

# *Cyclospora* in Produce: What is this all About?



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# Overview

What is *Cyclospora cayetanensis*



Learnings from previous outbreaks



Moving forward with awareness and prevention in mind!



# *Cyclospora* is not a bacterium

- It is a protozoan parasite
- Unlike bacteria, protozoa can **not** grow outside a host

There are still numerous remaining questions, challenges, and work needed to better understand and manage this pathogen.

Difficult to study without an animal or cell-culture laboratory model.



*Cyclospora cayetanensis* oocyst  
(8-10  $\mu\text{m}$ )

# Protozoan Parasites of Concern

- Protozoa are microscopic single-celled organisms, with low infectious dose
- Protozoa are resistant to *normal levels* of chemical disinfectants



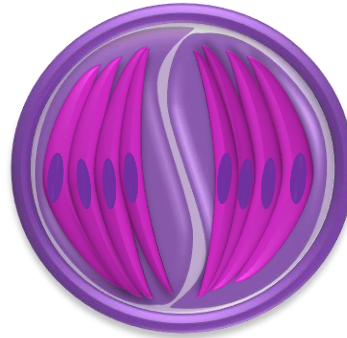
*E. coli*  
bacterium  
(~1  $\mu\text{m}$ )



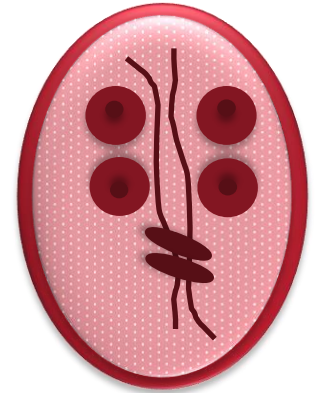
*Cryptosporidium*  
species  
oocyst (4-6  $\mu\text{m}$ )



*Cyclospora*  
*cayetanensis*  
oocyst (8-10  $\mu\text{m}$ )



*Toxoplasma gondii*  
oocyst  
(10-12  $\mu\text{m}$ )



*Giardia intestinalis*  
cyst  
(8-14  $\mu\text{m}$ )

# Protozoan Parasites and the Produce Industry

- **Challenges to Control**

- Low infectious dose (10 to 1000 oocysts)
- Robust nature and Extreme resistance to washing and chlorine
- Lack of awareness of public health significance, prevalence, persistence, and transfer in the environment
- Lack of understanding of complex life cycles
- Lack of standardized methods for detection and identification
- Detection methods may not detect low level contamination in the environment



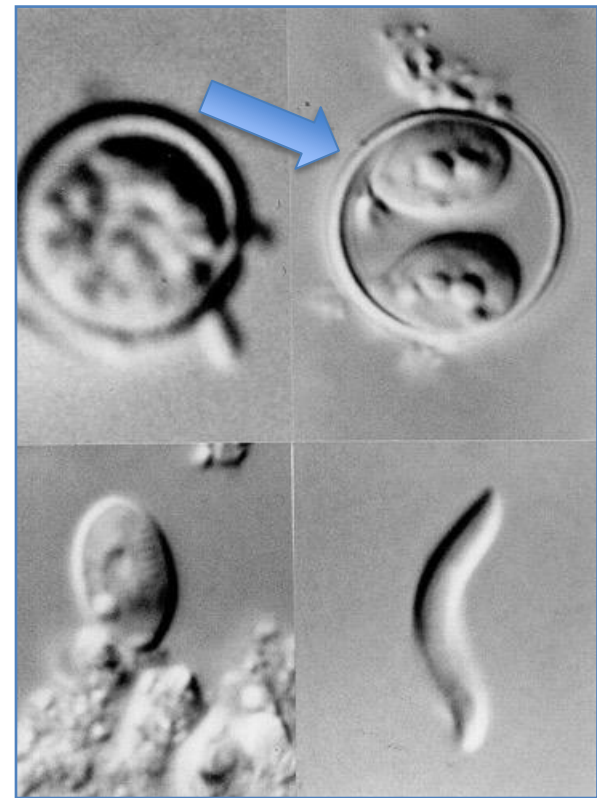
*Cyclospora cayetanensis* oocysts.  
Zhou *et al.*, 2011.



*Cryptosporidium*, *Giardia*,  
*Toxoplasma* (oo)cysts CDC.gov,  
scientificamerican.com

