Waterborne Disease Prevention: Detection of *Cryptosporidium* in Watersheds

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Waterborne Disease – Global Statistics

- ***663 million people*** lack access to improved water supply
- ***2.4 billion people*** lack access to improved sanitation
- Between *1.085 to 2.187 million deaths* each year due to diarrheal diseases can be attributed to the 'water, sanitation, and hygiene' risk factor
  - 90% of these deaths are among children under age 5

Burden of Waterborne Disease

- Water-related disease is the 2nd biggest killer of children worldwide (1st = acute respiratory infections)

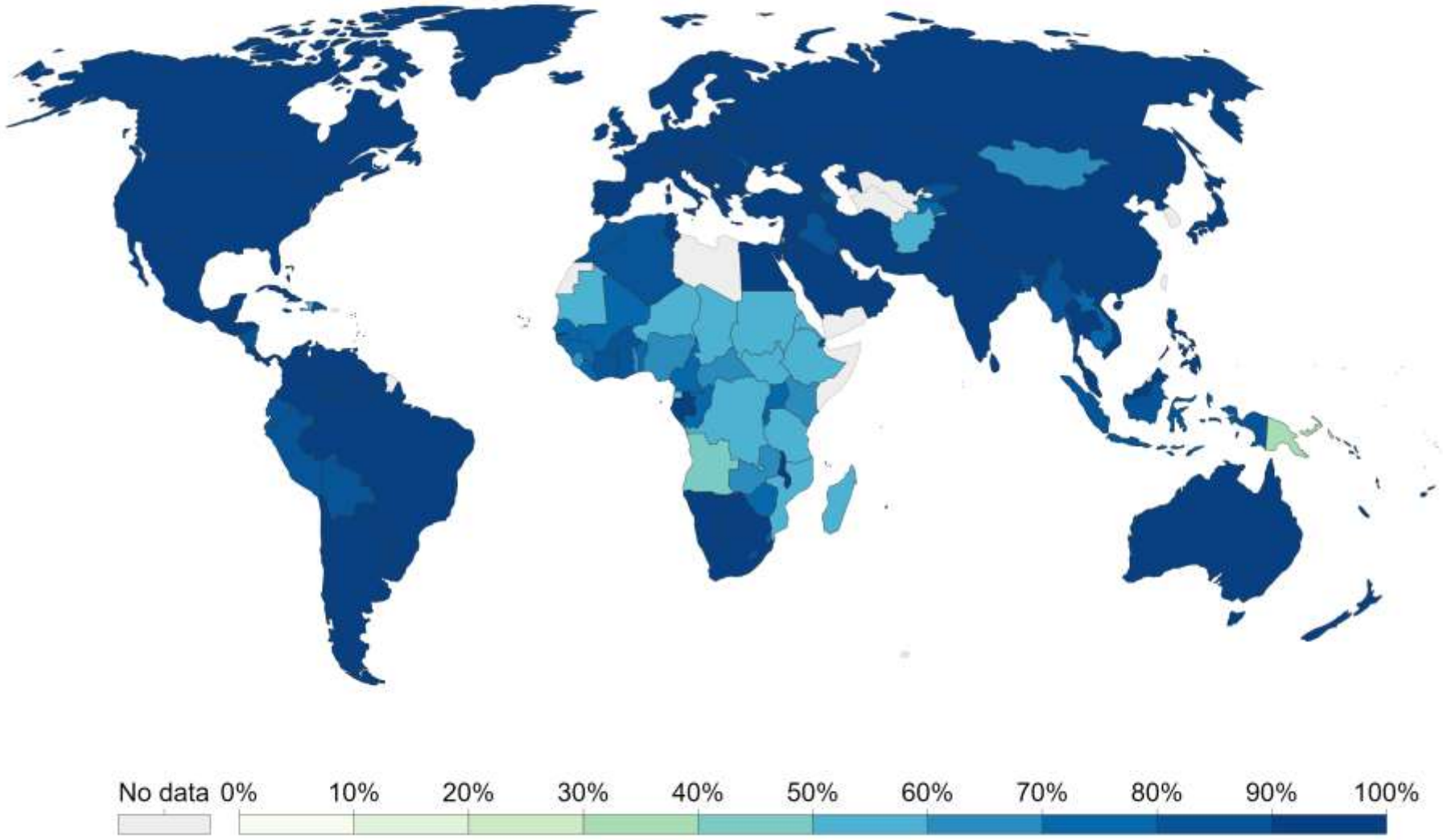
- At any one time:
  - half of the world's hospital beds are occupied by patients suffering from water-related diseases (WaterAid, 2008)
  - half of the population of the developing world is suffering from one or more diseases associated with inadequate water and sanitation (WaterAid, 2008)

- 443 million school days lost annually to water-related diseases

Share of the population with access to improved drinking water, 2015

An improved drinking water source includes piped water on premises (piped household water connection located inside the user's dwelling, plot or yard), and other improved drinking water sources (public taps or standpipes, tube wells or boreholes, protected dug wells, protected springs, and rainwater collection).

