Sources and prevalence of *Cyclospora cayetanensis* in Southeastern US water sources and growing environments

Contact

Dr. Mia C. Mattioli
Domestic WASH Lead, Environmental Microbiology & Engineering Laboratory
Waterborne Disease Prevention Branch
National Center for Emerging and Zoonotic Infectious Diseases
Centers for Disease Control and Prevention
kuk9@cdc.gov

Authors

Amy Kahler (Co-PI), Michael Arrowood (Co-PI), Andre Luiz Biscaia Ribeiro da Silva (Co-PI), Jessica Hofstetter

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Participating growers in Tift County, Georgia, and The Tifton Regional Wastewater Treatment Complex

Summary

This study will assess *Cyclospora cayetanensis* prevalence in the Southeastern coastal plain region throughout eight farms in Georgia representing growing practices across the Southeastern US (**Figure 1**). This region has several risk factors that warrant the evaluation of *C. cayetanensis* prevalence, including a farm worker population from *Cyclospora*-endemic areas, use of surface water for irrigation, and heavy rainfall during the vegetable season that could facilitate *C. cayetanensis* transport. *C. cayetanensis* prevalence in the agricultural environment will be assessed in water and on produce (via a proxy measurement) as well as in human sewage samples from municipal wastewater influents and on-farm portable toilets. This study will add to the understanding of *C. cayetanensis* contamination in US produce growing environments and provide information about contamination routes to produce within the Southeastern US.

Benefits to the Industry

- The data from this study will be the first step in determining the prevalence of *C. cayetanensis* in the Southeastern US growing region.
- Additionally, this study will provide much-needed information regarding the prevalence of *C. cayetanensis* oocysts in municipal wastewater and on-farm sewage, which is necessary to understand the risk of environmental contamination in the U.S., which is considered a non-endemic country.
- Finally, detection of *C. cayetanensis* in spent packinghouse wash or dump tank water would indicate contamination of harvested produce and highlight the potential risk of cross-contamination during post-harvest processing, which would inform necessary changes to water treatment and handling procedures for industry.

Objectives

1. Surveillance of *C. cayetanensis* prevalence in irrigation water ponds and on produce (via spent packinghouse water as a proxy)
2. Surveillance of *C. cayetanensis* prevalence in on-farm portable toilets and municipal wastewater influents

Methods

C. cayetanensis prevalence in the agricultural environment will be assessed by dead-end ultrafiltration (DEUF) of large-volume (50 L) irrigation water samples and spent packinghouse water from eight farms. DEUF sample concentrates will be subjected to DNA extraction and quantitative real-time PCR (qPCR) analysis for *C. cayetanensis* and the *Bacteroides* HF183 human fecal marker. Sewage from on-farm portable toilets and sewage influent to the Tifton Regional Wastewater Treatment Complex will be collected throughout the entire study period. DNA will be extracted from these samples and analyzed by qPCR for *C. cayetanensis*. Samples that test positive for *C. cayetanensis* by qPCR will be genotyped using a novel suite of eight nuclear and mitochondrial markers.

Results to Date

Method development and training has been completed for sample collection via grab samples and DEUF (**Figure 2**) and for sample processing techniques (*E. coli* quantification by IDEXX Colilert and Quanti-Tray 2000). Thickener and aeration basin municipal wastewater samples have been collected in parallel to compare and identify the sample type most amenable to our sample concentration methods. **Figure 3** shows microscopic images of C. *cayetanensis oocysts* and "imposter" organisms from Tifton wastewater and river water from a previous study. Methods for nucleic acid extraction from sludge samples were optimized by testing commercially available extraction kits against CDC's method using sewage sludge samples spiked with *C. cayetanensis* oocysts. The Qiagen AllPrep PowerViral DNA/RNA Kit® was found to give higher concentrations of DNA and RNA with higher purity than the in-house method.

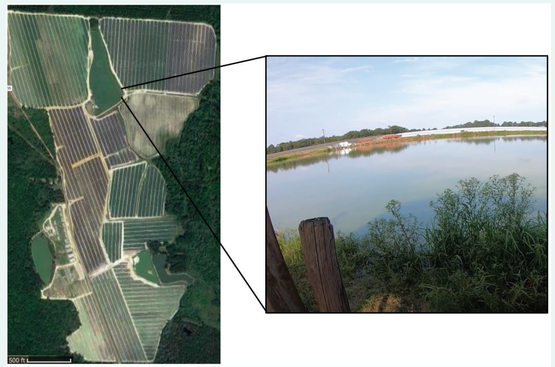

Figure 1. Irrigation pond on a farm of the collaborator grower in Tift County, GA.

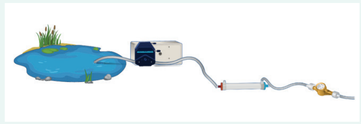

Figure 2. Illustration of dead-end ultrafiltration (DEUF) from an irrigation pond.

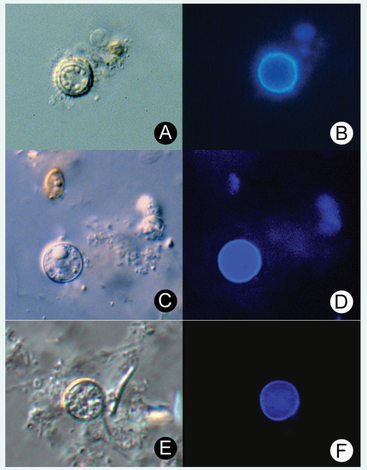

Figure 3. Microscopy images of *C. cayetanensis* oocysts and "imposter" organisms:
(A, B) River sample imposter organism – easily differentiated from *C. cayetanensis* by the spiky appearance via brightfield (A) but harder to see via fluorescence (B)
(C, D) *C. cayetanensis* oocyst
(E, F) Wastewater sludge sample imposter organism – more difficult to differentiate than A/B organism, but variable in size and with a slight color tone difference in the "wall" via brightfield (E), and the fluorescence image (F) is harder to differentiate