# Cyclosporiasis

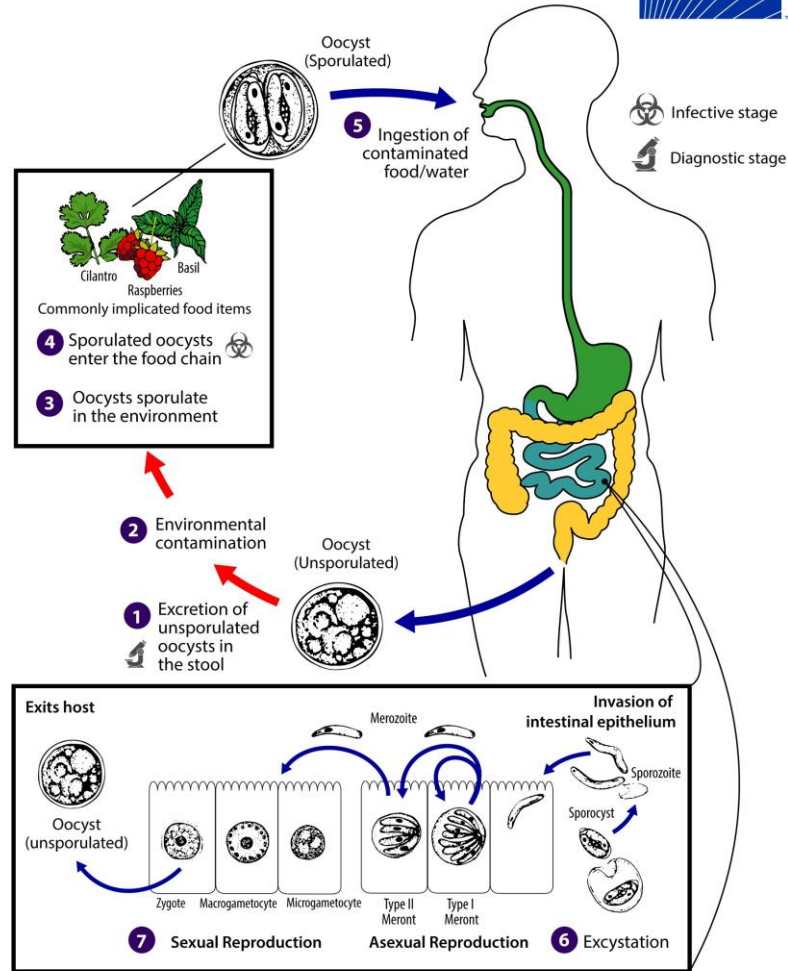
- *Cyclospora cayetanensis* infects the human intestine
- Spread by people ingesting contaminated produce or water
- *Cyclospora* needs time (typically at least 1-2 weeks) after being passed in feces to become infectious for another person
  - Sporulated oocysts are infectious
  - So it is unlikely that *Cyclospora* is passed directly from one person to another
- Time from ingestion to illness ranges ~2-14 days



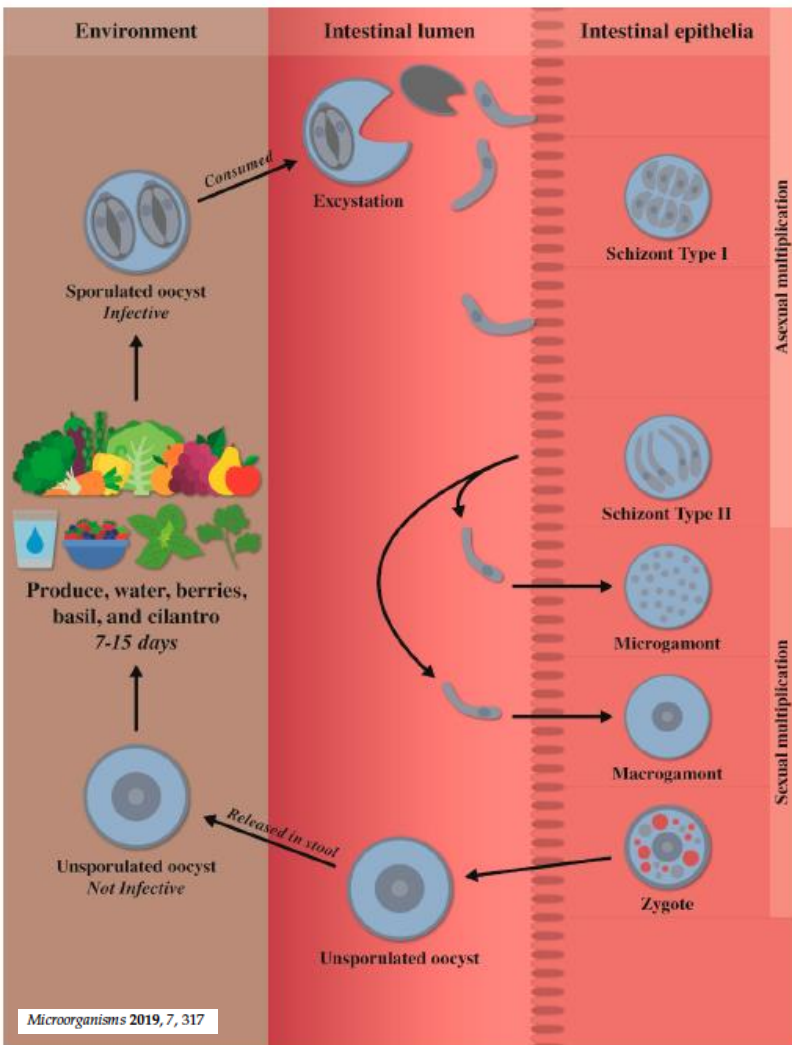
*Cyclospora cayetanensis* oocyst  
(8-10  $\mu\text{m}$ )



# Life Cycle







- If a susceptible human ingests sporulated oocysts in contaminated food or water, the sporozoites inside the sporocysts excyst in the gut lumen and invade the duodenum and jejunum where the sporozoites transform into trophozoites. Trophozoites subsequently form 2 types of schizonts (asexual multiplication).
  - Type I schizonts contain 8–12 merozoites. Type II schizonts contain 4 merozoites. Then, type II merozoites form gamonts (sexual multiplication results in microgamonts and macrogamonts).
- Microgamonts fertilize macrogamonts to form the zygote. Oocysts then are formed and excreted unsporulated in the feces.
- Unsporulated oocysts are not infectious—they need to sporulate to become infective for a host.
- Under laboratory conditions, at 22C and 30C, sporulation will take between 7 and 14 days to occur outside the host. A sporulated oocyst contains two sporocysts, each with two sporozoites.



