Mentored Undergraduate Summer Experience Summer 2021 Application

Faculty Member:

Email:

Professional Title:

Tenure Status:

Time at TCNJ:

Student Collaborators:

Student Email Addresses:

Requested Dollar Amount:

There is no need for IACUC or IRB approval as animals will not be manipulated and there are no human subjects. The IACUC office confirmed no approval is needed.

The faculty member has never received a MUSE grant and is finishing her first year as an Assistant Professor.

I) Intellectual Merit

Cryptosporidium parvum (*C. parvum*) is a protozoa which causes cryptosporidiosis, a parasitic infection resulting in gastrointestinal illness, mainly diarrhea, stomach cramps, dehydration, nausea, vomiting, fever, and weight loss. Typically, cryptosporidiosis is self-limiting in healthy individuals, but may be chronic or fatal in the immunocompromised. An estimated 748,000 cases of cryptosporidiosis occur annually in the United States, resulting in over $45 million in hospitalization costs. Roughly 30% of the adult population is seropositive for *Cryptosporidium.* Livestock, particularly cattle, are main reservoirs for *C. parvum*, causing a potential risk to those who work with cattle. **Our goal is to quantify the risk of cryptosporidiosis to dairy farmers in New Jersey.** The first step in mitigating risk is understanding risk.

This assessment will involve fecal sampling, DNA extraction, quantitative polymerase chain reaction (qPCR) for DNA quantification, and quantitative microbial risk assessment (QMRA). The project is ambitious, but feasible within the 8-week timeframe as laid out in the timeline below. Cow fecal samples will be collected from four dairy farms in New Jersey, which have agreed to participate in this study. Fresh fecal samples will be collected in sterile falcon tubes and transported back to The College of New Jersey (TCNJ) in coolers. Students will wait for a cow to defecate to ensure the sample is fresh but will collect the sample from the ground. Students will not have contact with the cattle and no animal manipulation will occur. Students will wear proper personal protective equipment (PPE) for sample collection, including closed toed shoes, long pants, gloves, and a facemask. Ten samples will be taken at each farm for a total of 40 samples for the project.

DNA will be extracted using the QIAamp DNA Stool Mini Kit with methods modified to optimize *Cryptosporidium* extraction as per Hawash (2014). The amount and purity of the extracted DNA will be assessed using a NanoDrop. The concentration of *C. parvum* will be quantified using qPCR. The qPCR protocol, including probes and primers, can be found in Appendix E. QMRA is a quantitative approach to assessing public health risk by estimating risk of infection and illness when a population is exposed to a pathogen in the environment. Student A is working on her public health capstone project in my lab during the spring term and will be developing the structure of the QMRA in the statistical program language, R. The QMRA can be populated with *C. parvum* quantification data and validated during MUSE.

My current project with the Philadelphia Water Department (PWD) assesses *C. parvum* contamination in surface water. This project will fund the purchase of primers, probes, standards, and chemicals needed for qPCR. Additional funding is needed for the QIAamp DNA Stool Mini Kit and for travel to the farms.

Summary Timeline	**Week**								**Post-Award**
Project Elements	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Fecal Sample Collection	X	X	X						
DNA Extraction			X	X	X				
Student Training			X	X					
Independent Processing				X	X				
qPCR				X	X	X	X		
Reagent Preparation				X					
Standard Curve Trouble Shooting				X	X	X			
Student Training				X	X	X	X		
Sample Processing						X	X		
QMRA							X	X	
Building dairy worker QMRA							X		
Comparing and validating results								X	
Write/submit manuscript for publication									X
NIOSH R03 Application									X

II) Role of the Students and Mentor

The students on this project, Student A and Student B, will be fully immersed in sampling, analysis, documentation, and final reports. They have been key contributors since the project's conception. Student A is working on her capstone project and Student B is volunteering in my lab during Spring 2021. I laid out the projects I am currently working on, current funding, and future grant opportunities and the students discussed their research interests. After spending some time with the literature, we designed this project together. The goal of this project is to provide experience in the rigor of conducting a scientifically sound experiment, laboratory skills, quantitative skills, and the process of publishing. While Student A and Student B have some laboratory experience, this project will be their first encounters with DNA extraction and qPCR. As such, they will require extensive training to become competent in these skills. I will be training both students in the field and lab throughout the project. We will be collecting samples together for at least the first week until the students are confident, and then they will go as a pair to collect samples. DNA extraction training will occur side by side for at least one week. As they become more independent, I will transition from actively training to observing. By week 5 they should be able to perform the DNA extraction themselves, though I will be in the lab for questions while they are working.

qPCR is a very sensitive process which cannot tolerate contamination. The students will learn how to prepare the reagents for qPCR in an aseptic and precise manner, how to create a standard curve, and how to run the qPCR assay. I will be developing and troubleshooting the qPCR assay for *C. parvum* over the spring term and both students will be involved. However, to truly learn qPCR and be able to execute the entire process independently they need dedicated time to be in the lab which the MUSE program will allow them. By week 6 I expect that the students will be able to create their own standard curves and by week 7 they should be able to run the qPCR assay from start to finish semi-independently (they will be able to ask me questions but will not require regular instruction).

