

Comparison of *Cryptosporidium* spp. detection by biofilms versus filtration in an urban water supply

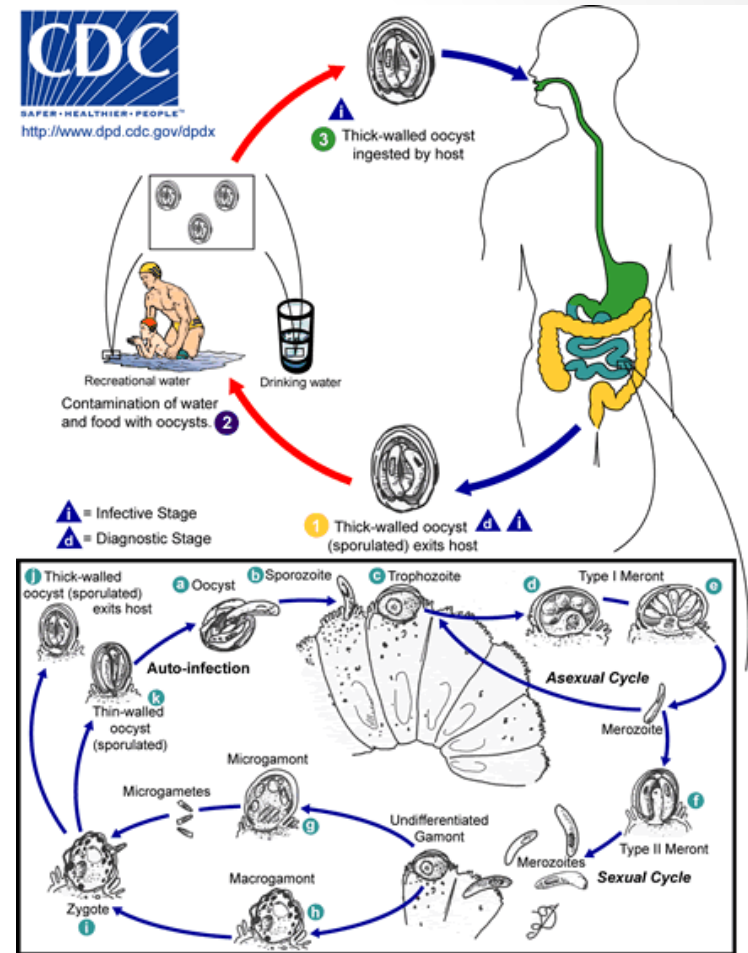
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Cryptosporidium

- Fecal-oral transmission
- Life-threatening for immunocompromised patients
- Challenge for water treatment plants
 - Oocysts 4-8 μm
 - Resistant to chlorine



<http://www.cdc.gov/parasites/crypto/biology.html>

EPA Method 1623

- 10 L sample
- “Snapshot” in time
- Variable recoveries
 - Seeded tap water: 23.5-71.2%
 - Raw source water: 19.5-54.5%
(McCuin & Clancy 2003)
- Expensive

Filtration



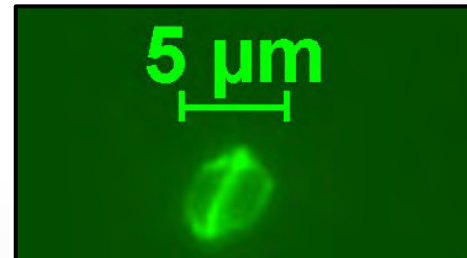
Elution



Immunomagnetic Separation (IMS)



Immunofluorescent Microscopy (IFA)