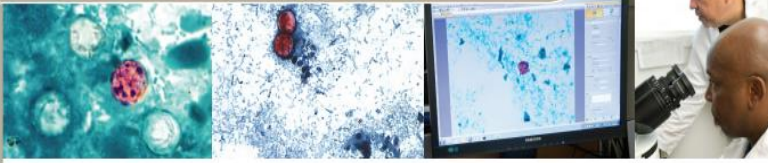
# Symptoms of cyclosporiasis

May last 5-10 days or up to 6 weeks or more

- Watery diarrhea (most common)
- Loss of appetite
- Weight loss
- Cramping
- Bloating
- Increased gas
- Nausea
- Fatigue
- May include vomiting, low-grade fever
  - Treatment with Trimethoprim/sulfamethoxazole (TMP/SMX)
  - *Sold under the trade names Bactrim, Septra, and Cotrim*
- Both immunocompetent and immunocompromised individuals can become sick
- Some people who are infected will be asymptomatic

# Clinical Detection

## Cyclosporiasis – Provider Fact Sheet



### How is cyclosporiasis treated?

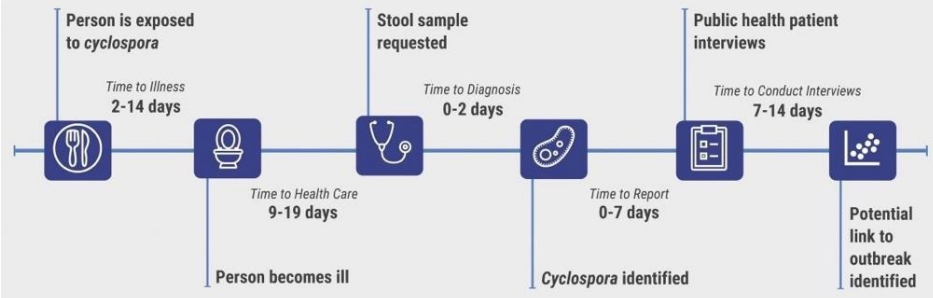
- Trimethoprim-sulfamethoxazole (TMP-SMX) is the treatment of choice.
- The typical regimen for immunocompetent adults is TMP 160 mg plus SMX 800 mg (one double-strength tablet), orally, twice a day, for 7–10 days. HIV-infected patients may need longer courses of therapy.
- No highly effective alternatives have been identified for persons who are allergic to (or are intolerant of) TMP-SMX.



### OUTBREAK INVESTIGATION TIMELINE

## Cyclospora Infection

The time from when a person is exposed to *Cyclospora* from contaminated food or water to the confirmation that he or she is part of an outbreak is typically about three to six weeks.



**Average Time: 3-6 weeks**



# The BioFire® FilmArray® Gastrointestinal (GI) Panel

## THE BIOFIRE GI PANEL MENU

Overall 98.5% Sensitivity and 99.2% Specificity<sup>8</sup>  
 Sample Type: Stool in Cary Blair medium



### BACTERIA:

- Campylobacter (jejuni, coli, and upsaliensis)*
- Clostridium difficile (toxin A/B)*
- Plesiomonas shigelloides*
- Salmonella*
- Yersinia enterocolitica*
- Vibrio (parahaemolyticus, vulnificus, and cholerae)*
- Vibrio cholerae*

- Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus (I, II, IV, and V)

### DIARRHEAGENIC E. COLI/SHIGELLA:

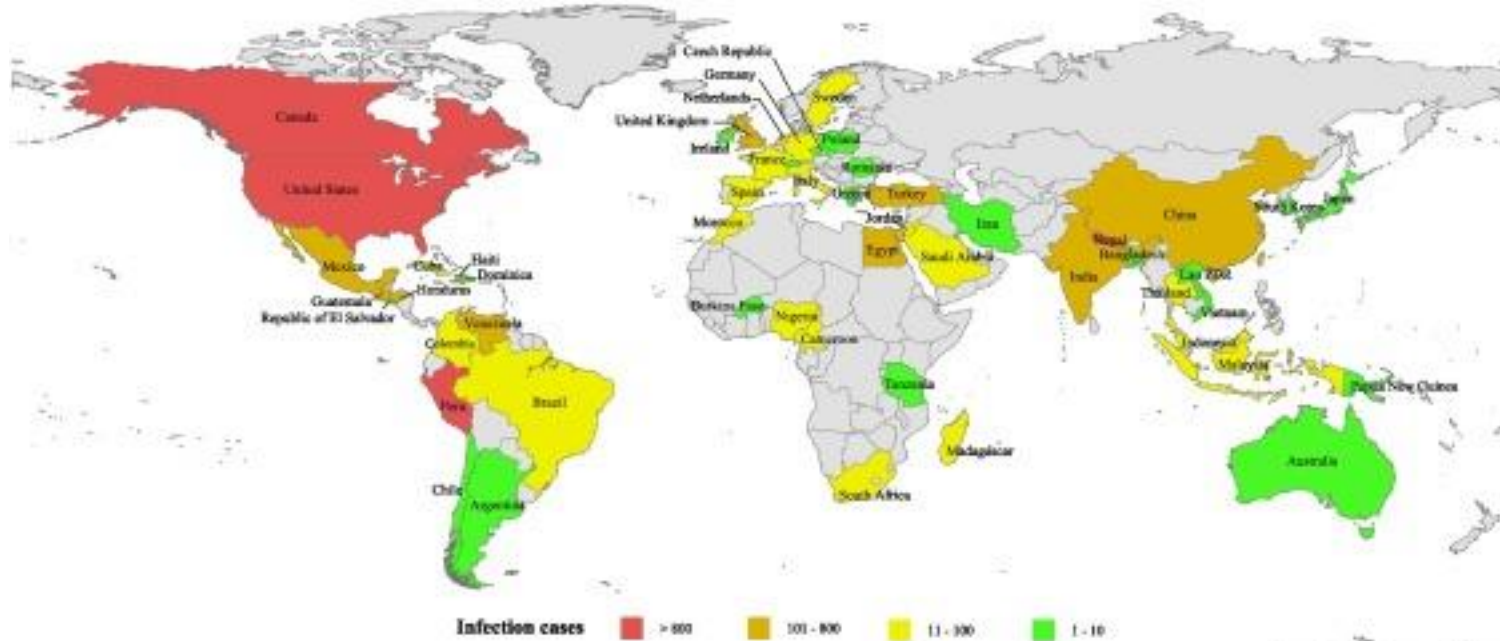
- Enteraggregative E. coli (EAEC)*
- Enteropathogenic E. coli (EPEC)*
- Enterotoxigenic E. coli (ETEC) It/st*
- Shiga-like toxin-producing E. coli (STEC) stx1/stx2*
- E. coli O157*
- Shigella/Enteroinvasive E. coli (EIEC)*