Student A will be taking the lead in developing the QMRA as she will be working on it over the spring term. Student B and I will assist her in populating the QMRA with the data from the qPCR and validating the results. Two students are requested for this study to process enough samples for publishable work. I do not believe collecting and processing 40 samples over the 8 weeks will be feasible for only one student, and fewer samples may result in the work being rejected for publication.

III) Broader Impacts

As a new faculty member at TCNJ I started my position during the pandemic and have not had the opportunity to work with students in my lab. The MUSE funding will allow me to get my lab up and running and work with two students who are dedicated to and excited about the research we are proposing. They are both thoughtful about their long-term career goals and this project can help provide them with skills and experience that will assist in reaching those goals. I chose a career in academia because I enjoy the teaching and mentoring aspects of research. I have sorely missed student engagement and interaction in my research since the pandemic. I am looking forward to being back in the lab and hoping this program will allow me to work full time with students who are as motivated, excited, and bright as Student A and Student B have shown themselves to be.

We are going to be applying for an Educational and Research Centers (ERC) grant through the Environmental Protection Agency (EPA) which will allow this research to continue through the 2021-2022 school year. The ERC grant will allow for more samples and a more rigorous analysis through the academic year but will not provide a summer salary or housing for students. MUSE will allow Student A and Student B to dedicate full time efforts to this work over the summer. The students will be presenting their work during the MUSE poster session as well as submitting it to the Society for Risk Analysis's Annual Meeting in December 2021 as first and second author. We will be submitting the results of this study to Microbial Risk Analysis for publication. We will be using the data as preliminary data for a Small Research Grant (R03) application through the National Institute of Occupational Safety and Health (NIOSH).

MUSE 2021 Budget

a) Student stipend $3,000 requested for 2 students - $6,000
b) Student house $1,732.24 requested for 1 student - $1,732.24
c) Faculty stipend $1,250, requested for 1 faculty - $1,250
d) Project-related expenses, as itemized below - $471
    a. QIAAmp DNA extraction kit - $294
    b. Sterile Falcon Tubes - $121
    c. 100 miles to travel to farms at $0.56/mile - $56

Total Project Cost: $9,453.24

Standard Operating Procedure: qPCR Analysis

DNA Extraction Samples: Page Number:

Prepared Reagents: Date Prepped: Page Number:
BSA
Primer/Probe Mix
Fwd Primer (300 nM): *Crypto* – CATGGATAACCGTGGTAAT
Rev Primer (300 nM): *Crypto* - TACCCTACCGTCTAAAGCTG
Probe (50 nM): *Crypto* TACCCTACCGTCTAAAGCTG

Materials: Manufacturer: Item #: Lot#:
PCR Grade Water
TaqMan Master Mix

Instructions:

1. Use the qPCR "tray set up" spreadsheet to create Crypto and Sketa22 Master Mix
2. The following volumes are required per reaction. In order to calculate the total volume of the master mix multiple each volume by the number of samples and a 10% safety factor

Reagent	**Reagent Concentration**	**Crypto Master Mix Volume per Reaction**	**Sketa22 Master Mix Volume per Reaction**
PCR Grade water			5 µL
BSA	0.2 mg/mL	µL	2.5µL
TaqMan® Environmental Master Mix	1 X	µL	12.5µL
Primer/probe mix	0.2uM/0.1uM	µL	3.0µL

3. Add the reagents in the following order: PCR Grade water, BSA, TaqMan Environmental Master Mix, and primer/probe mix.
4. Keep on ice until ready to use
5. Design Plate layout ensuring you have at least 3 method extraction blanks (MEB) and 3 non-template controls (NTC) in both Crypto and Sketa22 reactions. Keep in mind that Crypto and Sketa22 reactions will be on separate plates:

Crypto	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												

H												

Sketa22	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

6. In the template addition hood:
    a. Add 19 µL of the Crypto Master Mix to each well designated for the adeno reactions
    b. Add 6 µL of the template DNA to designated adeno wells, use PCR grade water for the NTC wells
    c. Add 23 µL of the Sketa22 Master Mix to each well designated for the Sketa22 reactions
    d. Add 2 µL of template DNA to designated Sketa22 wells, use PCR grade water for the NTC wells
    e. Seal the plates with optical adhesive PCR tape
7. Vortex the plates briefly by dragging the plate along the for vortex at the highest speed
8. Centrifuge plate at 10,000xg for 2 minutes
9. Place the Crypto plate into qPCR instrument and set amplification controls for:
    a. 94℃ for 10 minutes
    b. 45 cycles of 95℃ for 5 seconds, 60℃ for 10 seconds and 72℃ for 5 seconds
10. Place the Sketa22 plate into the qPCR instrument and set amplification controls for:
    a. 95℃ for 10 minutes
    b. 40 cycles of 94℃ for 10 seconds and 54℃ for 30 seconds, and 72℃ for 10 seconds