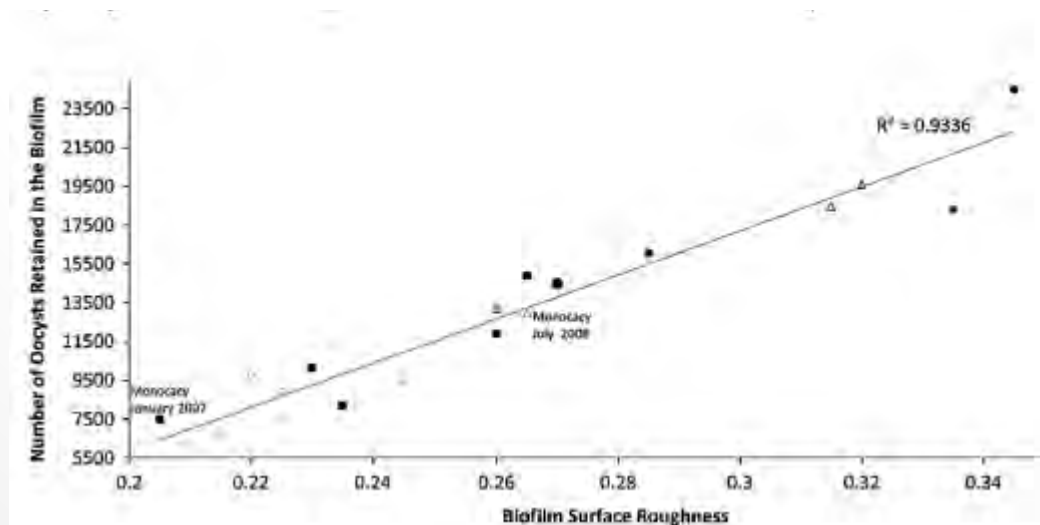
Biofilms

- *C. parvum* attaches to (and persists in) biofilms

(Helmi et al. 2008; Howe et al. 2002; Rogers & Keevil 1995; Searcy et al. 2006; Wolyniak-DiCesare et al. 2012; Wolyniak et al. 2009; Wolyniak et al. 2010)

- Some fraction of oocysts remain attached even after oocysts are removed from feed (Wolyniak et al. 2009; Wolyniak et al. 2010)

- Retention of attached oocysts correlates strongly with biofilm roughness (Wolyniak-DiCesare et al. 2012)



Biofilms

- Biofilms sampled from benthic rocks collected upstream and downstream of WWTP

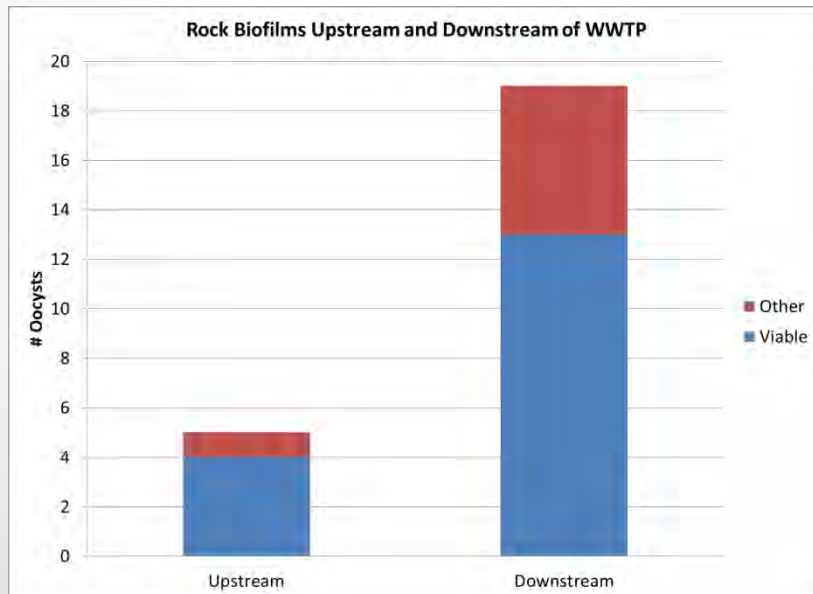
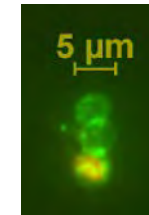
Rocks Scraped (6 up/6 down)



IMS



IFA/Fluorescent *in situ* Hybridization (FISH)



Viable = viable *C. parvum* or *C. hominis*

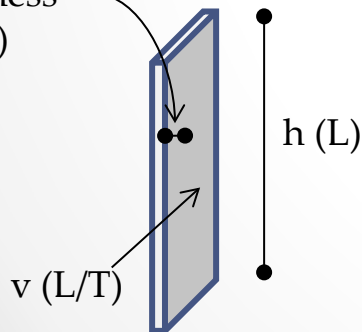
Other = nonviable *C. parvum* or *C. hominis* OR viable/nonviable other *Cryptosporidium* species