### PARASITES:

- Cryptosporidium*
- Cyclospora cayatanensis*
- Entamoeba histolytica*
- Giardia lamblia*



# *Cyclospora cayentanensis* global distribution



Trends in Parasitology

Li, Zhang and Zhang, 2021

## Outbreaks

## Other occurrences

'77 1st 3 documented cases of infection with "undescribed coccidian" diagnosed in  
'78 Papua New Guinea

'79

'80

'81

'82

'83 1st documented cases in Haiti ("Big Crypto"), in patients with AIDS

'84

'85 1st documented case in Peru ("*Cryptosporidium muris*-like object")

'86 1st documented U.S. cases, in 4 travelers returning from Haiti and Mexico

'87

'88

'89 1st documented cases in Nepal, in 55 foreigners

1st documented U.S. outbreak (Chicago)

'90

'91 Name "cyanobacterium-like or coccidian-like body (CLB)" used

'92

'93 Organism confirmed to be a coccidian parasite

Waterborne outbreak (Pokhara, Nepal)

'94 Name *Cyclospora cayetanensis* proposed

'95 Trimethoprim-sulfamethoxazole shown to be effective

'96 Phylogenetic relationship to *Eimeria* species shown

'97

'98

'99

Multiple foodborne outbreaks in North America linked to various types of fresh produce

# Testing and Detection?

- At this time testing methods are primarily restricted to research purposes.
- Methodology continues to evolve, including the validation of different food and environmental matrices, reagent variability, and the inclusion of appropriate positive and negative controls.



From Clinical Infectious Diseases 31(4):1040–1057.  
© 2000 by the Infectious Diseases Society of America.

# Testing and Detection

3 published methods summarized in the FDA's Bacteriological Analytical Manual (BAM)

- (2004) BAM Chapter 19a: Detection of *Cyclospora* and *Cryptosporidium* from Fresh Produce: Isolation and Identification by Polymerase Chain Reaction (PCR) and Microscopic analysis
- (2017) Chapter 19b: Detection of *Cyclospora cayetanensis* in Fresh Produce using real-time PCR
- New (2020)! BAM 19c: Dead-end Ultrafiltration for the Detection of *Cyclospora cayetanensis* from Agricultural Water



[www.fda.gov](http://www.fda.gov)

Bacteriological  
Analytical Manual  
(BAM)





