Developing Time Integrated Technology to Detect Cryptosporidium in Waterways

Sean McKinney
Dr. Jillian Maxcy-Brown
Dr. Frances O'Donnell
Dr. Chingming Wang

WATERS
WATER AND THE ENVIRONMENT'S RESILIENCY AND SUSTAINABILITY

Author Information:
Sean McKinney
Contacting Author e-mail
SPM0036@auburn.edu
Phone: (251)-229-3511

Introduction

- In Alabama, 247 of the 548 impaired waterways are listed due too high concentrations of pathogens and bacteria[1], indicating fecal contamination that poses health risks to individuals exposed to these waters.
- One of the most significant pathogens affecting humans and animals is *Cryptosporidium.*
- Cryptosporidium causes Cryptosporidiosis, a diarrheal illness with 823,000 cases[2] and 200,000 child deaths[4] annually.
- Can pollute through failed waste water management.
- Due to the low permeability of the clay soils found in the Black Belt region of Alabama, failing systems are much more common.
- More risk for cryptosporidium outbreak.
- Lower income area makes it harder to find funding for consistent sampling

Background

The current method for testing for pathogens in surface water, EPA Method 1623, involve grab samples, capturing a sample only representative of a single moment of time. Additionally, these grab samples can cost up to $160 per sample, often too expensive for low-income areas consistently sample. To address this, we developed a time integrated sampling method which could significantly reduce costs.

Previous lab work has shown that while *Cryptosporidium* oocysts attach to the stomach wall when ingested by humans, they can also attach to naturally grown biofilm[3]. Additional research has been done to the practicality of this discovery in field work, and it has been found to capture similar numbers of oocysts as the traditional EPA method[3]. We built on this previous research by designing a reusable sampling device that can collect multiple samples at a time.

Site

The location chosen for the deployment of the sampling device was Parkerson Mills Creek in Auburn Alabama. This location was chosen due to its close proximity to both cattle and swine farms. We believed this would give us the best chance of detecting *Cryptosporidium* oocysts.

Traditional Methods

Water Quality
Water quality samples were tested using a handheld sonde device. Parameters tested were:
- pH
- Turbidity
- Pressure
- Temperature
- Fluorescent Dissolved Organic Matter
- Dissolved Oxygen
- Specific Conductance

Additionally grab samples were taken and used to test for total coliforms and E coli.

EPA Method 1623.1
The traditional EPA method uses filters to catch oocysts from stream water. The filters used in this process can be seen below.



Figure 1: Envirocheck Filter

Microscopy
Once the sample is processed through the filter it is then dried and dyed on a microscope slide and analyzed using fluorescent microscopy. A picture of an oocyst analyzed with the EPA Method can be seen below.

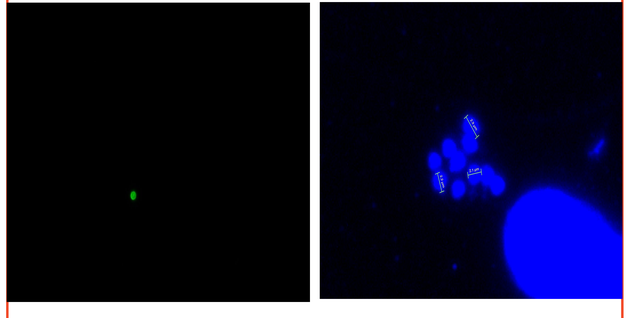


Figure 3 and 4: *Cryptosporidium oocysts analyzed with Microscopy method*

New Methods

Preliminary Tests
The first task faced in the design process was choosing a material for biofilm growth. The materials tested are listed and shown in the below photo:

- Stainless steel
- HDPE
- Smooth Rubber
- Textured Rubber
- Polycarbonate



Figure 5: Preliminary Biofilm Test

The samples were left in the stream for one week to grow biofilm. After removal, the biofilm growth was compared using dry mass comparison and Image J analysis. Of the 5 materials tested, textured rubber yielded the most biofilm growth. Dry mass comparison showed the textured rubber yielded almost 0.009 g/cm^2 compared to the next highest mass, stainless steel, only grew 0.003 g/cm^2.