Biofilm Sampler

- Monitor stream quality over time
- Inexpensive
- Quantitative data is attainable given stream velocity



w = oocyst thickness (L)



t = duration of time deployed

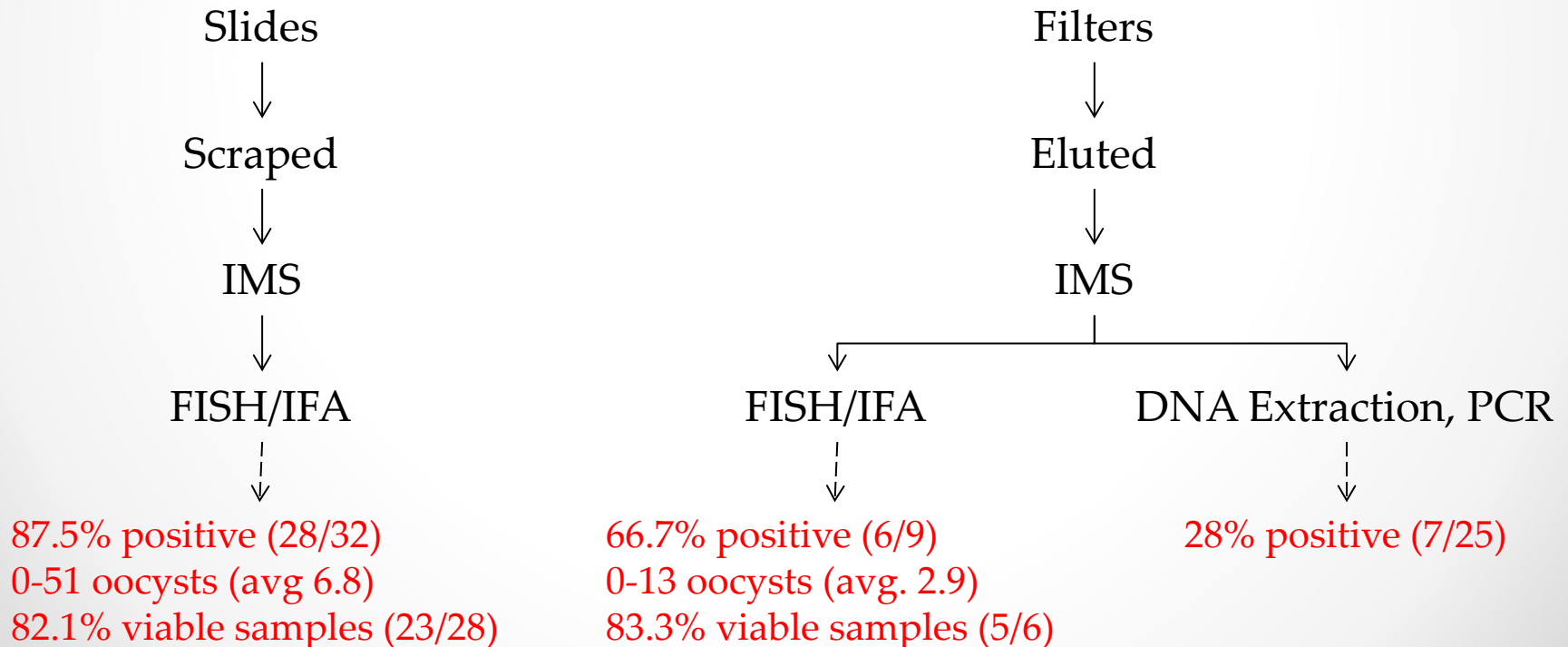
n = number of slide surfaces



$$\text{Oocyst concentration} = \sum_{i=1}^n \frac{\# \text{ oocysts counted}}{v \cdot h \cdot w \cdot t}$$

Sampling Results 2009-2011

- Samples collected at WTP intake Sept. 2009- Feb. 2011
- Slides recovered and processed on same day that water was filtered for EPA Method 1623 (slides in water 7-48 d)



Sampling Results 2013-2014

- Samples collected at (i) WTP intake and (ii) stream influenced by misconnected sewer laterals, July 2013-March 2014
- Slides recovered and processed on same day that water was filtered for EPA Method 1623 (slides in water 10-24 d)

Sampling Results 2013-2014

WTP

Slides
↓
Scraped
↓
IMS
↓
FISH/IFA

41% positive (7/17)

0-8 oocysts

None viable

Filters
↓
Eluted
↓
IMS
↓
FISH/IFA

29% positive (5/17)

0-3 oocysts

None viable

Stream

Slides
↓
Scraped
↓
IMS
↓
FISH/IFA

50% positive (5/10)

