</i>	<i>Toxoplasma</i>	<i>Campylobacter</i>	Norovirus	Kuru
<i>Toxoplasma</i>	<i>Acanthamoeba</i>	<i>Shigella</i>	Polio	Bovine Spongiform Encephalopathy



Seasonality of the disease



What is/are the host organism for *Cyclospora cayentanensis*?

0
Cow

0
Bird

0
Human

0
Dog

0
All of the Above

The host organism for *Cyclospora cayetanensis* is humans

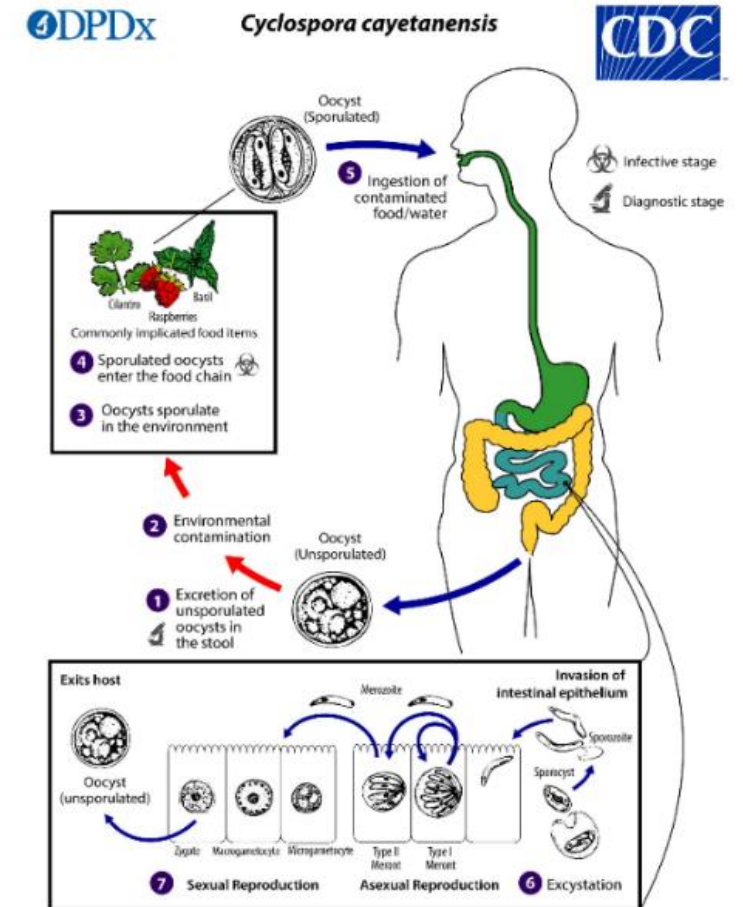
Human Feces/ Human to Human Contact

As regulators what do we do?

- www.afdo.org/wp-content/uploads/2021/05/Investigating-Fresh-Produce-Cyclospora-Outbreaks.pdf

What should growers do?

- <https://edis.ifas.ufl.edu/publication/FS440>



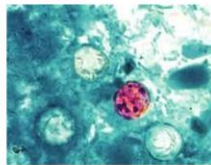
Investigating Fresh Produce *Cyclospora* Outbreaks



Foodborne illness investigations involving *Cyclospora* are more complicated because they are centered around a parasite rather than traditional pathogens. Inspectors are often challenged when investigating *Cyclospora* outbreaks due to the lack of resources and guidance specific to the farm. This guidance is designed to provide considerations, preventive opportunities, and challenges involved in *Cyclospora* related foodborne illness outbreaks.

Introduction

Foodborne illness outbreaks (when two or more people get sick from eating the same food) of *Cyclospora cayetanensis* (referred to as *Cyclospora* in this document) in produce have been linked to various produce items including berries, mesclun lettuce, snow peas, basil, and cilantro. Foodborne illness outbreak investigations involving fresh produce are typically centered around bacterial and viral pathogens. In the case of *Cyclospora*, the agent of contamination is a parasite, not a bacterial or viral pathogen (disease causing microorganism). As a result, it is critical to understand the differences of an investigation and the life cycle of *Cyclospora*, which will facilitate the appropriate communication with farmers, growers, and workers.



Humans, not animals, are the only known source of *Cyclospora*. When investigations occur for a *Cyclospora* parasitic infection outbreak, they focus on the potential of contamination by human waste. Due to the nature of outbreaks, sometimes they are not reported to public health officials until several weeks after the occurrence. Because of the hardness of the parasite, and its extended survival in the environment, it is important to understand the life cycle of *Cyclospora*, and how contamination of fresh produce might occur.

