Comparison of Two Gene Targets for the Prevalence of *Cyclospora cayetanensis* in Irrigation Waters from US Southeast Fresh Produce Growing Regions

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Cyclospora cavetanensis is a human-specific coccidian parasite that causes the seasonal diarrheal illness Cyclosporiasis. It can infect humans through consumption of fresh produce such as lettuce and basil. As C. cayetanensis has been found to also be present in irrigation waters, its prevalence must be determined in the agricultural waters of fresh produce growing regions where oocysts can transmit to and contaminate fresh produce. The Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM 19C) method was used for the detection of C. cavetanensis oocysts in 288 water samples using the 18S rRNA target. Ten liter samples were collected using Dead-End Ultrafiltration (Hollow fiber ultrafilter Rexeed-25S; Asahi Kasei Medical Co.), followed by sieving (100 um, 70 um, and 40 um) and concentration at 4,000 x g for 45 min. DNA extraction with the FASTPrep 24, FastDNA SPIN Kit for Soil and purification with the QIAquick® PCR Purification Kit was performed in preparation for real-time PCR using primer sets for either the 18S rRNA or the recently developed Mit1C. Results with the 18S rRNA show a positive rate of 43% (124/288). However, the 18S rRNA has the potential of generating false positives due to possible cross reactivity with *Eimeria* found in the same family. To address this concern a more recent gene target was developed by the FDA targeting the mitochondrial region called the Mit1C. The present study has tested 16 out of the 124 positive samples with the Mit1C with none resulting positive. Preliminary results indicate downstream sequencing will be necessary to confirm true positives and determine the sensitivity and specificity of the 18S rRNA and Mit1C targets in a water matrix. This would determine which gene marker is more reliable to the detection of C. cayetanensis in irrigation waters.