Our World in Data

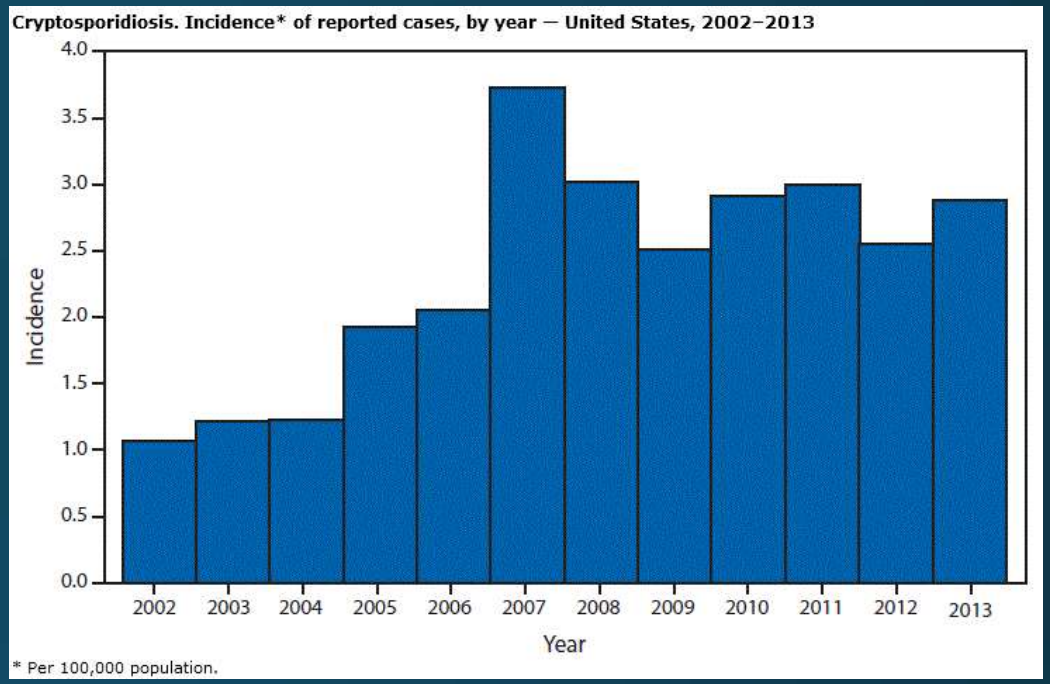


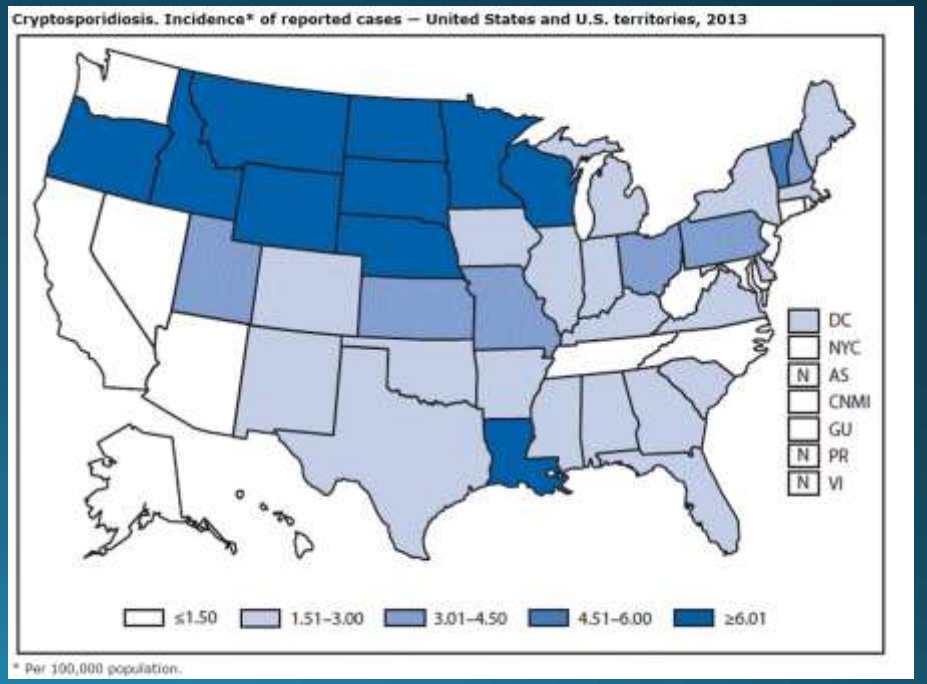
Source: World Bank – WDI

OurWorldInData.org/water-access-resources-sanitation/ • CC BY

Cryptosporidium parvum

- Protozoan parasite
- Obligate intracellular pathogen
- Primarily infects small intestine
- Forms oocysts
  - Survive 2-6 months without host
  - Resistant to disinfection
  - Killed by ozone, desiccation
- Found worldwide