UF IFAS Extension

FSHN22-6
<https://doi.org/10.32473/edis-FS440-2022>

Prevention of *Cyclospora* Contamination and Transmission on the Farm¹

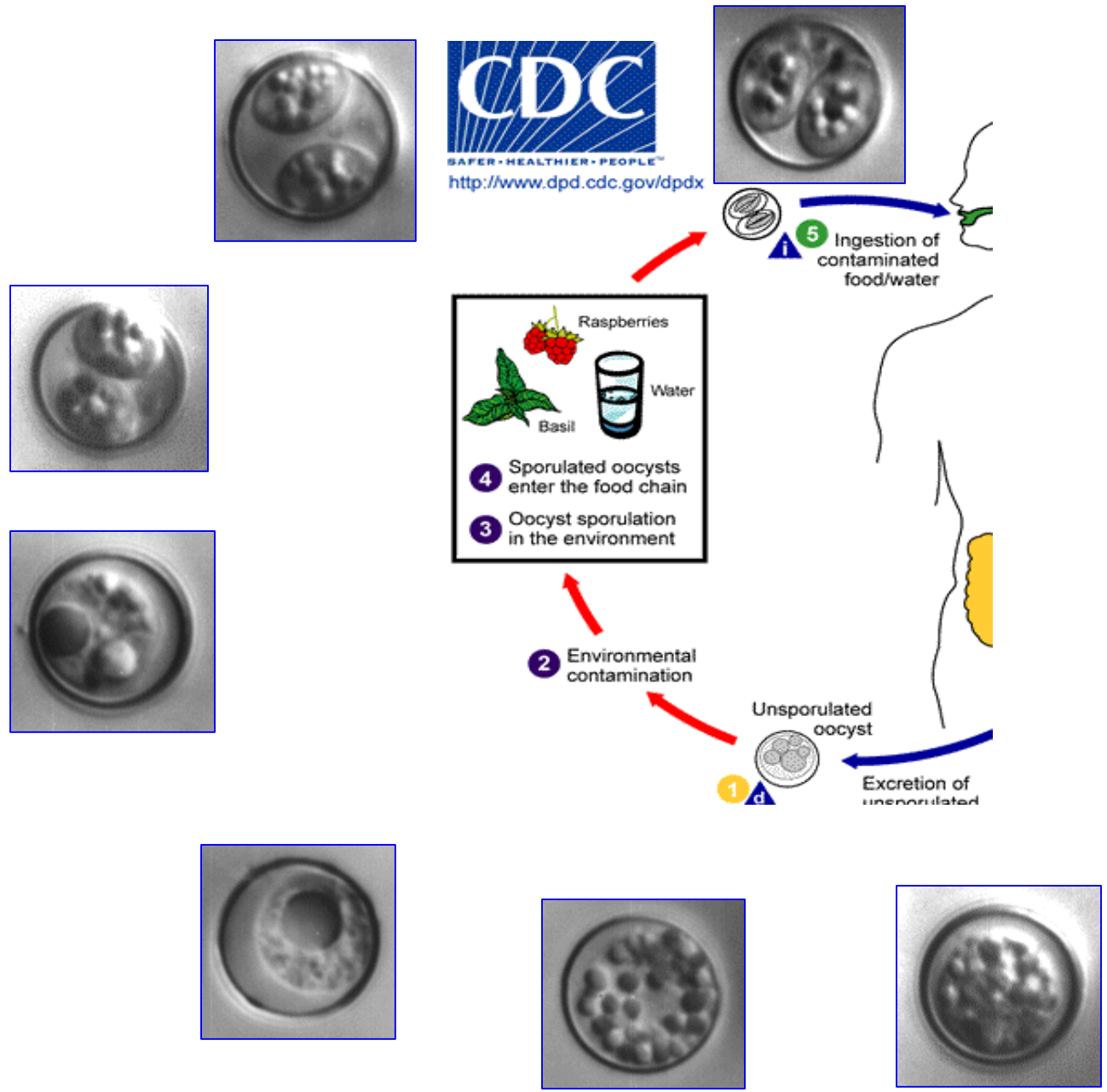
Taylor L. O'Bannon, Michelle D. Danyluk, Keith R. Schneider, and Matthew D. Krug²

This EDIS fact sheet is intended for fresh produce growers to provide education on preventing transmission and contamination of *Cyclospora* on the farm.

