Optimizing Detection Methods for *Cyclospora cayetanensis*

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Introduction:

Cyclospora cayetanensis is a parasite that causes gastrointestinal illness called cyclosporiasis. The foodborne outbreaks are associated with produce and irrigation water. In the stool of infected people, the oocysts must be sporulated (mature) to be infective which takes about 1-2 weeks. This means it cannot be passed directly from person-to-person but is passed through direct contact with the parasite in the stool. My project is specifically looking at *C.cayetanensis* in water sources such as pond and irrigation water. There are published and validated FDA detection methods that can be used to detect *C.cayetanensis* but due to issues like discontinuation of supplies and ease-of-use, the methods need to be improved upon. Another issue is that an infected person is likely to shed a low number of oocysts so multiple stool samples must be collected and the detection method must have high resolution at low concentrations.

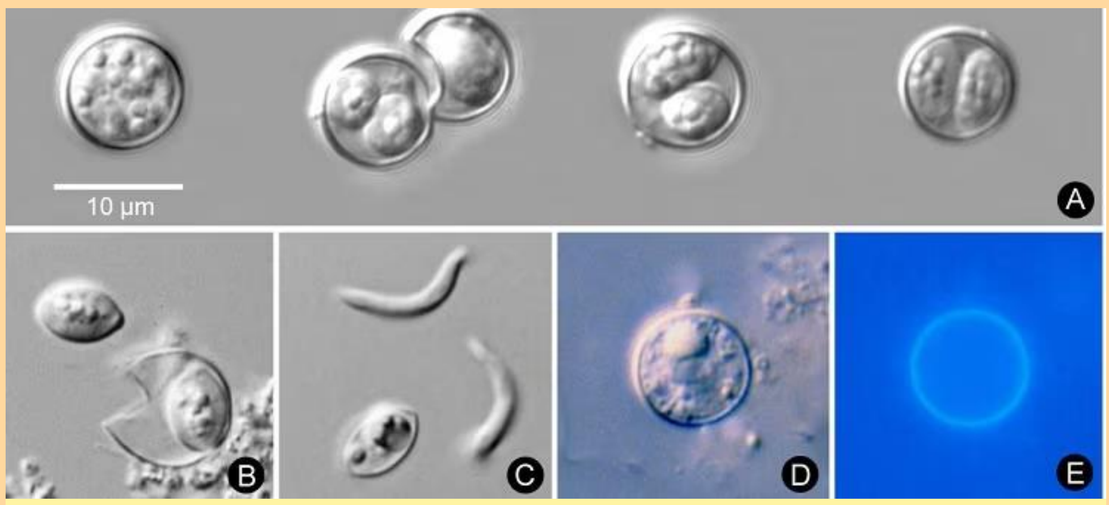
Site Information:

Site name: Joint Institute for Food Safety and Applied Nutrition (JIFSAN)

Address: 8301 Muirkirk Road, Laurel, MD

My supervisor: Mauricio Durigan

Mission: JIFSAN is a joint program between the University of Maryland (UMD) and the Food and Drug Administration (FDA). The mission is to improve public health, food safety, and applied nutrition. The project I worked on was specifically looking at detection methods for *Cyclospora cayetanensis* in water sources.



Cyclospora cayetanensis at various stages. A) includes an unsporulated oocyst (left) next to sporulated oocysts. B) and C) show the ruputued oocyst to release the infective stage of the parasite. D) shows an oocyst while E) shows the same oocyst under ultraviolet light. Image from https://www.cdc.gov/parasites/cyclosporiasis/index.html

Bibliography:

Durigan, M., H. Murphy, K. Deng, et al. 2020. BAM 19c: Dead-end Ultrafiltration for the Detection of *Cyclospora cayetanensis* from Agricultural Water. *Bacteriological Analytical Manual (BAM)*.

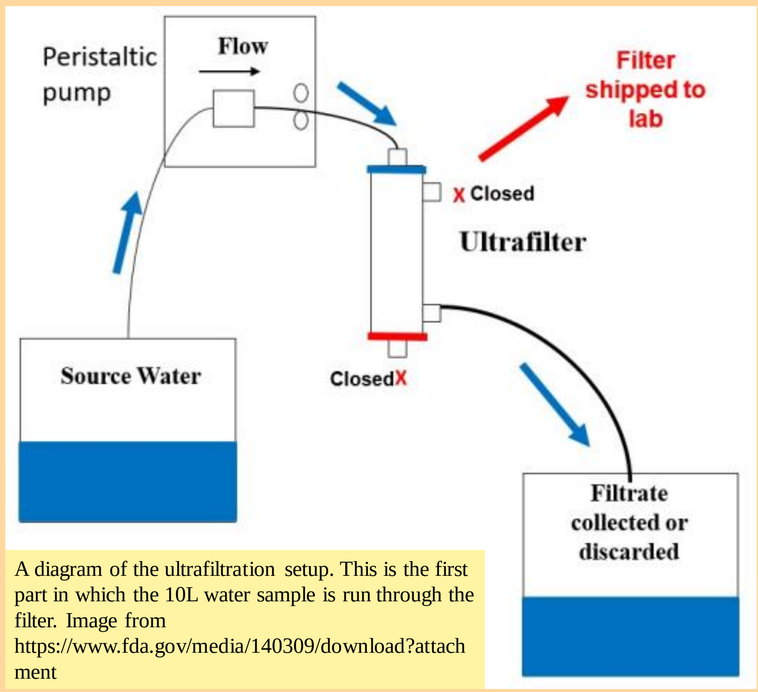
Murphy, H.R., S. Almeria, and A.J. da Silva. 2017. BAM Chapter 19b: Molecular Detection of *Cyclospora caytanensis* in Fresh Produce Using Real-Time PCR. *Bacteriological Analytical Manual (BAM)*.

Methods:

There were three main parts to the process: water filtration, DNA isolation and purification, and real-time qPCR. For water filtration, we collected pond water into carboys and spiked 10L aliquots with known concentrations of *C.cayetanensis*. We then ran these each through their own filter to catch the *C.cayetanensis* and then ran them in the reverse (backflush) to collect what was filtered out of the water (Durigan 2020). The collected samples were then isolated and purified into smaller volumes of DNA samples. Those DNA samples were finally analyzed using real-time qPCR which could tell us if *C.cayetanensis* was detected in those samples (Murphy 2017).

Results:

This research is consistently ongoing as there are still ways to optimize it, but we were able to achieve some results. We were able to validate a new brand-name water filter that could be used in the published method. It gave similar concentrations in collected samples as the previously used filter. We also validated another enzyme that could possibly be used in the PCR methodology. We had to test various concentrations and cycle lengths to try and achieve similar results to the currently published enzyme.



A diagram of the ultrafiltration setup. This is the first part in which the 10L water sample is run through the filter. Image from https://www.fda.gov/media/140309/download?attachment

Discussion:

The previously published brand-name filter was discontinued in the U.S. so there was an urgency to find a new filter. The filter and enzyme were validated in only our lab, so it still needs to go through multi-lab validation to be officially added to the published methodology. Other resources, like the enzyme, may be become unavailable in the future so we are going to continue to test other available resources that achieve similar results. This will allow for less confusion and issues with the methodology as multiple resources will be able to achieve similar detection results. Other future work includes measuring the exact concentrations of samples, rather than just the PCR results, and sequencing the DNA so we know more exact values. These exact values will allow us to optimize the methodology to have a high resolution at the low concentrations often found in clinical samples.

Acknowledgments:

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