AFECCT: Assessing filtration efficacy for *Cyclospora* control

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Summary

Contamination with oocysts of the enteric parasitic pathogen *Cyclospora cayetanensis* compromises produce safety, endangering public health and the reputation of the produce industry. Water contaminated with human fecal material may serve as a source of such contamination, so filters warrant evaluation as tools to mitigate risk. Because *C. cayetanensis* pose danger and are difficult to procure in numbers required for research, we established filter conditions and performance using surrogate parasites (*Eimeria* parasites derived from chickens). Filters composed of sand alone, or of sand mixed with zero-valent iron (ZVI) proved highly effective in removing parasites from water. ZVI improved filter performance in removing oocysts from water. Scaled-down ZVI-sand filters now enable validation with *Cyclospora*.

Objectives

1. Determine filtration efficacy for *Eimeria*.
2. Determine how well *Eimeria* models *Cyclospora* undergoing filtration.
3. Evaluate filter performance in field deployments.

Methods

Experimental methods are depicted in **Figure 1**.

- Briefly, filters (**Figure 2**) containing either sand alone, or 50/50 sand/ZVI were inoculated with 500,000 oocysts of *Eimeria tenella* in 400 mL water and rinsed with another 600 mL until 1 L was collected (**Figure 3**).
- Filters were backflushed, rinsed with another liter of water, and employed in three successive challenges of 500,000 oocysts each (total inoculum – 1.5M oocysts).
- 0.5-micron capillary filters were used to concentrate the effluent to 10–30 mL (**Figure 4**).
- Sedimentation and centrifugation of thus concentrated oocysts were employed before suspending oocysts in <0.5 mL for microscopic examination and enumeration.

Results to Date

Sand, alone, impeded the transit of *Eimeria* parasite oocysts (derived from chickens and resembling those of *Cyclospora*); adding ZVI to sand (in a 50/50 ratio) improved filter performance (**Figure 5**).

- Sand alone blocked passage of >95% of 1.5 million oocysts inoculated in three successive challenges of 0.5 million oocysts in 1L of water, each; sand/ZVI filters blocked passage of at least 99.98% of such oocysts (**Figure 5**).
- Larger ZVI-sand columns (**Figure 2A**) performed comparably or better.

Scaled-down filters now enable evaluation of filter efficacy on *Cyclospora*, available in only small quantities for laboratory evaluation. Scaled-down filters, designed to allow passage of sufficient numbers of oocysts, now enable evaluation of infectivity among *Eimeria* parasites that have transited filters.

Benefits to the Industry

Industry may benefit from simple filters capable of substantially reducing parasite loads in water.

- Sand filters, filters containing ZVI and sand, and capillary filters all show promise in mitigating parasite risk.

Long-term filter performance under field conditions merits evaluation. If filters deliver sustained protection under real-world conditions, low-cost filtration of irrigation and/or wash water may enhance produce safety. Backflushing filters may reintroduce parasites to water sources, so the deposition of backflushed water warrants care. Future study will determine whether filters provide additional risk mitigation by reducing infectivity of the few parasites that transit such filters.

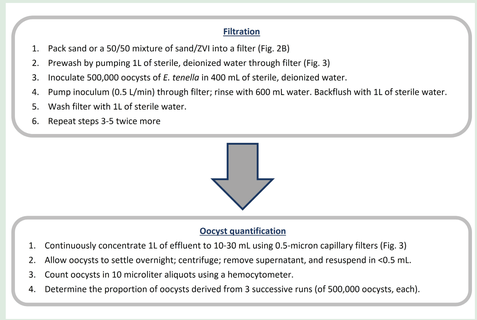

Figure 1. **Experimental overview**

Figure 2. **Example test filters**:
(A) 12″ × 4″ (L × dia), 2,471-mL capacity, ~1L pore vol; (B) 4.25″ × 2″ (L × dia), 219-mL capacity, ~100mL pore vol.

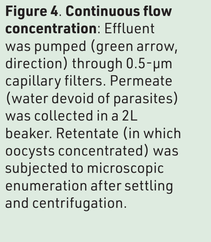

Figure 4. **Continuous flow concentration**: Effluent was pumped (green arrow, direction) through 0.5-µm capillary filters. Permeate (water devoid of parasites) was collected in a 2L beaker. Retentate (in which oocysts concentrated) was subjected to microscopic enumeration after settling and centrifugation.

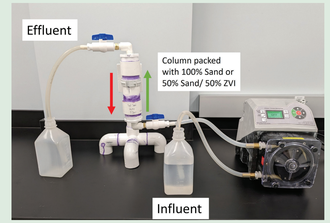

Figure 3. **Setup for filtration experiment**: Inoculated water (influent) was pumped (0.5 L/min) through filters – 100% sand (0.45–0.55 mm particle size) or 50% sand/50% ZVI (0.43–60 mm particle size); 3 successive runs were performed on a given filter. Green arrow indicates direction of flow; red arrow indicates backflush.

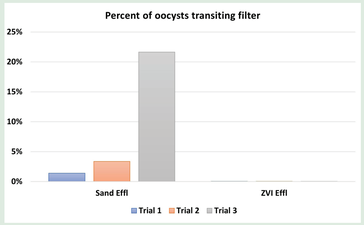

Figure 5. **Filter efficacy – oocysts recovered from effluent of 100% Sand or 50% Sand/50% ZVI**: 500,000 oocysts were inoculated into 400 ml of influent water, and 1 L of total effluent was collected. The same filter for each condition was used for three consecutive filter runs.