## LEARNINGS FROM OUTBREAKS

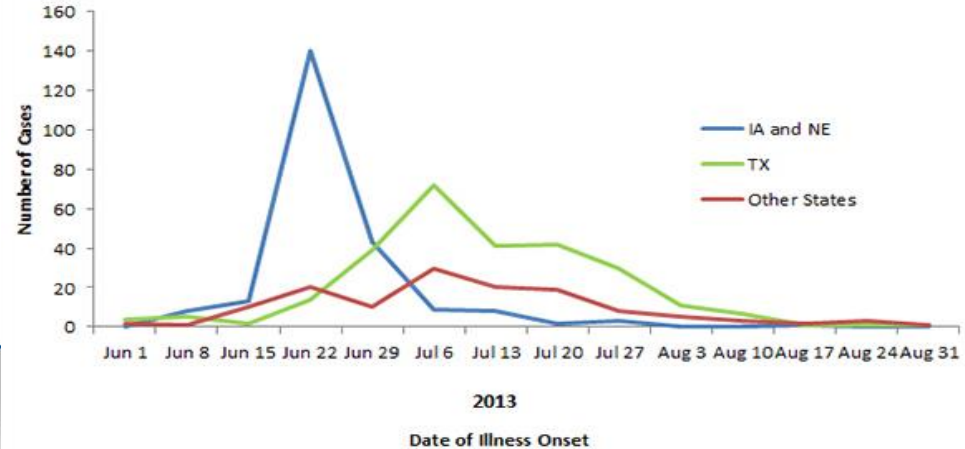
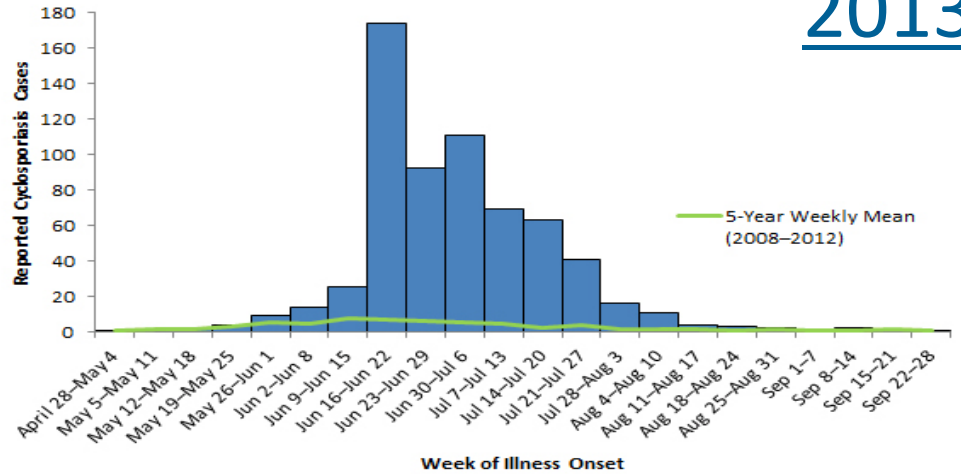
# Outbreaks in the 1990s - early 2000s

- Imported produce
- Berries, snow peas, herbs, lettuce
- Sometimes “stealth” ingredients
  - Raspberry coulis
  - Basil in the vinaigrette dressing
- Traceback was often difficult
- Less advanced molecular diagnostics