0-1 oocyst

None viable

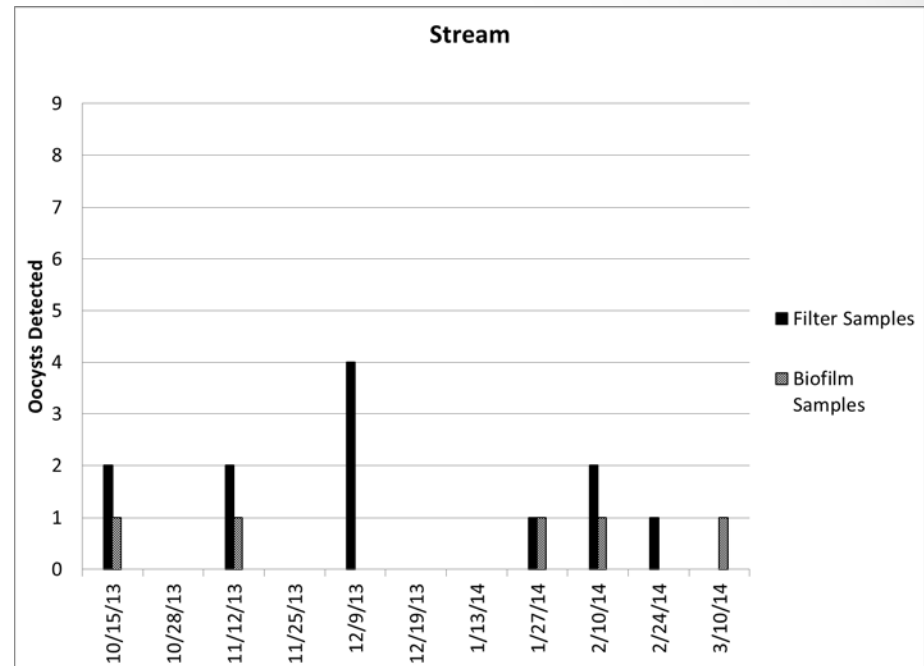
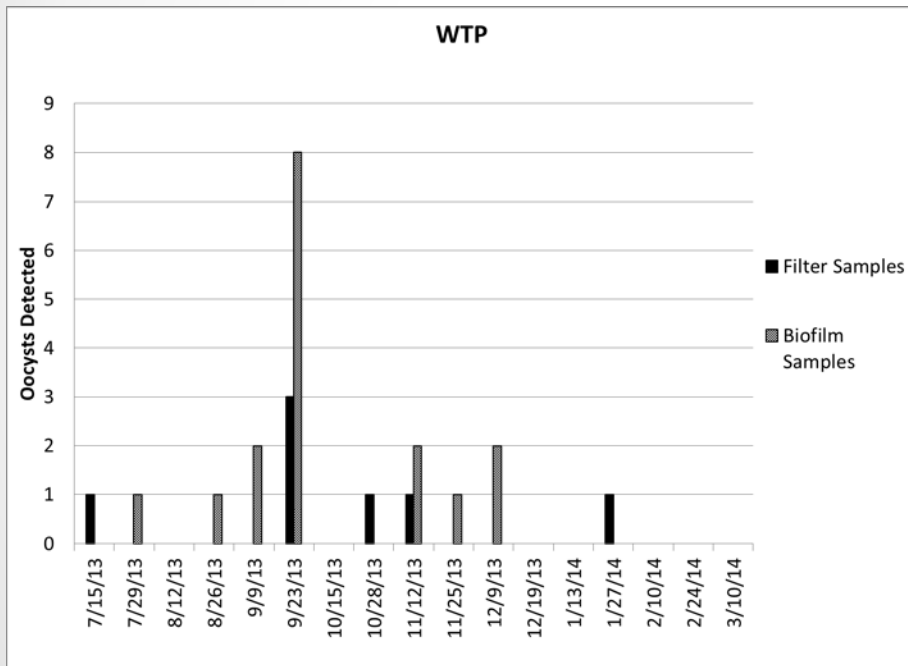
Filters
↓
Eluted
↓
IMS
↓
FISH/IFA

55% positive (6/11)

0-4 oocysts

None viable

Sampling Results 2013-2014



Preliminary Conclusions

- Benthic biofilm sampling may provide historic look at water quality conditions along the length of a waterway
- Oocysts detected in slide biofilms at least as often as in filtered water samples
- Biofilm monitoring is much less expensive than filtration
 - Could permit more frequent monitoring at more locations in the watershed
 - Could be used to identify point sources of oocysts along the length of a waterway

Disclaimer: This is research in progress and the methodology described here is continuing to be developed. Conclusions are preliminary at this point.



Acknowledgements

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