Recent outbreaks of *Cyclospora cayetanensis* (*Cyclospora*) linked to fresh produce have prompted a closer look into prevention strategies on the farm. *Cyclospora cayetanensis* is the only species of *Cyclospora* that causes disease in humans, known as cyclosporiasis. Unlike bacteria, parasites such as *Cyclospora* require a human host to survive and multiply. *Cyclospora* is spread when human feces containing *Cyclospora* oocysts (egg-like life stage) are released into the environment in or around agriculture growing regions. Due to the oocysts' thick-walled "outer shell," *Cyclospora* survives in the environment for weeks before sporulating (i.e., maturing), at which time they become infectious. People become infected with *Cyclospora* when food contaminated

preventive measures can help reduce the risk of contamination of fresh produce on the farm:

- Worker training:
 - Ensure worker training materials are appropriate for workers' native language, cultural practices, education level, and background.
 - Train workers on health and hygiene principles, including proper handwashing, identification of foodborne illness symptoms, and the importance of not working when sick.
 - Train workers on how to properly use toilet facilities provided by the farm, including the sanitary disposal of toilet paper inside the toilet, and emphasizing handwashing after use.
- Health, Hygiene, and Sanitation:



Cyclospora cayetanensis

1308

THE NEW ENGLAND JOURNAL OF MEDICINE

May 6, 1993

CYCLOSPORA SPECIES — A NEW PROTOZOAN PATHOGEN OF HUMANS

YNÉS R. ORTEGA, M.S., CHARLES R. STERLING, PH.D., ROBERT H. GILMAN, M.D.,
VITALIANO A. CAMA, D.V.M., AND FERNANDO DÍAZ, M.D.

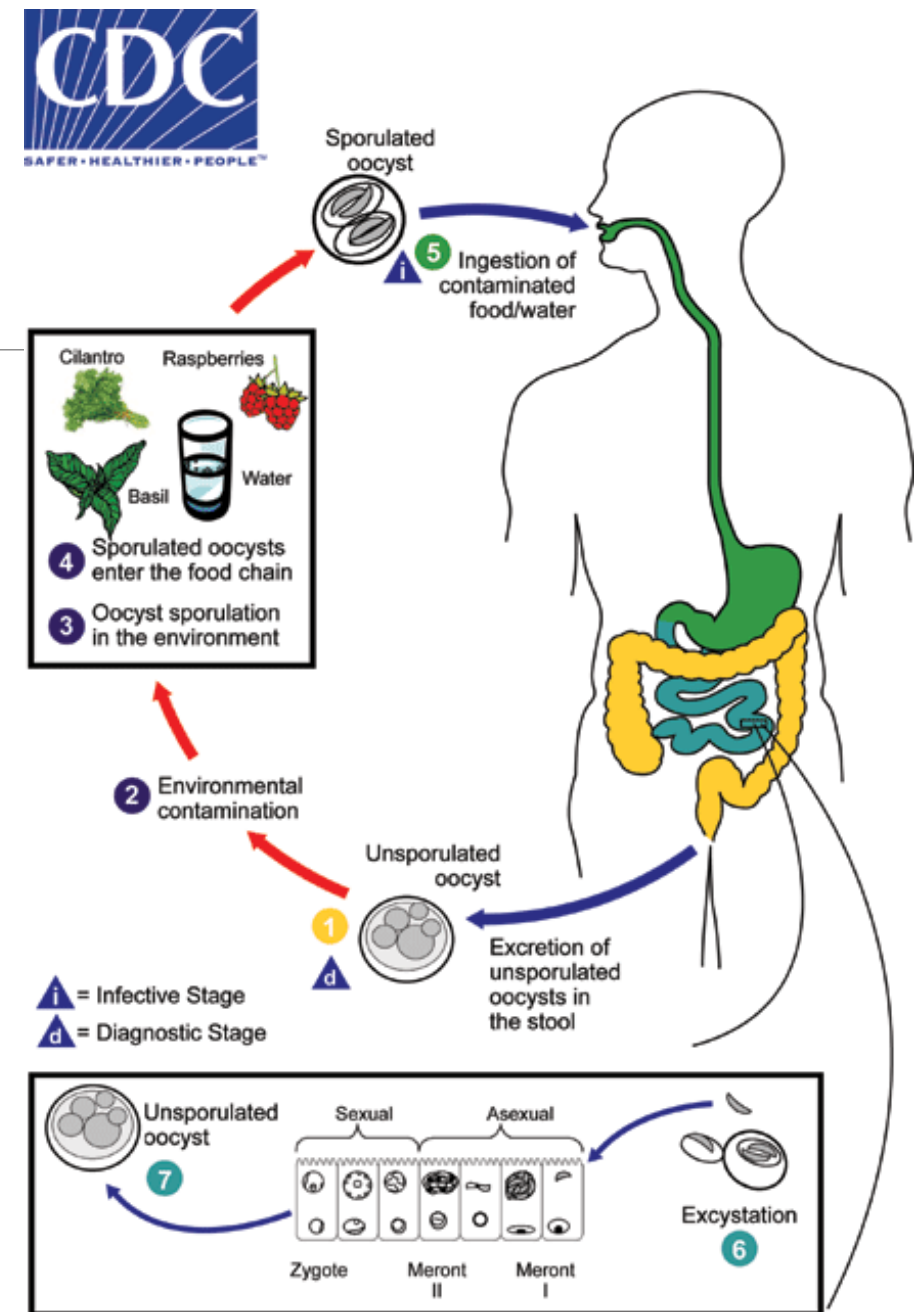
Abstract Background. Organisms referred to as "cyanobacterium-like bodies" have now been identified worldwide in the feces of both immunocompetent and immunocompromised patients with diarrhea. Organisms with a similar appearance have been isolated from Peruvian patients since 1985. From 1988 to 1991 we studied prospectively two cohorts of infants and young children infected with this organism. We now attempt to identify it.

