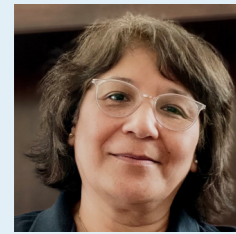


The prevalence of *Cyclospora* in water and produce



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Authors

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Summary

Foodborne outbreaks of cyclosporiasis have often been reported in the U.S. in the past 8 years, with most of the cases occurring in April to August. In 2018, locally produced vegetables were implicated in two large outbreaks associated with fresh produce, specifically vegetable trays. In 2020, 701 people were associated with a *Cyclospora* outbreak. The likely source of this outbreak was bagged salad mix containing lettuce, carrots, and red cabbage produced in the U.S. To address the limited information on the prevalence and persistence of *Cyclospora* in the environment we are performing systematic surveys of water and fresh produce. This testing will help determine if *Cyclospora* is present in the environment and if it follows the seasonality observed in the human population.

Objectives

1. Compare three molecular tools for the detection of *Cyclospora* in water. [completed]
2. Examine the presence of *Cyclospora* oocysts in waters in Florida and California.
3. Perform a systematic survey of fresh produce in Florida based on the current knowledge of the distribution of this parasite in the state.

Methods

Water samples were collected from canals in Florida and rivers in California (**Figure 1**), with 3 samples obtained from each sampling point. Water samples of 20 liters each were filtered using hollow fiber filters and tested with PCR for the presence of *Cyclospora*; presumptive positive samples were prepared for sequencing. Selected samples will be further examined by metagenomic analysis.

Three samples of romaine lettuce, basil, cilantro, and parsley were obtained from markets and farmers markets in Florida in 2020 during the months of June, July, August, October, and December, and in 2021 during February and April. Samples (100 g) were washed in filter bags with 100 ml of elution buffer. The wash solution was concentrated by centrifugation and the pellets examined for the presence of *Cyclospora*.

Results to Date

Water samples (n=187) and vegetables (n=333) from Florida were collected during the months of September 2020 to April 2021. Water turbidity for the Florida samples varied from 1.78 to 59.8 NTU, temperature varied from 17.4 to 29.6°C, and water flow ranged from 0 to 0.1 m/s.

Water samples in California were collected from June 2020 to April 2021, with 231 water samples collected to date (**Table 1**). Water turbidity for the California samples varied from 3.14 to 323 NTU, temperature varied from 8.8 to 22.1°C, and water flow ranged from 0 to 0.3 m/s. Unlike in Florida, water at various sampling points in California was not always available for sampling in the dry season (**Figure 2**). Samples are currently being evaluated by PCR and presumptive positive samples with DNA sequencing.

Benefits to the Industry

Understanding the prevalence of *Cyclospora* oocysts in surface waters (rivers or ponds/lakes) where agriculture is intensive will allow the industry to focus prevention efforts in given locations. We will also be able to determine if other organisms may be present locally that could influence the specificity of detection assays that could influence environmental surveys. A simple and rapid method to collect samples in agricultural settings will be available for the fresh produce industry.

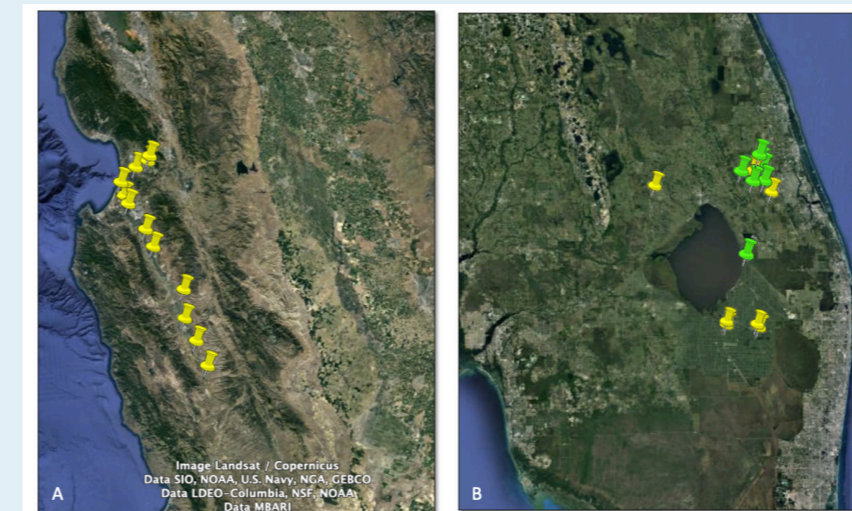


Figure 1. Water sampling sites: A) California, B) Florida. Image by Google Earth Pro.



Figure 2. A sampling point in California in December 2020 (A) and February 2021 (C), and a sampling point in Florida in December 2020 (B) and February 2021 (D).

Table 1. Water and vegetable samples collected by state, month, and sample type

Sampling dates	Water				Vegetables	
	Florida (n)		California (n)		Florida (n)	
	samples	locations	samples	locations	samples (n)	markets
June-20	ND	ND	24	12	ND	ND
July-20	ND	ND	34	12	ND	ND
August-20	ND	ND	29	11	ND	ND
October-20	43	10	54	9	90	8
December-20	48	12	29	7	75	7
February-21	48	12	46	12	72	7
April-21	48	12	35	10	96	8
Total samples collected to date	187	46	251	73	333	30
Proposed	266	19	266	19	1000	

ND = sampling was not performed during these months due to the Covid-19 pandemic. As result, travel was not authorized.

*Future scheduled sampling dates in 2021: May, June, July, August, and October