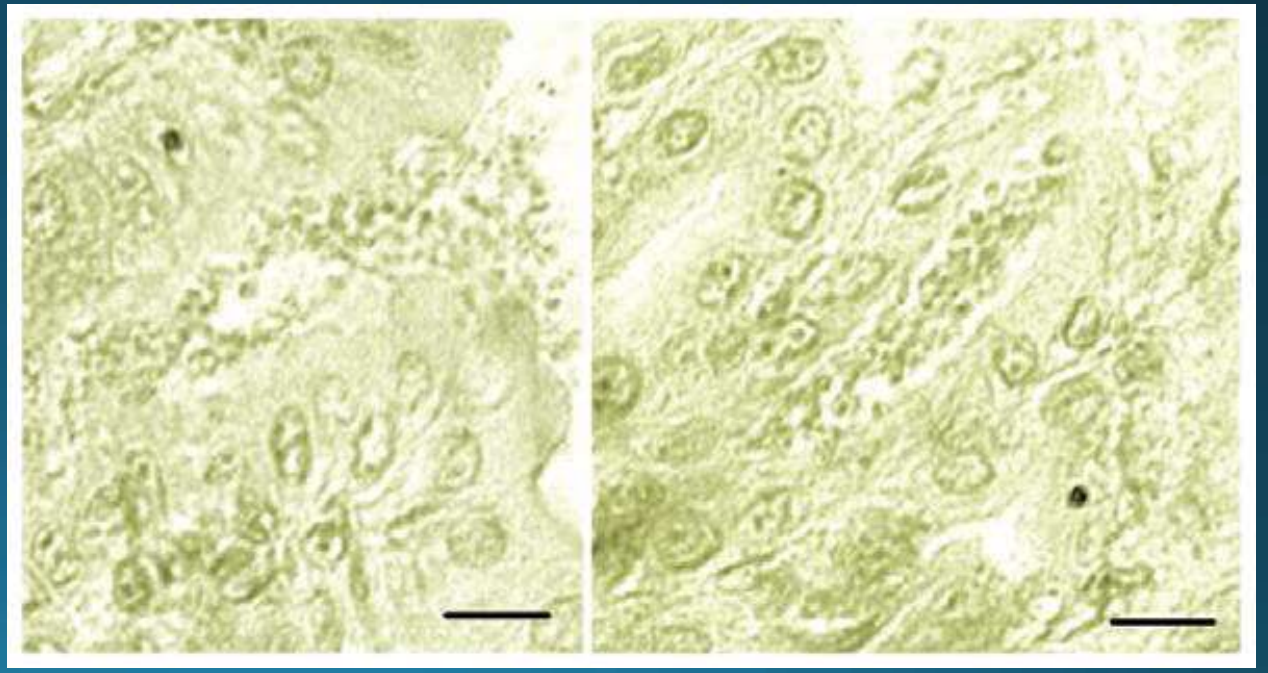


Adams, D., Fullerton, K., Jajosky, R., Sharp, P., Onweh, D., Schley, A., ... & Kugeler, K. (2014). Summary of Notifiable Infectious Diseases and Conditions—United States, 2013. *MMWR. Morbidity and mortality weekly report*, *62*(53), 1-119.