Methods. Fecal samples were collected weekly from the children and examined with the use of acid-fast staining and staining with a monoclonal antibody specific for cryptosporidium. Stools positive for cyanobacterium-like bodies were preserved in potassium dichromate and exposed to conditions allowing coccidian sporulation and excystation. Both unsporulated and sporulated oocysts were fixed by freeze-substitution techniques and then examined by electron microscopy.

Results. Organisms isolated from the feces of Peruvian patients and two patients from the United States were identified as belonging to the coccidian genus *Cyclospora*, after sporulation and excystation of the oocysts according to standard techniques. Complete sporulation occurred within 5 to 13 days in oocysts maintained in potassium dichromate at 25 or 32°C. Complete excystation resulted in the liberation of two sporozoites from the two sporocysts within each oocyst (cryptosporidia have four naked sporozoites within each oocyst). The presence of organelles characteristic of coccidian organisms was confirmed by electron microscopy.

Conclusions. We have identified organisms of the genus *Cyclospora* that are remarkably similar to cryptosporidia in their morphologic features and the diarrheal disease that they produce in humans. The complete life cycle and epidemiology of this new protozoan parasite remain to be described. (N Engl J Med 1993;328:1308-12.)

- *Cyclospora cayetanensis* is the only species of the genus known to infect humans.
- Fresh produce contaminated with sporulated oocysts.
- Direct person-to-person transmission is unlikely.



How can you decrease Cyclospora concentrations on produce?