Table: Summary of U.S. foodborne outbreaks of cyclosporiasis, 2000–2017

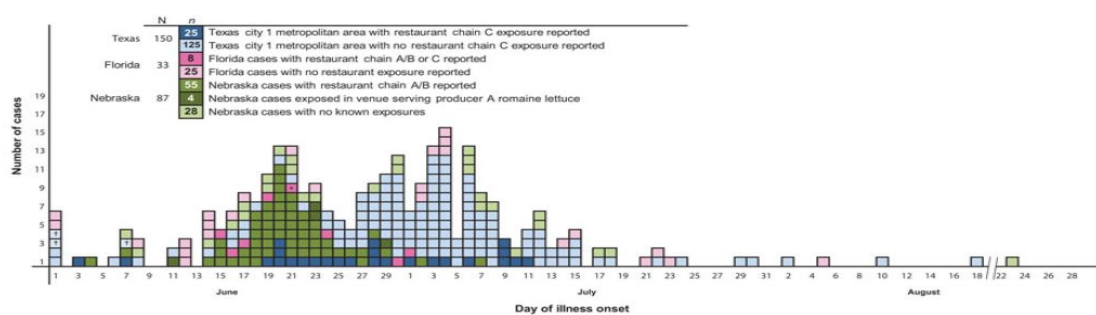
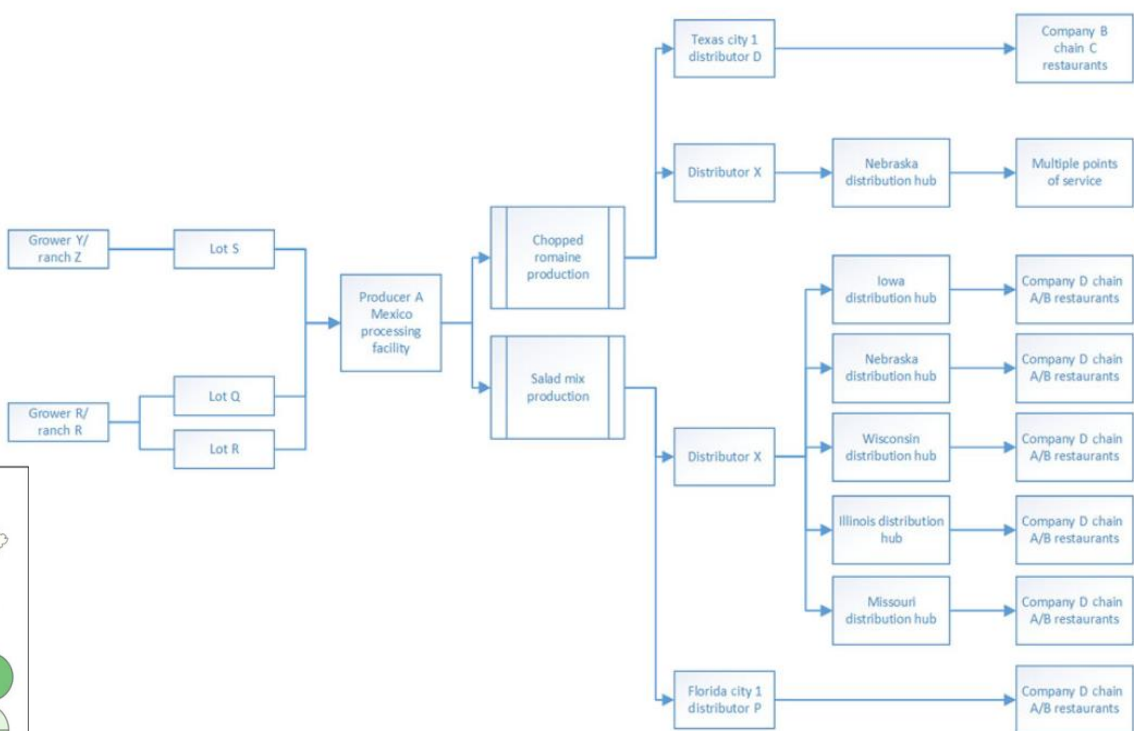
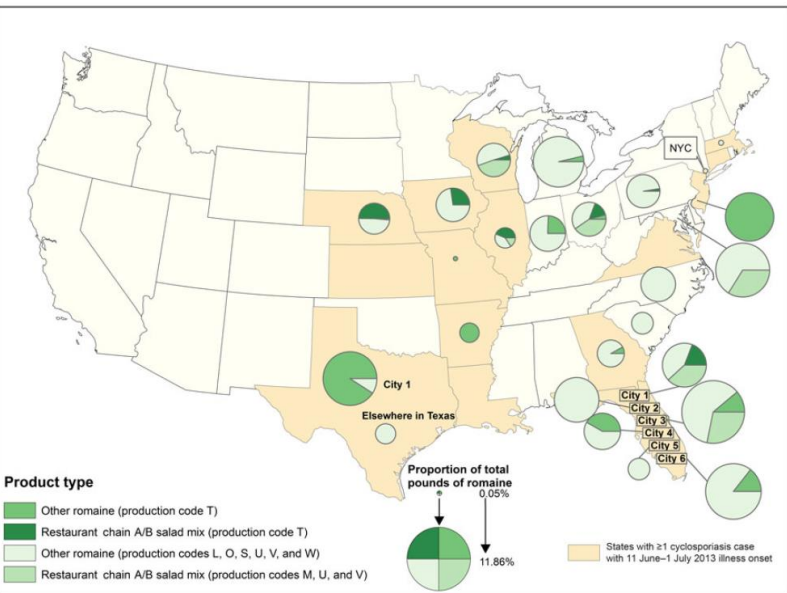
Year(s)*	Month(s)*	Jurisdiction(s)*	No. of cases†	Food vehicle and source, if identified‡
2000	May	Georgia	19	Raspberries and/or blackberries (suspected)
2000	June	Pennsylvania	54	Raspberries
2001	January–February	Florida	39	
2001	January	New York City	3	
2001–02	December–January	Vermont	22	Raspberries (likely)
2002	April–May	Massachusetts	8	
2002	June	New York	14	
2004	February	Texas	38	
2004	February	Illinois	57	Basil (likely)
2004	May	Tennessee	12	
2004	May–June	Pennsylvania	96	Snow peas from Guatemala ¶
2005	March–May	Florida	582 ¶	Basil from Peru
2005	May	South Carolina	6	
2005	April	Massachusetts	58	
2005	May	Massachusetts	16	
2005	June	Connecticut	30	Basil (suspected)
2006	June	Minnesota	14	
2006	June	New York	20	
2006	July	Georgia	3	
2008	March	Wisconsin	4	Sugar snap peas (likely) ¶
2008	July	California	45 ¶	Raspberries and/or blackberries (likely)
2009	June	District of Columbia	34	
2011	June	Florida	12	
2011	July	Georgia	88**	
2012	June–July	Texas	16	

- A total of 631 persons infected with *cyclosporiasis* were reported from 25 states.
- Most persons became ill between mid-June and mid-July.
- Eight percent (8%) of ill persons were hospitalized, and no deaths reported.
- 2 outbreaks identified: IA and NE linked to salad mix and TX linked to cilantro; both grown in Mexico



# Multistate product traceforward investigation to link imported romaine lettuce to a US cyclosporiasis outbreak – Nebraska, Texas, and Florida, June–August 2013

B. F. BUSS<sup>1,2\*</sup>, M. V. JOSHI<sup>1,3</sup>, J. L. DEMENT<sup>4</sup>, V. CANTU<sup>5</sup> AND T. J. SAFRANEK<sup>1</sup>





## ENVIRONMENTAL ASSESSMENT: 2013 Cyclosporiasis outbreak in Iowa and Nebraska – Findings and Recommendations

November 2013



Taylor Farms de Mexico provides medical care to all of their employees. Ill employees are sent home, and a company nurse performs home visits to confirm the employee's recovery and that no other household member exhibits symptoms prior to the employee returning to work. The table below provides a synopsis of individual harvest crew member medical care within the outbreak time of interest. No diagnosis was recorded by the firm as to the probable cause of the employee illnesses.