History

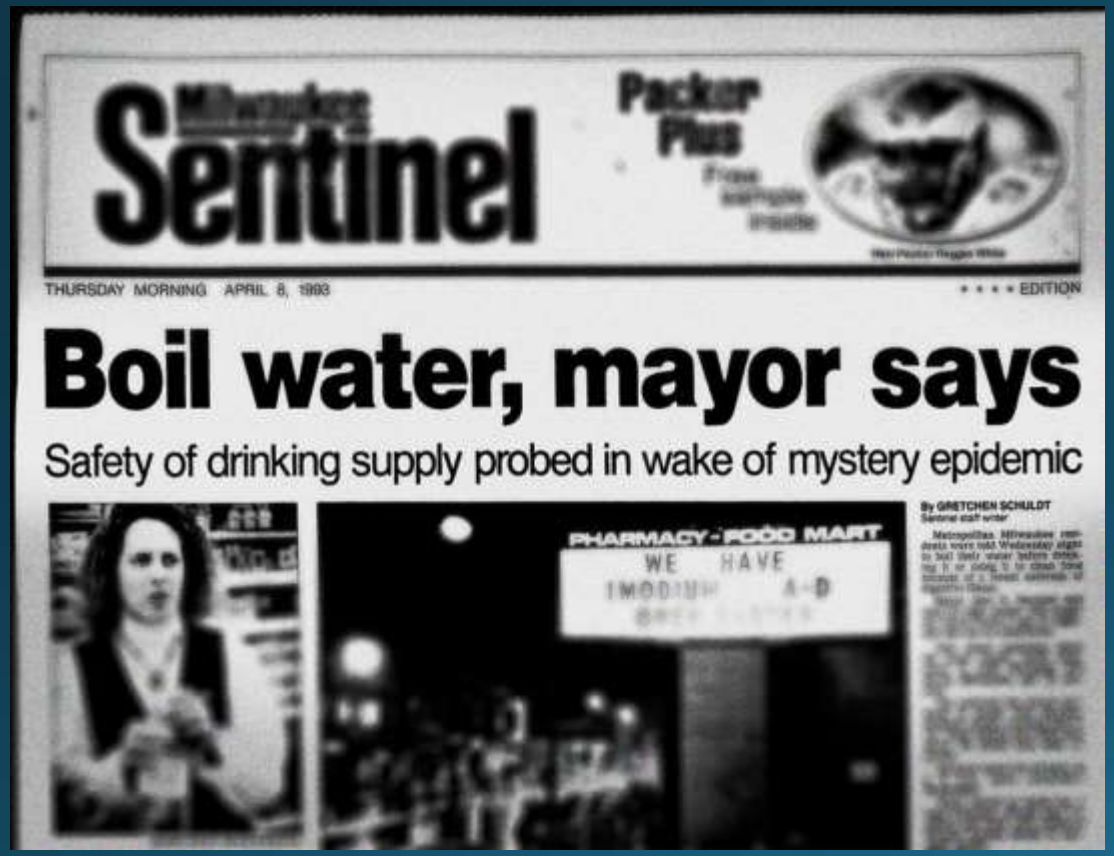
- 1912 - Discovered by Prof. Ernest Tyzzer (1875 – 1965)
  - American parasitologist

- Outbreaks associated with
  - Drinking water
  - Food
  - Swimming pools
  - Lakes
  - Hospitals (nosocomial) - HIV wards, pediatric hospitals