0

Chemical Sanitizers

0

Drying

0

Heating

0

Freezing

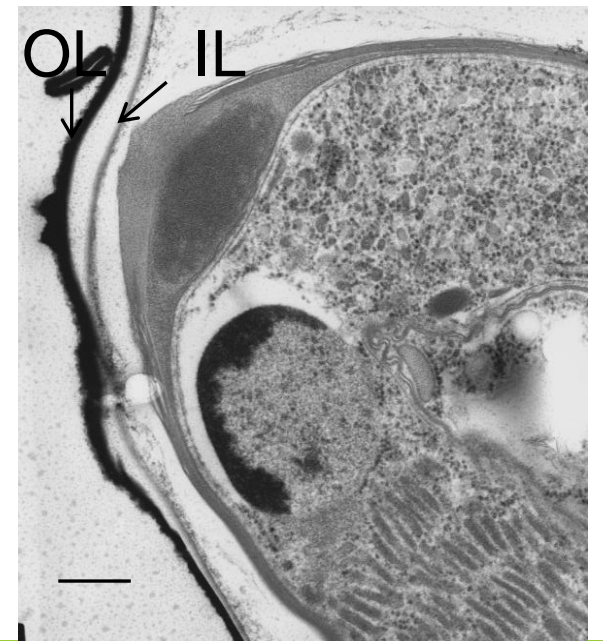
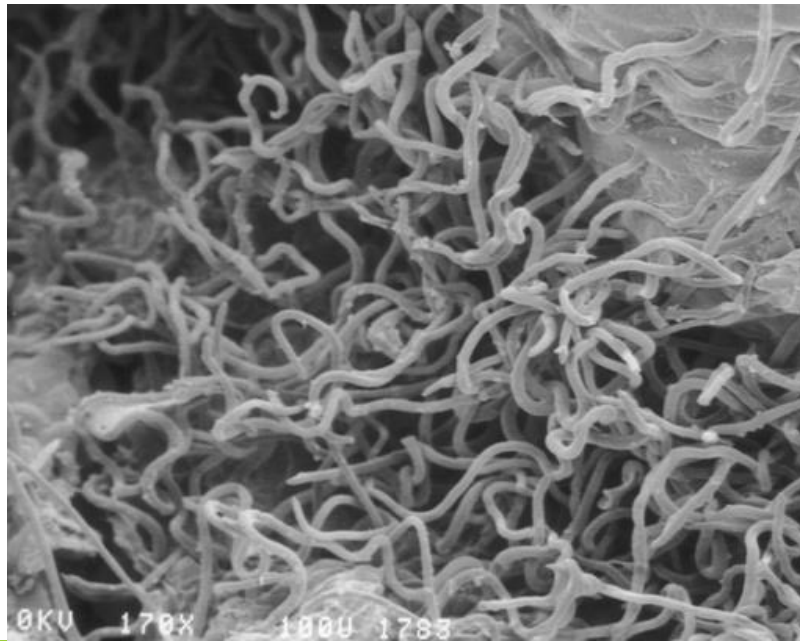
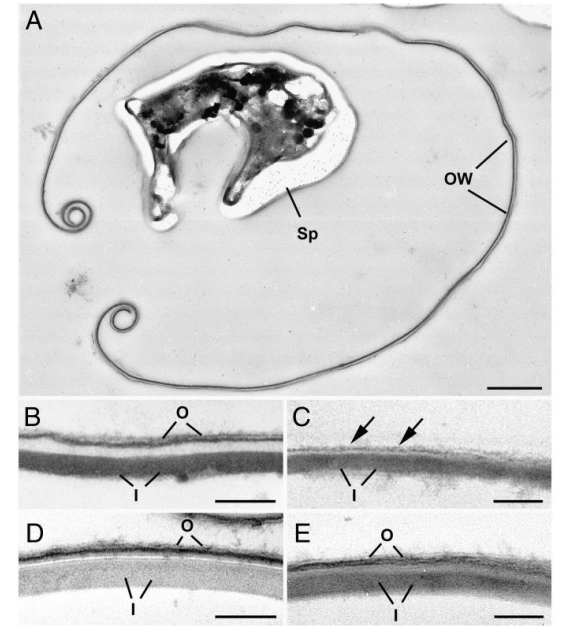
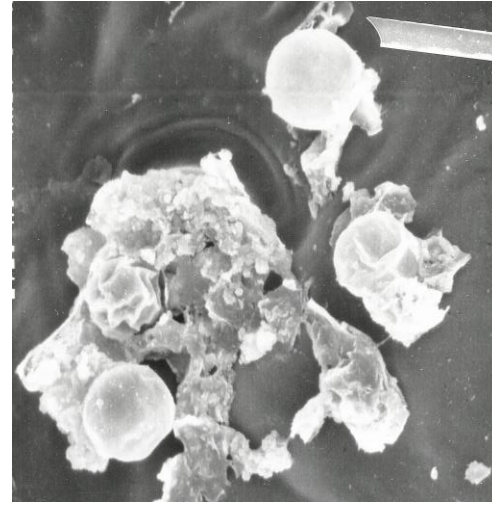
0

All of the above

0

None of the above

Login to edit this Menti



Why is Cyclospora so hard to detect in the environment and in food samples? Choice

0

Only grows in humans

0

DNA is hard to isolate from Protozoa

0

Genome is larger and more complicated than bacteria or virus

0

Present in small concentrations

0

All of the above

FDA's Bacteriological Analytical Manual

BAM CHAPTER 19b



Matrix	Seeding Level	Positive samples (80 tested)	% positive
cilantro	0	0	0.0%
	5	25	31.3%
	10	64	80.0%
	200	80	100.0%
raspberries	0	0	0.0%
	5	40	50.0%
	10	72	90.0%
	200	80	100.0%



Also approved for shredded carrots, basil, blackberry, shredded cabbage, romaine lettuce, and parsley.

Research Paper
Evaluation of an Improved U.S. Food and Drug Administration Method for the Detection of *Cyclospora cayetanensis* in Produce Using Real-Time PCR

