Adapting USEPA Protocol for Detection and Enumeration of *Giardia* and *Cryptosporidium* in an Agricultural Setting

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Introduction

Two protozoan parasites that are of concern related to water quality are *Giardia* and *Cryptosporidium*. Both organisms are transmitted as the infective cysts or oocysts are passed by an infected human or animal in their feces. Symptoms of the diseases caused by these organisms include diarrhea, vomiting, dehydration, and lethargy (Baron, 1996; USDA AWPL, 2004). These infections tend to be self-limiting for healthy individuals, however, if the host is seriously immunocompromised, life-threatening complications may arise (Kneen and Lemley, 2004).

As a result of water run-off being contaminated by feces, *Giardia* and *Cryptosporidium* are found in most surface water supplies (Schijven et al., 2004). Varying levels may be found in water sources near agricultural facilities, sewage treatment plants, or wildlife areas such as forests as a result of shedding from infected hosts (USEPA, 2000). As a result of the health threat these parasites pose, special water treatment is key to preventing outbreaks. The USEPA has established guidelines for both *Giardia* and *Cryptosporidium* removal from public water supplies being 99.9% and 99% respectively (USEPA, 2000).

New methods for effective water treatment have been investigated with human waste such as the Ecological Treatment System (ETS) designed by Todd and Josephson (Todd et al, 2003) referred to as an Advanced Ecologically Engineered System (AEES) that utilizes plants and water tanks to reduce levels of waste components that are of concern. This system is now being applied to animal agriculture in the hopes that it will provide an alternate means for handling waste produced by the animals and providing a renewable water source for the producer.

This project focuses on the detection of parasite levels present in the animal waste entering the ETS and how effective it is in the cyst and oocyst reduction.

Objectives

1. To determine if USEPA Method 1623 and the Filta-Max® system are adequate methods to recover and elute *Giardia* cysts and *Cryptosporidium* oocysts from a filtered sample of water and manure sludge

2. To determine the level of initial and final *Giardia* cysts and *Cryptosporidium* oocysts while utilizing the ETS designed by Todd and Josephson (Todd et al., 2003).

Materials and Methods

Adaptations on the United States Environmental Protection Agency (USEPA) Method 1623 (USEPA, 2001) were made to accommodate the different sample source from the original method, but Method 1623 was the basis and majority of the experimental design. Three areas were investigated and validated: the enumeration of stock *Giardia* and *Cryptosporidium*, evaluation of filtering and elution with the Filta-Max® system, and assessment of staining the organisms.

Changes made were:

- the sample source (surface water vs. manure)
- sieving the samples before filtration
- sugar floatation with antibody and DAPI staining
- creation of a qualitative standard curve for both organisms

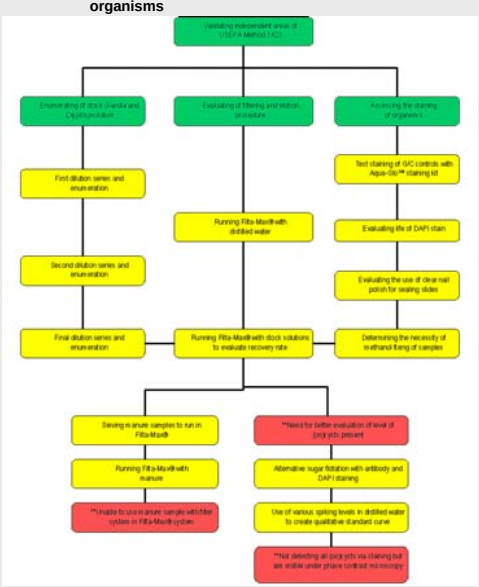

Figure 1. Research flow chart to outline and summarize the order of events for validation and adaptations on USEPA Method 1623. Green boxes denote main objectives. Yellow boxes denote steps executed in process of validation. Red boxes denote problems or pitfalls of USEPA Method 1623 with animal agriculture waste.

Results and Conclusions

Enumeration of stock organisms

- A more concentrated dilution scheme for the spiking suspensions offered a more accurate and precise enumeration of the organisms.

Evaluating of filtering and elution procedures

- The Filta-Max® system worked well for running distilled water and the stock suspensions to evaluate recovery rates.
- Recovery rates were lower than expected and sensitivity of the system was questioned.
- Manure samples, even after sieving, damaged the filter because of particulate size and load.
- It will be impossible to use this system to evaluate the initial and final level of *Giardia* cysts and *Cryptosporidium* oocysts of the ETS as a result of its inability to handle the particulate size and load.

Assessing the staining of organisms

- The Aqua-Glo™ G/C direct, FL, staining kit stained samples very well when concentration of the organisms was successful.

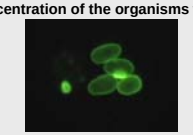

Figure 2. Direct immunofluorescence stain of positive control with Aqua-Glo™ G/C Direct, FL, staining kit viewed with UV microscopy. Small organism on left is *Cryptosporidium* oocyst and organisms on right are *Giardia* cysts.

- The alternate sugar floatation with antibody and DAPI staining also proved a successful method for qualitative staining of the sample.

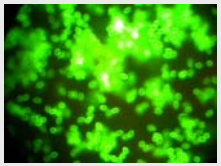

Figure 3. Direct immunofluorescence with alternate sugar flotation of known-positive *Giardia* sample viewed with UV microscopy. Organisms are *Giardia* cysts.

- A qualitative standard curve was not created because it was discovered that not all the cysts and oocysts were being stained. Many were seen under phase contrast microscopy, but when the same slide was placed under UV microscopy, fewer were visualized.

Future Research

Why were the cysts and oocysts not detected in the staining, but were visible under phase contrast microscopy?

Are there proteins that are used in the antibody tagging that are present on "fresh" cysts and oocysts that were degraded on the "aged" cysts and oocysts?

How does this reflect upon USEPA Method 1623 and detection of "aged" cysts and oocysts in the environment that may still be infective but undetected?

Proposed future study for these questions

- use fresh stock suspensions to inoculate negative fecal samples
- allow samples to be exposed to environmental conditions and age for various time periods
- process each sample and visualize with both UV microscopy and phase contrast microscopy
- compare visualization with the two microscopy methods to time elapsed between the samples

What filtration and elution system could handle the particle size in the manure from the manifold of the ETS?

How can this ETS and the parasite load be evaluated?

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