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2716703/

Milwaukee Outbreak, 1993



Milwaukee Sentinel

Packer Plus

THURSDAY MORNING APRIL 8, 1993 **** EDITION

Boil water, mayor says

Safety of drinking supply probed in wake of mystery epidemic

By GRETCHEN SCHULDT

PHARMACY-FOOD MART

WE HAVE

IMODIUM A-D

https://onmilwaukee.com/living/articles/ebolainmilwaukee.html

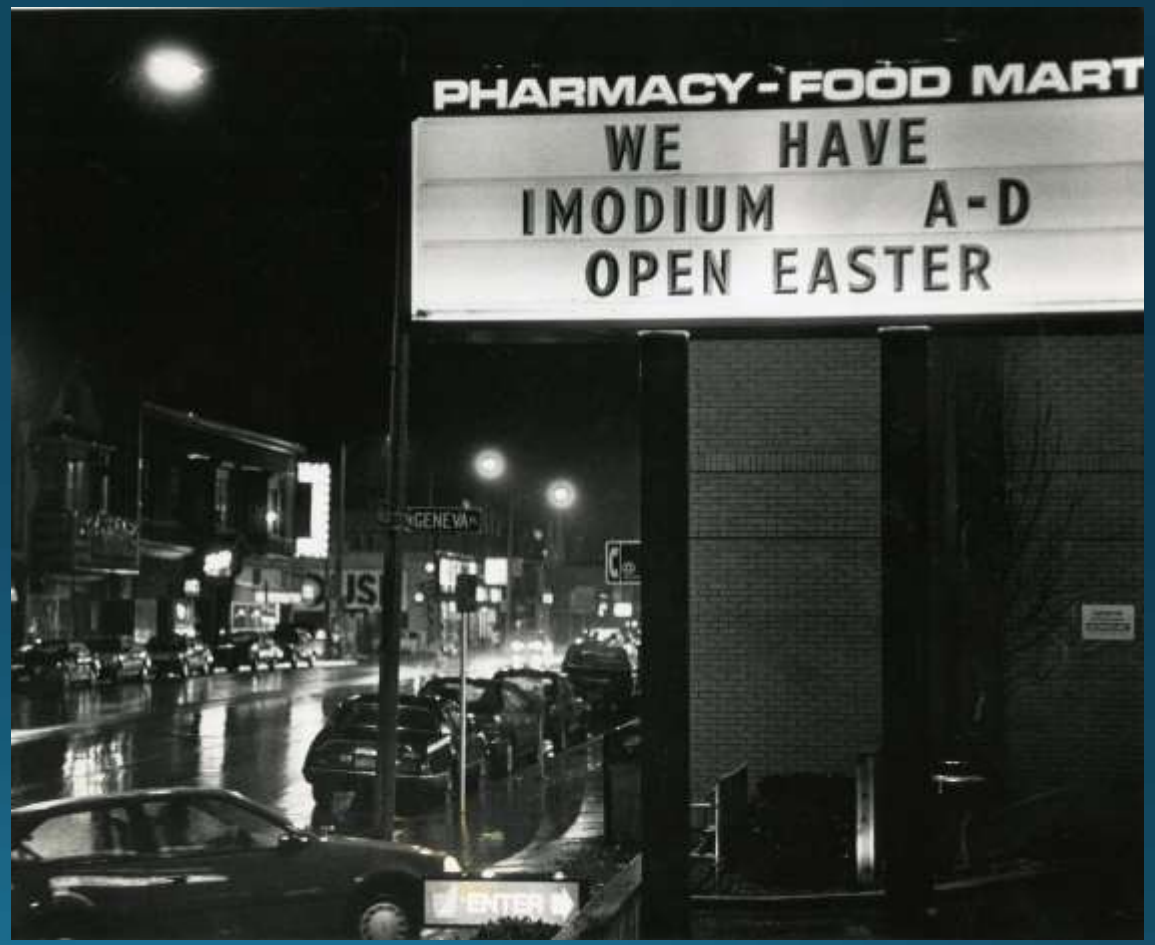


Image Credit: Jeffrey Phelps, Milwaukee Journal Sentinel

Cryptosporidium Life Cycle

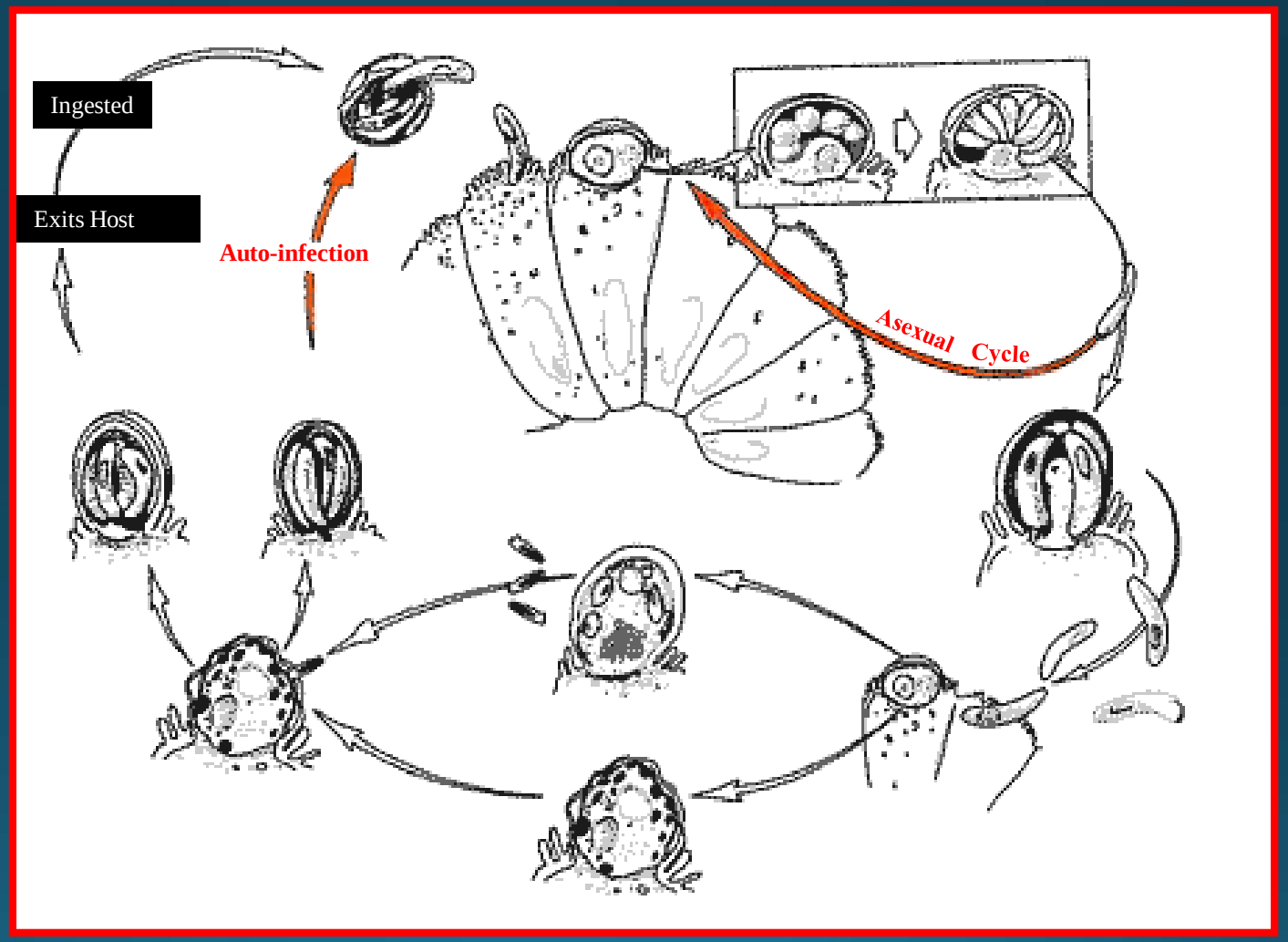
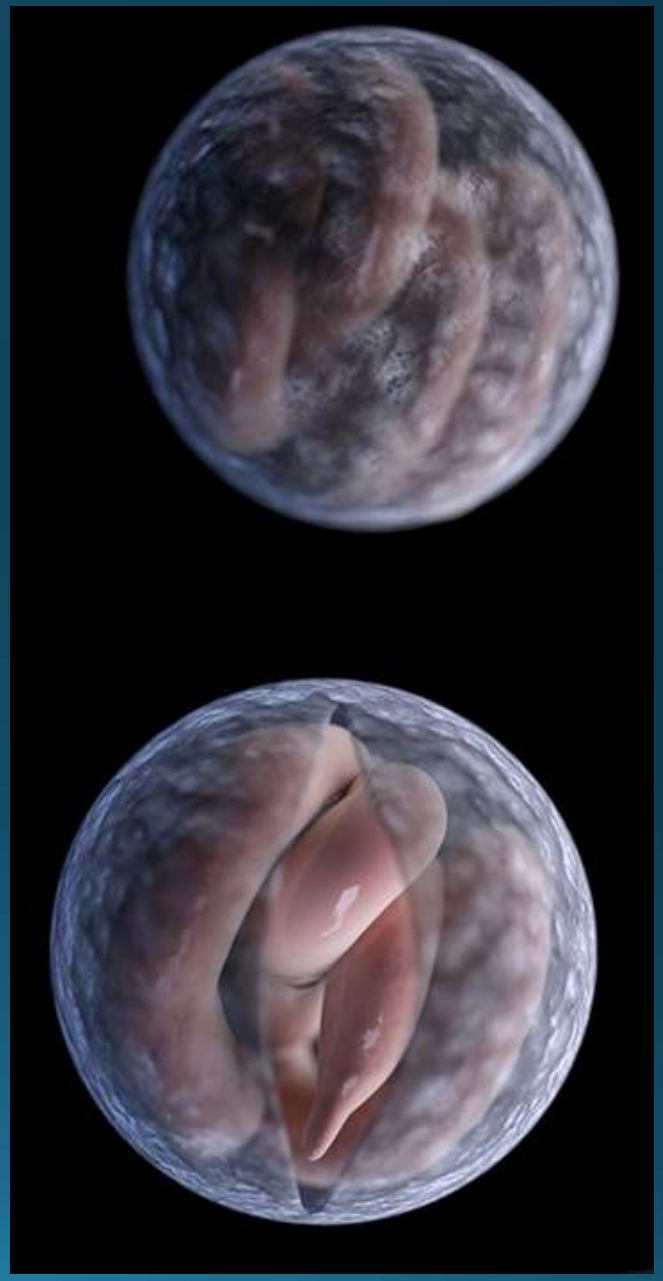


Image Credits: http://www.hominis.mic.vcu.edu/Cparvum_sporozoites.html; http://www.esemag.com/archive/0199/crypto.htm; Current & Blagburn, 1990

Cryptosporidiosis

- Fecal-oral transmission
- ID_{50} = 132 oocysts (DuPont et al., 1995)
  - dependent on isolate (9 → 1042 oocysts)
  - <10 oocysts can initiate infection
- Symptoms: severe watery diarrhea, fever, nausea, vomiting, weight loss, abdominal cramps
  - appear 2-10 days post-infection
  - duration ~2 weeks (prolonged for immunocompromised)
  - may be asymptomatic
- Underdiagnosed and underreported
- No cure



https://www.cdc.gov/parasites/crypto/index.html

U.S. EPA Method 1623.1

Limitations

- 10 L sample
- "Snapshot" in time
- Variable recoveries
  - Seeded tap water: 23.5-71.2%
  - Raw source water: 9.5-54.5%

