Sources and prevalence of *Cyclospora cayetanensis* in Southeastern U.S. irrigation water sources and growing environments

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References

1. Durigan, M., et al., 2020. BAM Chapter 19c: Dead-End Ultrafiltration for the Detection of *Cyclospora cayetanensis* from Agricultural Water. US Food and Drug Administration.
2. Murphy, H., et al., 2017. BAM Chapter 19b: Detection of *Cyclospora cayetanensis* in fresh produce using real-time PCR. US Food and Drug Administration.
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Summary

This study is assessing *Cyclospora cayetanensis* prevalence throughout farms in Georgia representing growing practices across the Southeastern U.S. The 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 transport *C. cayetanensis* into surface waters. *C. cayetanensis* prevalence in the agricultural environment is being assessed in irrigation water and on produce, as well as human waste samples from municipal wastewater influents and on-farm portable toilets. This study will add to the understanding of *C. cayetanensis* contamination in U.S. produce growing environments not previously evaluated and provide information about contamination routes to produce within the Southeastern U.S.

Objectives

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

Methods

C. cayetanensis prevalence in the agricultural environment is being assessed from water samples (50 L) from 8 irrigation ponds and spent packinghouse water (20 L). Irrigation water samples are collected monthly during fallow periods and every two weeks during growing and harvest periods. Packinghouse water is collected weekly during harvest periods. Sample concentrates are subjected quantitative real-time PCR (qPCR) analysis for *C. cayetanensis* and human-specific fecal source tracking markers, Bacteroides HF183 and crAssphage. Waste from on-farm portable toilets and municipal sewage influent at the local municipal wastewater treatment plant is collected bi-monthly and tested for *C. cayetanensis* by qPCR. All samples that test presumptively positive for *C. cayetanensis* by qPCR will be further analyzed by molecular and microscopic methods to verify the qPCR result (**Figure 1**).

Results to Date

To date, 88 irrigation pond samples have been collected, and 32 of these samples have been analyzed by qPCR for *C. cayetanensis* (**Table 1**), HF183, and crAssphage (**Table 2**). Nine irrigation pond samples were presumptively positive for *C. cayetanensis* (**Table 1**), and HF183 and crAssphage were detected in 2 and 1 pond samples, respectively (**Table 2**). A co-detection of *C. cayetanensis* and HF183 occurred in October 2020. Verification of presumptive-positive *C. cayetanensis* samples is ongoing. To date, 16 dump tank water samples have been collected during a harvesting period. Eleven of these samples have been analyzed by qPCR, with no detections for *C. cayetanensis*, HF183, or crAssphage. Additionally, 26 portable toilet samples and 46 municipal wastewater samples have been collected. Waste samples have not yet been analyzed.

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. The study will also provide much-needed information regarding the carriage of *C. cayetanensis* oocysts in communities living near and working on produce farms, which is necessary to understand the risk of environmental contamination in the US. Finally, detection of *C. cayetanensis* in spent packing house dump tank water would indicate contamination of harvested produce, which would inform potential changes to produce washing, handling, and storage procedures to prevent cross-contamination of oocysts to unaffected produce and reduce the incidence of illness.

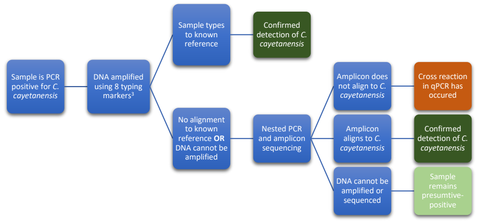

Figure 1. Laboratory workflow for verification of a *C. cayetanensis* qPCR presumptive-positive result.

Table 1. *C. cayetanensis* qPCR results for irrigation pond samples in 2020. Molecular *C. cayetanensis* results are listed as "Detected" based on the FDA BAM Methods 19b and 19c criteria[1,2]. All molecular results will undergo further verification described in Figure 1 to confirm presence for *C. cayetanensis*.

Pond [a]	Sampling Month			
	September	**Oct 6-8**	**Oct 20-22**	**Early-Nov**
A1	Not Detected	Not Detected	Not Detected	Not Detected
A2	**Detected**	Not Detected	Not Detected	Not Detected
A3	**Detected**	Not Detected	**Detected**	**Detected**
A4	Not Detected	Not Detected	**Detected**	Not Detected
B1	Not Detected	Not Detected	Not Detected	**Detected**
B2	Not Detected	**Detected**	Not Detected	Not Detected
B3	**Detected**	Not Detected	**Detected**	Not Detected
B4	Not Detected	Not Detected	Not Detected	Not Detected

[a] Ponds are identified as 1-4 for Growers A and B.

Table 2. Human-specific fecal source tracking marker qPCR results for irrigation pond samples.

Pond [a]	Sampling Month			
	September	**Oct 6-8**	**Oct 20-22**	**Early-Nov**
A1	Not Detected	Not Detected	Not Detected	Not Detected
A2	Not Detected	Not Detected	Not Detected	Not Detected
A3	Not Detected	Not Detected	Not Detected	Not Detected
A4	Not Detected	Not Detected	**HF183**	**HF183**
B1	Not Detected	Not Detected	Not Detected	Not Detected
B2	Not Detected	Not Detected	Not Detected	Not Detected
B3	Not Detected	Not Detected	Not Detected	Not Detected
B4	**crAssphage**	Not Detected	Not Detected	Not Detected

[a] Ponds are identified as 1-4 for Growers A and B.