### TFM HARVEST CREW INDIVIDUAL PRESCRIPTIONS

Date of Visit	Medication provided	Crew
3/6/2013	trimetropin sulfa metazol	C
4/24/2013	dicloxacillin	A
4/24/2013	dicloxacillin	A
5/8/2013	ampicillin	E
5/22/2013	dicloxacillin	T
5/29/2013	dicloxacillin	Driver
6/26/2013	<i>loperamide</i>	E
7/3/2013	<i>loperamide</i>	O
7/10/2013	<i>loperamide</i>	S
7/10/2013	ampicillin	T
7/24/2013	<i>loperamide</i>	X
7/31/2013	dicloxacillin	T
8/7/2013	trimetropin sulfa metazol	O*
8/7/2013	trimetropin sulfa metazol	O*

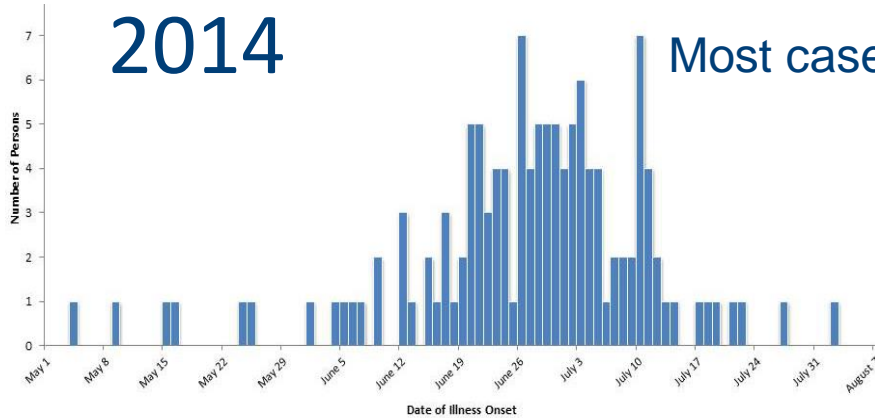
Colors indicate patients of the same crew.

Medications were provided based on availability.



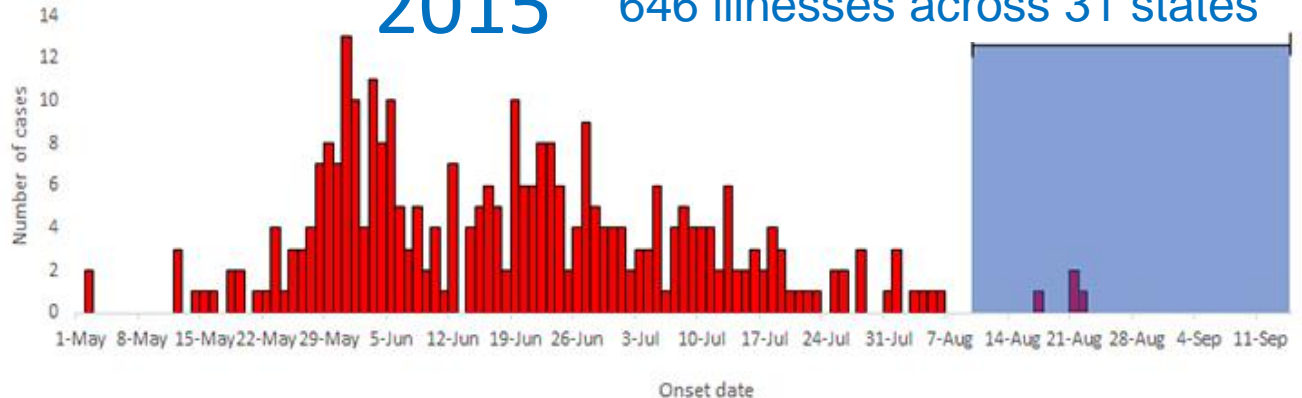
# Moving forward notice trends in seasonality

## 2014



## 2015

646 illnesses across 31 states





# Partnership to promote food safety

## Initiated July 2014, implemented 2015, continues today



<https://www.fda.gov/food/international-cooperation-food-safety/fda-senasica-cofepris-food-safety-partnership>

- In 2016, CDC reports a total of 384 laboratory-confirmed cases of cyclosporiasis across 25 states. No reported connection or outbreak.
- In 2017, CDC reported 1,065 laboratory-confirmed cases of cyclosporiasis across 40 states. No specific vehicle of interest or potential source identified.

# 2018

- 250 laboratory-confirmed cases from 4 states linked to consumption of pre-packaged Del Monte Fresh Produce vegetable trays (broccoli, cauliflower, carrots, dill dip).
- 511 laboratory-confirmed cases from 15 states reported consuming salads from McDonald's restaurants in the Midwest
- All illnesses reported May-August

## Domestically Acquired

- 2, 299 laboratory-confirmed cases by 33 states (no history of international travel)
- All illnesses reported May-August

# 2019



- 241 people with laboratory-confirmed *Cyclospora* infections and who reported eating fresh basil had been reported from 11 states. Epidemiologic evidence and product distribution information indicated that fresh basil exported by Siga Logistics de RL de CV of Morelos, Mexico was a likely source of this outbreak.

## Domestically Acquired

- 1,696 laboratory-confirmed cases of cyclosporiasis were reported to CDC by 33 states. Illnesses from May-August.

# 2020 and 2021

- As of September 23, 2020, a total of 701 people with laboratory-confirmed *Cyclospora* infections associated with this outbreak were reported from 14 states. Illness onset ranged from May-July.
  - Epidemiologic evidence and product traceback indicated that bagged salad mix containing iceberg lettuce, carrots, and red cabbage produced by Fresh Express was a likely source of this outbreak.

## Domestically Acquired

- As of September 23, 2020, 1,241 laboratory-confirmed cases of cyclosporiasis in people who had no history of international travel during the 14-day period before illness onset have been reported to CDC by 34 states. Onset dates May-August.
- As of September 28, 2021, 1,020 laboratory-confirmed cases of cyclosporiasis in people who had no history of international travel during the 14-day period before illness onset have been reported to CDC by 36 states. Onset dates May-August.