  (McCuin & Clancy 2003)
- Expensive

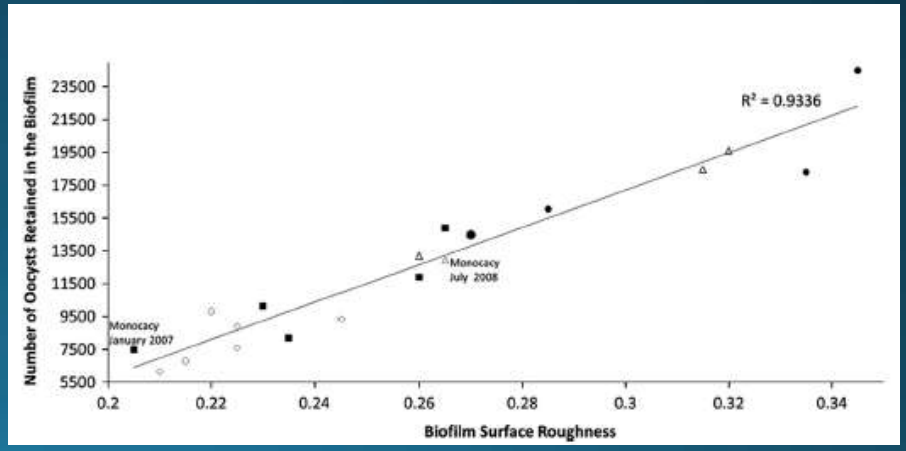
1. Filtration
   ↓
2. Elution
   ↓
3. Immunomagnetic Separation (IMS)
   ↓
4. Immunofluorescent Assay (IFA)

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)

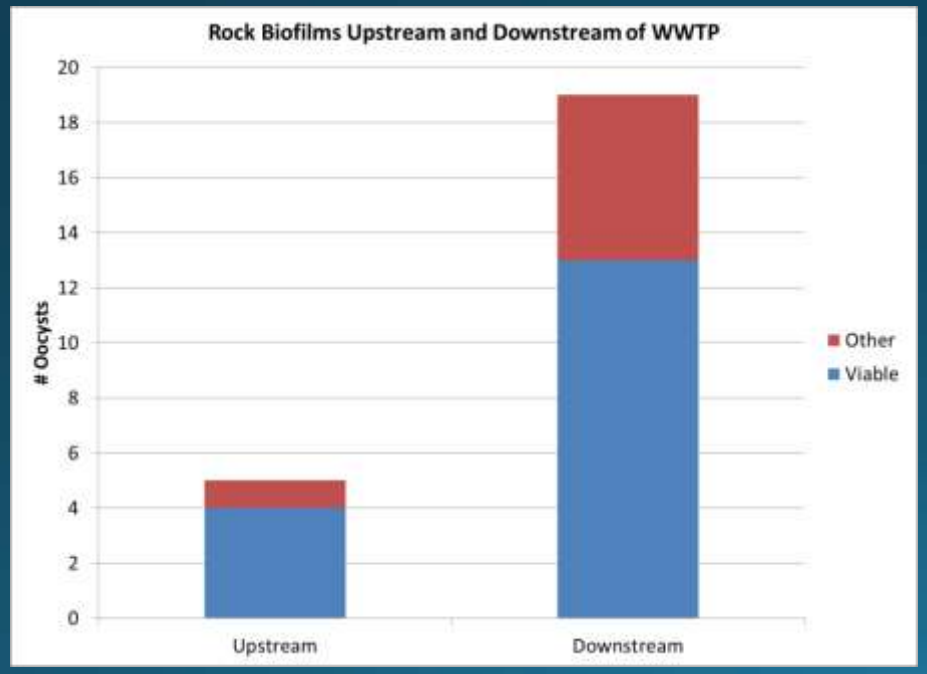
- Some fraction of oocysts remain attached even after oocysts are removed from feed
  (Wolyniak et al. 2009; Wolyniak et al. 2010)

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

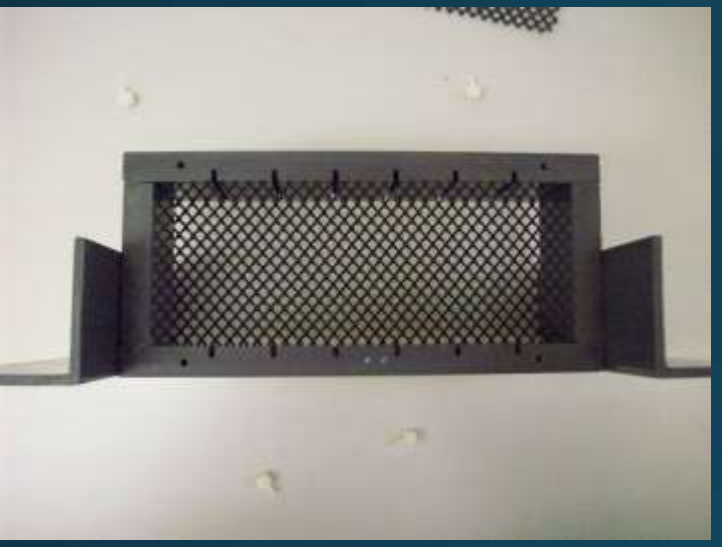


Benthic Rock Biofilm Sampling

- Benthic rock biofilm sampling may identify point sources of oocysts along the length of a waterway
  - May provide historic look at water quality conditions



