Analysis of the presence of *Cyclospora* in waters of the Mid-Atlantic States and evaluation of removal and inactivation by filtration

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Summary

This project aims to address concerns surrounding freshwater availability and safety, as more outbreaks of foodborne illness caused by parasites have been linked to water used in produce production. *Cyclospora cayetanensis* has presented a unique challenge to the scientific community in understanding its persistence, transfer, and detection in the environment. Cases of domestically acquired foodborne illness associated with *C. cayetanensis* have drastically increased in the past five years. Zero valent iron (ZVI), a by-product of the steel industry, is affordable and has been shown to be effective in removing and neutralizing bacterial, viral, and chemical contaminants from water. The efficacy of ZVI on parasites has not been studied but shows great potential in filtration applications.

Benefits to the Industry

There are increasing concerns regarding groundwater quality and availability for produce irrigation. It is crucial to evaluate non-traditional sources of agricultural water, like surface and reclaimed water. Protozoan parasites, including *Cyclospora cayetanensis*, have been isolated in drinking, irrigation, surface, and reclaimed waters and are recognized as waterborne pathogens. The results of this work will provide a better understanding of testing and of the presence of *C. cayetanensis* in non-traditional agricultural waters, including surface and reclaimed waters in the Mid-Atlantic States.

Objectives

1. Confirmation of the presence of *Cyclospora cayetanensis* in presumptive positive environmental water samples using the Sanger (dideoxy) sequencing method (and nested PCR amplification, RFLP analysis)
2. Evaluation of zero valent ion (ZVI) and sand filtration in the removal and inactivation of *C. cayetanensis* surrogates in artificial agricultural water

Methods

Environmental water samples from the Mid-Atlantic States were collected in accordance with a modified EPA 1623.1 method, "Cryptosporidium and Giardia in Water by Filtration/IMS/FA" (**Figure 1**). The method was modified to exclude immunomagnetic separation (IMS) and fluorescent antibody (FA) testing, as there are no widely accepted IMS or FA methods for *C. cayetanensis*. The confirmation of the presence of *C. cayetanensis* in presumptive positive environmental water samples will be performed using the method 19a:9, "PCR Analysis," from the U.S. Food and Drug Administration Bacteriological Analytical Manual (proposed by Orlandi *et al.*, 2003). The FDA BAM method utilizes nested PCR amplification and RFLP analysis. In addition to the proposed method, sequencing will be performed for further confirmation of genus and species of isolated parasites.

Results to Date

To date all presumptive positive water samples have been prepared for nested PCR amplification, and all primers have been tested for specificity and sensitivity. Proposed primers are listed in **Table 1**. Primers were tested using DNA from *C. cayetanensis*, *Eimeria acervulina*, *E. maxima*, and *E. tenella* as positive controls, and nuclease-free water and DNA from *Cryptosporidium parvum* as negative controls. Negative water samples were spiked to test for inhibitors and other factors that could interfere with PCR.

Images of the gels indicating primer and method testing are shown in **Figure 2**. Preliminary results indicate that the nested PCR method increases sensitivity of the detection. The limit of detection for *C. cayetanensis* decreased from 250 genomic units to 2.5 genomic units.

Figure 1. Shani Craighead (graduate research assistant) filtering a water sample collected earlier that day using a modified EPA 1623.1 method.

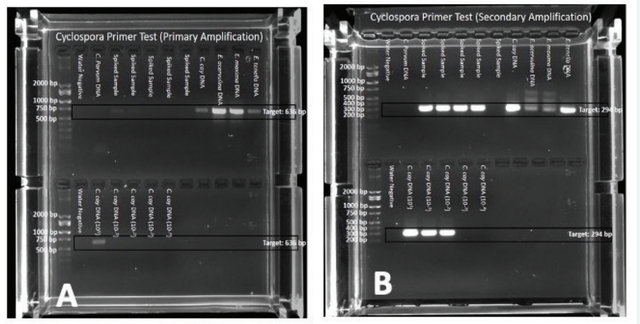

Figure 2. Gel images of PCR preliminary testing for primary (A) and secondary (B) amplification primers for the detection and differentiation of *Cyclospora* and *Eimeria* (primers listed in Table 1).

Table 1. DNA Primer Sequences for *Cyclospora*-specific PCR Amplification (18S rRNA gene)

Primer Code	Primer Specificity	Primer Sequence (5'-3')	Amplicon Size (bp)	Application
F1E (Forward)	*Cyclospora* and *Eimeria* spp.	TACCCAATGAAAACAGTTT	636	Primary Amplification
R2B (Reverse)		CAGGAGAAGCCAAGGTAGG		
F3E (Forward)	*Cyclospora* and *Eimeria* spp.	CCTTCCGCGCTTCGCTGCGT	294	Nested Amplification
R4B (Reverse)		CGTCTTCAAACCCCCTACTG		
CC719	*Cyclospora cayetanensis*	GTAGCCTTCCGCGCTTCG	298	Nested Amplification
PDCL661	*C. cercopitheci*, *C. colobi*, *C. papionis*	CTGTCGTGGTCATCGTCCGC	361	
ESSP841	*Eimeria* spp.	GTTCTATTTTGTTGGTTTCTAGGACCA	174	

Adapted from: Orlandi *et al.*, 2003