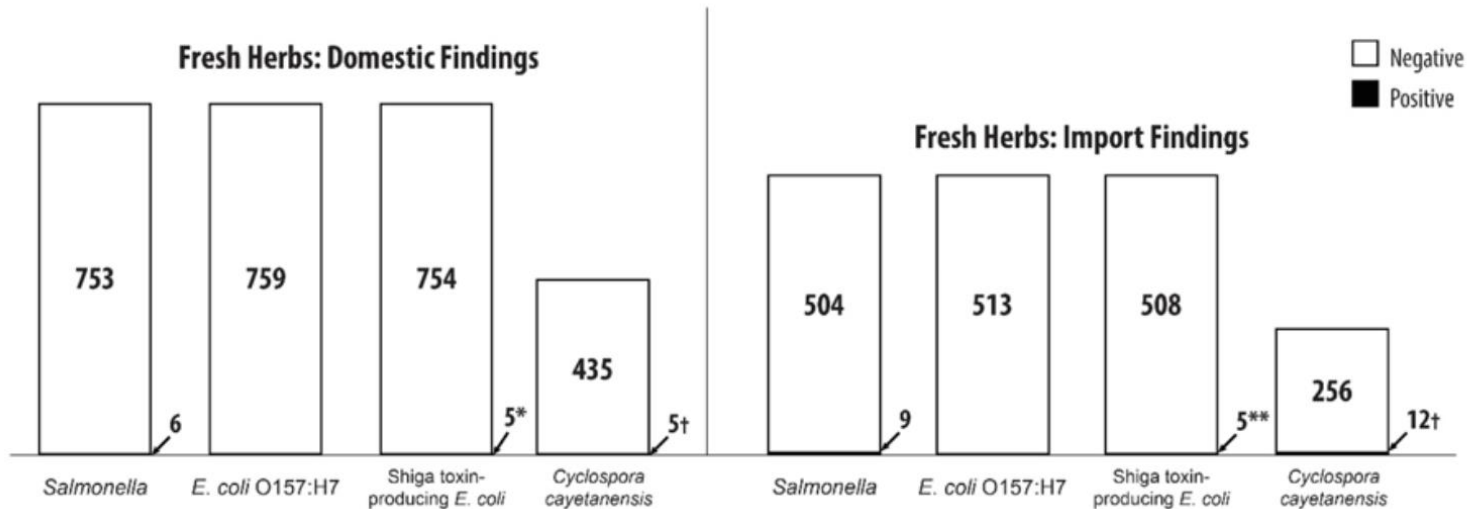
# Moving forward... prevention awareness and future research

- This is a complex problem, but solutions do not need to be complex
- *You* can decrease the risk
- Surveillance studies suggest that *Cyclospora* oocysts may be present in surface waters at varying degrees
- Oocyst presence could be associated with ineffective sewage treatment or contamination and the role of portable toilets is being explored

# FDA Herb Sampling

## Microbiological Surveillance Sampling: FY18-21 Fresh Herbs (Cilantro, Basil & Parsley) and Processed Avocado and Guacamole Assignments

- As of March 31, 2020, the agency had collected and tested 1,272 samples of fresh herbs (759 domestic and 513 imported). The agency plans to collect 1,600 fresh herbs samples (761 domestic, and 839 of international origin) under this assignment.





# Key Industry Controls

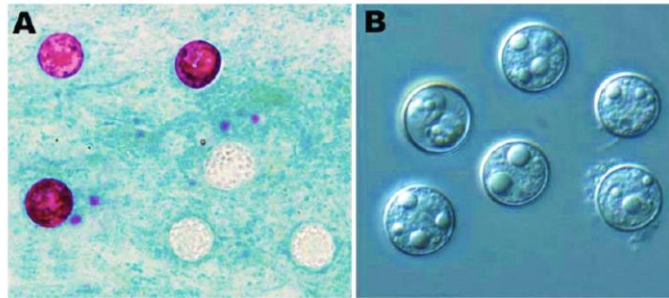
- Attention to handwashing, sanitation of restrooms and portable toilets, avoiding use of sewage sludge, managing sewage spills etc.
- It is important that controls against human fecal contamination are implemented across the supply chain, since oocysts can be introduced by people at any stage.
- The persistence of Cyclospora in the environment is unknown
- The triggers for sporulation are not well understood (temperature, acidity, etc)
- Focus on preventive measures





# The future of testing and detection?

- Not yet feasible or widely available
- Many tests are under development
- Focus on molecular diagnostics and testing platforms



**Microscopic appearance of *Cyclospora cayentanensis* oocysts**

Source: Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 10, October 2011



# What about research efforts???

- Great industry support and interest
  - Center for Produce Safety supporting academic and government researchers
  - FDA scientists and industry, with collaborative efforts lead by United Fresh and PMA
- Many critical research questions remain
  - Surveillance studies
  - Infective dose and factors affecting sporulation
  - Environmental persistence
  - Role of water and soil on transmission
  - Existence of potential reservoirs

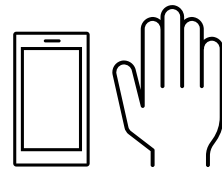




# Focus on People!



Education and Training  
Open Communication



# Thank You



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