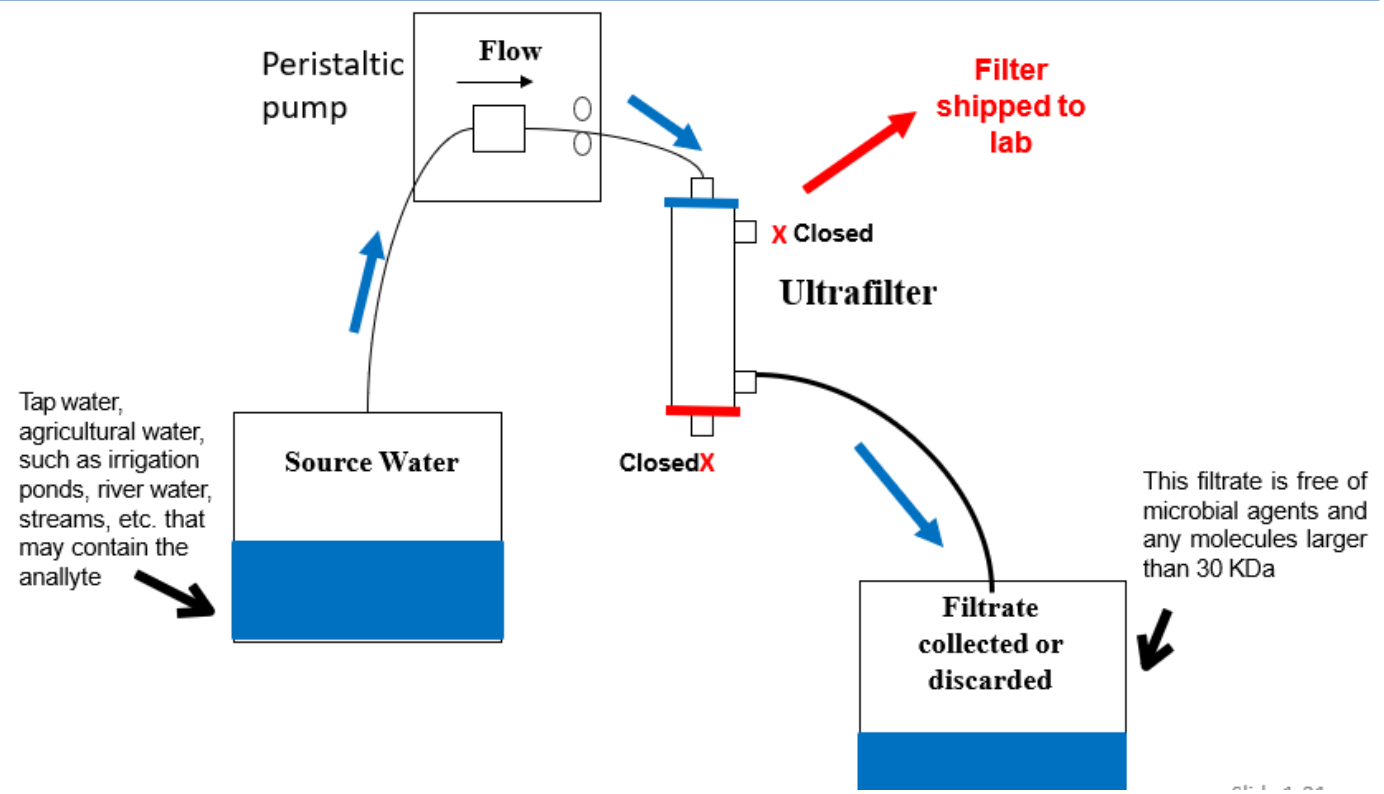
HELEN R. MURPHY,* SEULGI LEE,† AND ALEXANDRE J. DA SILVA

Journal of Food Protection, Vol. 80, No. 7, 2017, Pages 1133–1144
 doi:10.4315/0362-028X.JFP-16-492
 Published 2017 by the International Association for Food Protection
 Not subject to U.S. Copyright

