The prevalence of *Cyclospora* in water and produce

Contact

Ynes R. Ortega
Center for Food Safety
University of Georgia
ortega@uga.edu

Authors

Ynes R. Ortega (PI), Michael Aaron, Alexander Studebaker

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Summary

In the past six years, a large number of cyclosporiasis cases have been reported in the US. Historically, *Cyclospora* outbreaks have been associated with the ingestion of contaminated imported fresh produce. In 2018, more than 2,200 laboratory-confirmed cases of cyclosporiasis were reported using culture-independent diagnostic tools. That same year, two large outbreaks were associated with fresh produce and vegetable trays (cauliflower, broccoli and carrots), implicating vegetables produced in the US. These outbreaks raised several questions about *Cyclospora* in the US. The prevalence and persistence of *Cyclospora* in the environment and in the US (water and soil) are unknown. We improved water sample collection and evaluated molecular detection assays in order to detect *Cyclospora* oocysts in water and fresh produce.

Benefits to the Industry

Understanding the prevalence of *Cyclospora* oocysts in surface waters (rivers or pond/lake) where agriculture is intensive will allow the industry to focus prevention efforts in given locations and for commodities. In addition, we will be able to recommend a simple and rapid method to collect samples in agricultural settings for monitoring purposes and interventions when necessary. Testing for *Cyclospora* using the mitochondrial DNA either by using qPCR or conventional PCR will provide faster and more accurate results. And because this method has the capability to discriminate oocyst isolates, we will be able to determine if we can effectively source track.

Objectives

To optimize natural surface water sample collection and to examine the presence of *Cyclospora cayetanensis* oocysts in water samples, including fresh produce – for this, two molecular detection methods were evaluated.

Methods

Water samples were collected using a transfer pump, and samples were filtered with a Sawyer hollow fiber filter (**Figure 1**). The pump is operated using a 6V battery, and the filtration of a 20-liter water sample is achieved within 20 min.

DNA extractions of samples positive for *Cyclospora cayetanensis* were achieved using the FastDNA Spin Kit for Soil. DNA was amplified using two assays: the first method amplified a 501 bp of the 18SRNA gene, and the second method amplified a product of about 357 bp of the mitochondrial genome. Products were electrophoresed in 1.5% agarose and observed for the presence/absence of amplified products. Assays were run in triplicate and repeated two or three times.

Results to Date

The sampling procedure using hollow fiber filters was optimized to filter 20 liters of natural surface water; the procedure takes an average of 15–20 minutes and can be done in the field. The concentrate from the filters can be easily recovered in the laboratory (or the field) and sent for analysis.

The team also determined that the two assay methods were comparable to detect *Cyclospora* oocysts (**Figure 2**). The assay targeting the 18S RNA gene (501 bp) by nested PCR, could detect 35 of 36 (97%) of the samples examined, and the assay targeting the mitochondrial DNA (~350 bp) could detect 34 of 36 samples (94%). This method has the advantage to discriminate isolates of different locations. The amplified product can be sequenced.

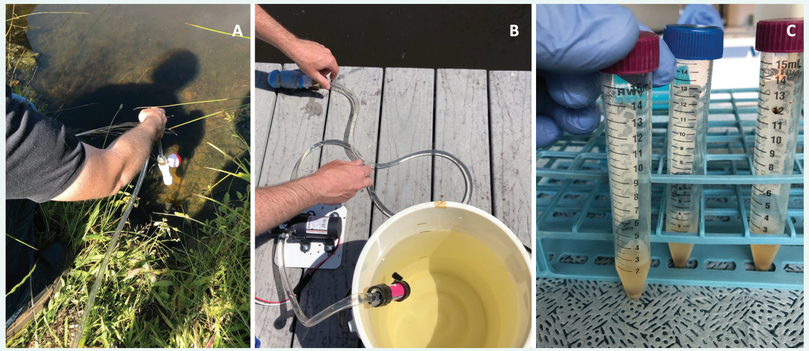

Figure 1. (A) Water collection from pond, (B) complete filtration system including prefilter and hollow fiber filter, and (C) tube containing eluent from hollow fiber filtration of 20-liter sample of pond water.

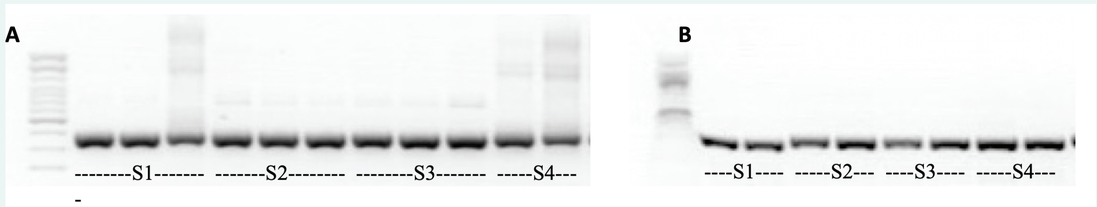

Figure 2. Amplified products for samples S1 to S4, using two different assays: (A) 500 bp nPCR (18S RNA), and (B) 350 bp PCR (mitochondrial).