Sampler Design
The sampler was designed using three pieces of 3" diameter PVC pipe connected using a 3-way tee fitting. The two bottom PVC pipes were designed to hold 1.5" diameter PVC pipes cut in half used to hold samples,. On top of the sampler a removable pressure cap is installed for future additions of calcium. The sample design can be seen below in figure 1.

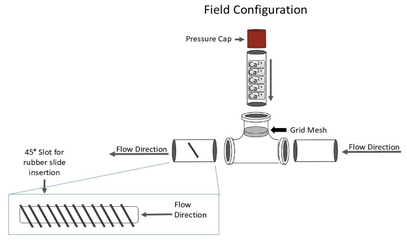


Figure 6: Sample Design





Figure 7 and 8: Sampler in the field

Lab Analysis
The samples were left in Parkerson Mills Creek for 2 weeks to grow biofilm and collect cryptosporidium oocysts before being retrieved. Once retrieved samples were analyzed using PCR analysis to detect any presence of cryptosporidium oocysts.

Discussion

- More textured materials grew more biofilm, in this case textured rubber
- The cost to build the sampling device is $100.
- Device is capable of collecting multiple samples as well as being reused with the only additional cost being the sample material.
- Device is significantly more affordable compared to traditional methods ($160/ sample)
- Anticipate that future testing will continue to show the developed device is more effective and inexpensive compared to filtration testing.
- Future plans include sharing results and methods with local testing centers.

References

1. ADEM. (2024). 2024 Alabama 303d List. https://adem.alabama.gov/programs/water/wquality/2024AL303dList.pdf
2. CDC. (2024). Cryptosporidiosis NNDSS Summary Report for 2019. Waterborne Disease and Outbreak Surveillance Reporting. https://www.cdc.gov/healthy-waterdata/documentation/cryptosporidiosis-nndss-summary-report-for-2019.html
3. Jellison, K., Cannistraci, D., Fortunato, J., & McLeod, C. (2020). Biofilm Sampling for Detection of Cryptosporidium Oocysts in a Southeastern Pennsylvania Watershed. Applied and Environmental Microbiology, 86(23), 1–12. https://doi.org/10.1128/AEM.0139920/SUPPL_FILE/AEM.01399-20-S0001.PDF
4. Sarkhosh, T., Zhang, X. F., Jellison, K. L., & Jedlicka, S. S. (2019). Calcium-Mediated Biophysical Binding of Cryptosporidium parvum Oocysts to Surfaces Is Sensitive to Oocyst Age . Applied and Environmental Microbiology, 85(17). https://doi.org/10.1128/aem.00816-19
5. Sow, S. O., Muhsen, K., Nasrin, D., Blackwelder, W. C., Wu, Y., Farag, T. H., Panchalingam, S., Sur, D., Zaidi, A. K. M., Faruque, A. S. G., Saha, D., Adegbola, R., Alonso, P. L., Breiman, R. F., Bassat, Q., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., … Levine, M. M. (2016). The Burden of Cryptosporidium Diarrheal Disease among Children < 24 Months of Age in Moderate/High Mortality Regions of Sub-Saharan Africa and South Asia, Utilizing Data from the Global Enteric Multicenter Study (GEMS). PLOS Neglected Tropical Diseases, 10(5), e0004729. https://doi.org/10.1371/JOURNAL.PNTD.0004729
6. He, J., Dougherty, M., Zellmer, R., & Martin, G. (2011). Assessing the Status of Onsite Wastewater Treatment Systems in the Alabama Black Belt Soil Area. Environmental Engineering Science, 28(10), 693–699. https://doi.org/10.1089/EES.2011.0047

Acknowledgements

Thank you to Dr. Maxcy-Brown for leading this project and to Dr. O'Donnell, Dr. Ojeda, Dr. Wang, and Sierra Stubbs for expertise and contributions to this project. This project was funded by Alabama Water Resources Research Institute.