<https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm553445.htm>

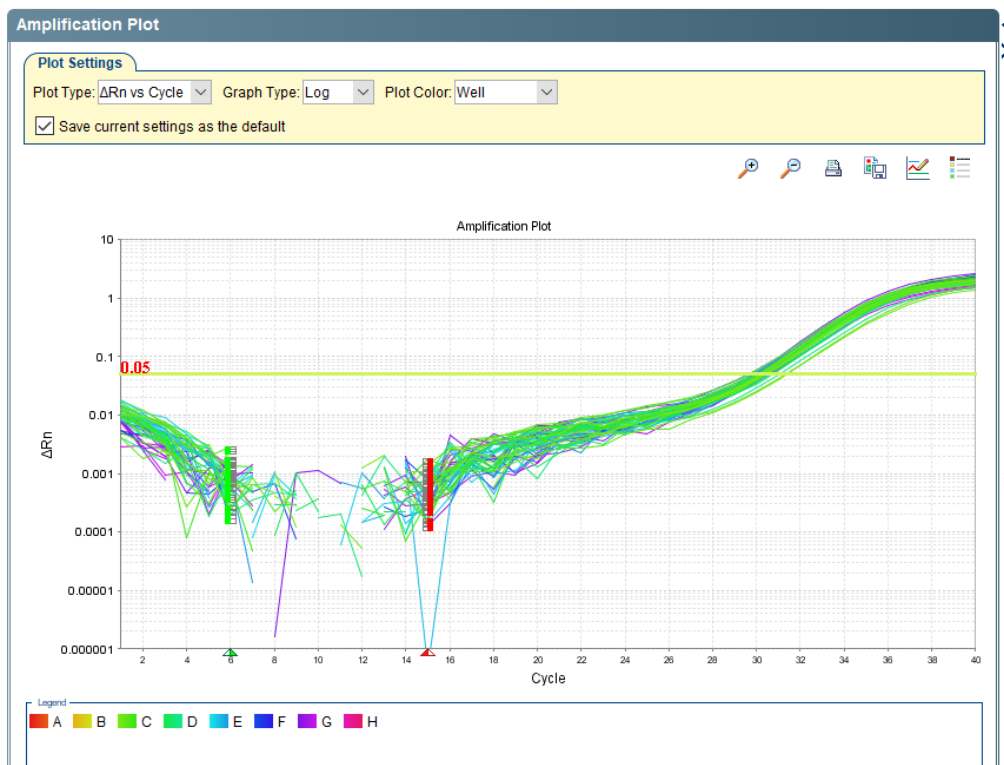
Detection of *Cyclospora cayetanensis* in Agricultural water

Dead-end Filtration Setup



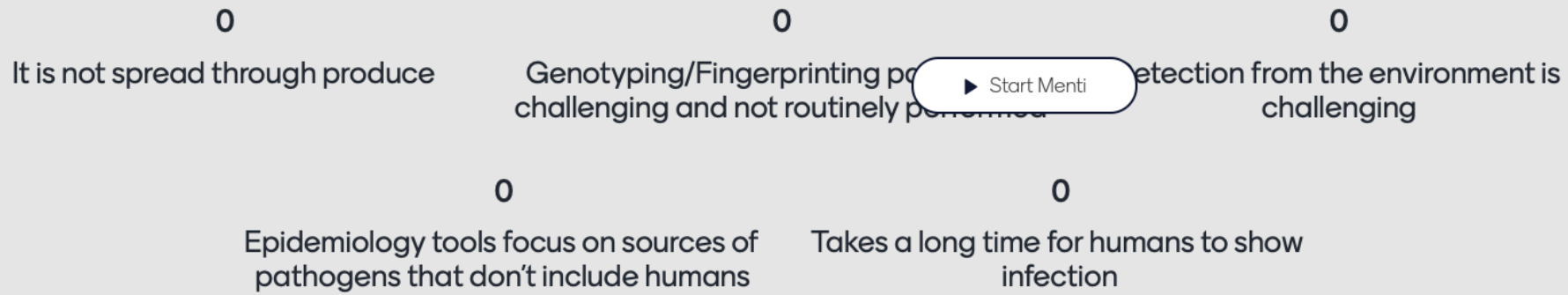
Development and Validation of New Molecular Tools for Detection of *C. Cayetanensis* in Produce and Agricultural Water

New Mit1C marker



- **Same sensitivity** in comparison to the current 18S marker.
- *In silico* analysis showed **improved specificity** based on more mismatches when compared to related organisms.

Join at menti.com | use code **7319 3359**



Development of Sequencing-based Method for Detection of *C. cayetanensis*

METHODS - WORKFLOW

FDA's *C. cayetanensis* BAM method protocol were followed to recover oocysts from produce, extract the parasite's DNA, and perform molecular detection using qPCR analysis for comparison with the new amplification method.

1. Primer Design

A total of 8 PCR primer pairs were designed in selected regions of the *C. cayetanensis* mitochondrial genome.

SNPs and InDel loci of 2014 outbreak strains



Table 1. SNPs detected in *C. cayetanensis* positive samples from oocysts diagnosed in 2014

2. PCR Optimization

Clinical and Food samples

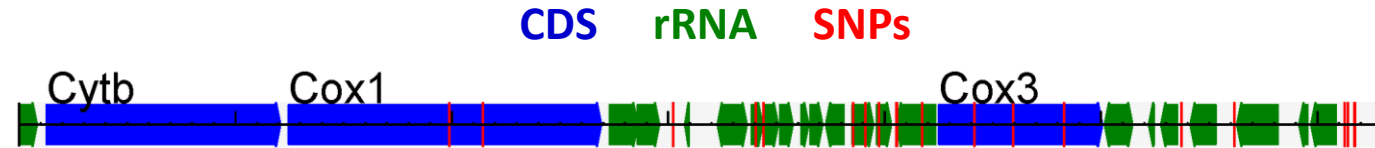
Selection of the most specific and sensitive primers

Size range between 182 and 688bp

Annealing temperatures varying between 60°C and 68°C



Mitochondrial Genome

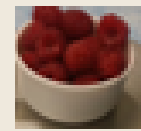


3. Identification of the detection limit

Food matrices



25g of cilantro

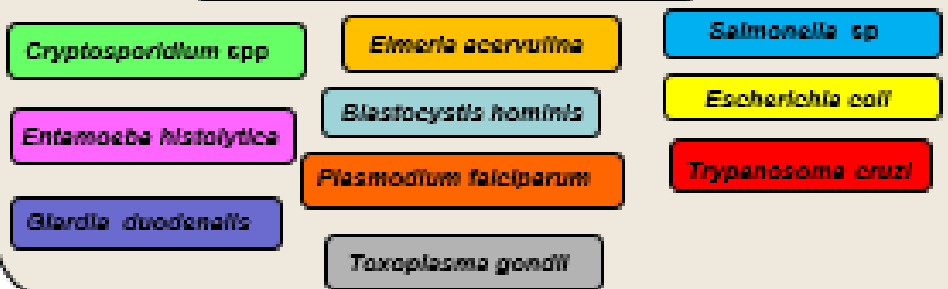


50g of raspberries

Seeded with 5 (n=4), 10 (n=4), 20 (n=3) and 200 (n=3) oocysts of *Cyclospora cayetanensis*

4. Exclusivity Panel

81 other *Cyclospora* (non-*C. cayetanensis*)



Join at menti.com | use code **7319 3359**

▶ Start Menti

Waiting for responses ...



FDA Cyclospora Task Force

CTF Email: CyclosporaActionPlan@fda.hhs.gov

Resources:

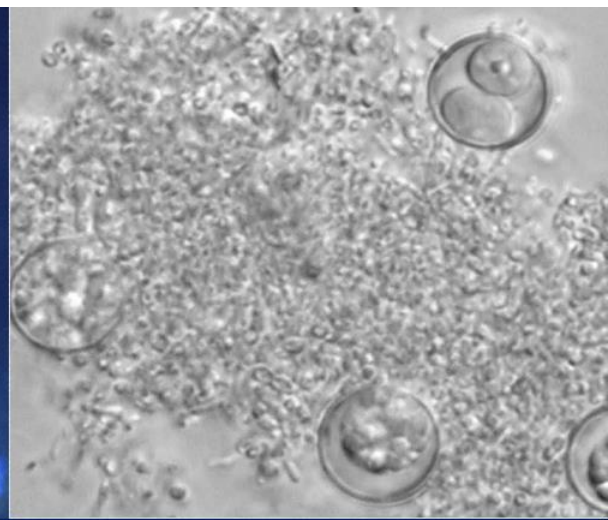
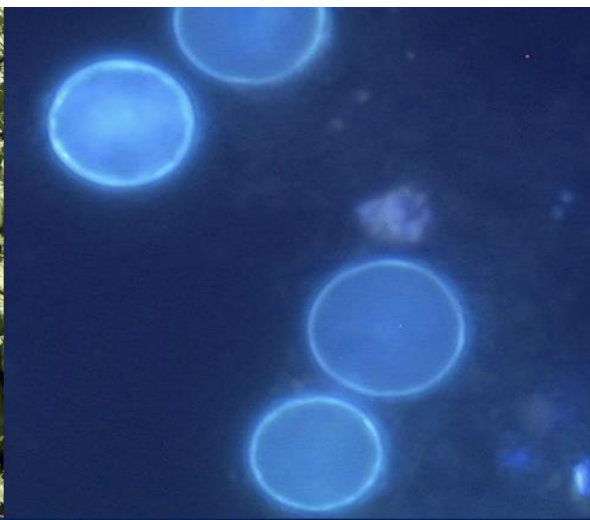
Cyclospora Prevention, Response and Research Action Plan:

<https://www.fda.gov/food/foodborne-pathogens/cyclospora-prevention-response-and-research-action-plan>

FDA's Cyclospora Task Force Continues Efforts

<https://www.fda.gov/food/conversations-experts-food-topics/fdas-cyclospora-task-force-continues-efforts>





International *Cyclospora* Conference

March 7 – 8, 2024

Emory Conference Center Hotel,

Atlanta, GA

Registration closes on February 26, 2024

Conference and
hotel registration at:



Ortega@uga.edu
Dylan.white@uga.edu