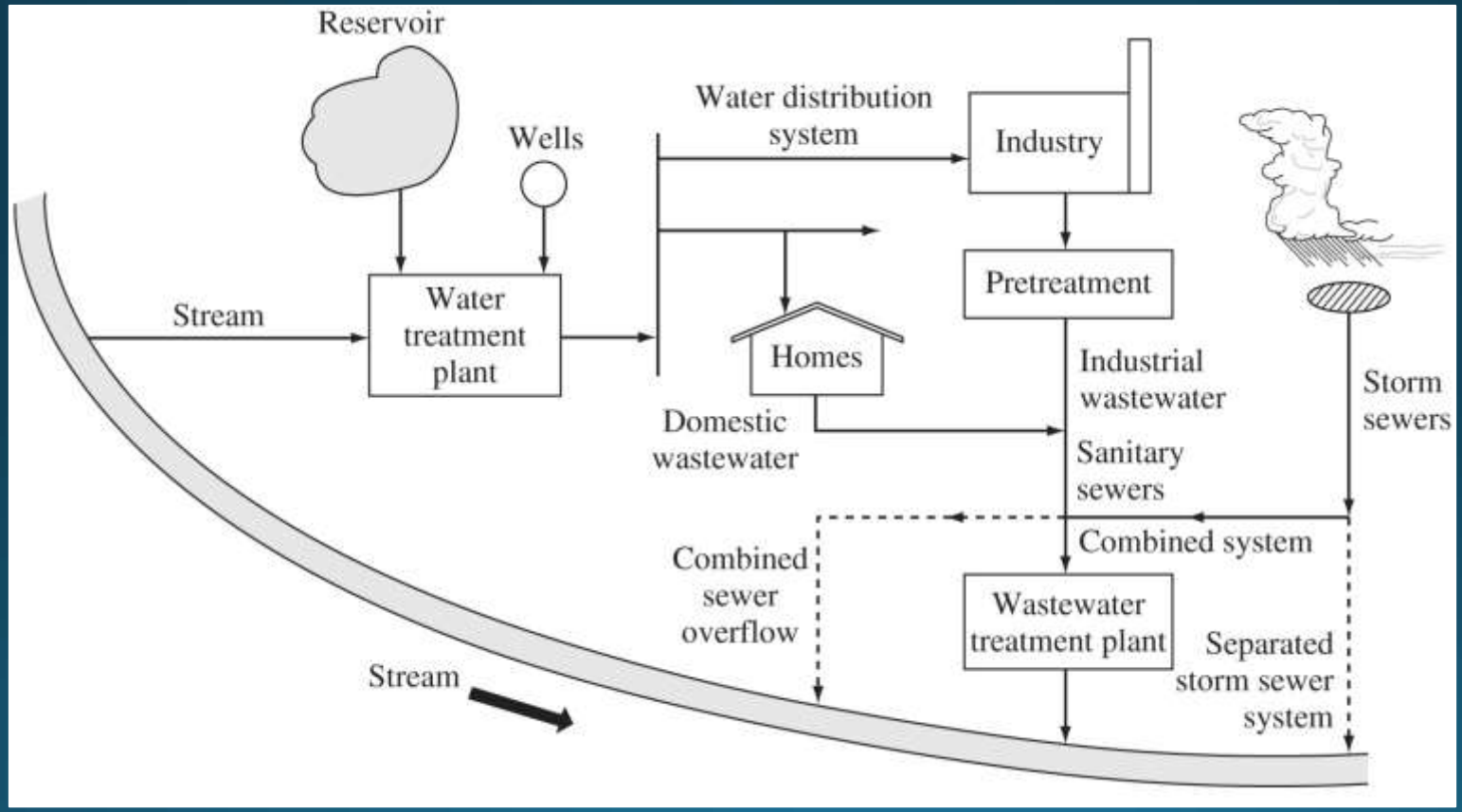
Biofilm Sampler




2 Sample Locations:

- Water Treatment Plant intake
- Stream impacted by defective sewer laterals

Water & Wastewater Treatment Systems



Sampling Results (10-13 months)

WTP (July - August)

Slides → Scraped → IMS → FISH/IFA

$\bar{x}$=14.7 d
s.d. 2.9 d
range: 10-24 d
n=28

36% positive (10/28)
0-8 oocysts
None viable

Filters → Eluted → IMS → FISH/IFA

$\bar{x}$=85.1 L
s.d. 30.3 L
range: 21-142 L
n=28

32% positive (9/28)
0-3 oocysts
None viable

Stream (October - August)

Slides → Scraped → IMS → FISH/IFA

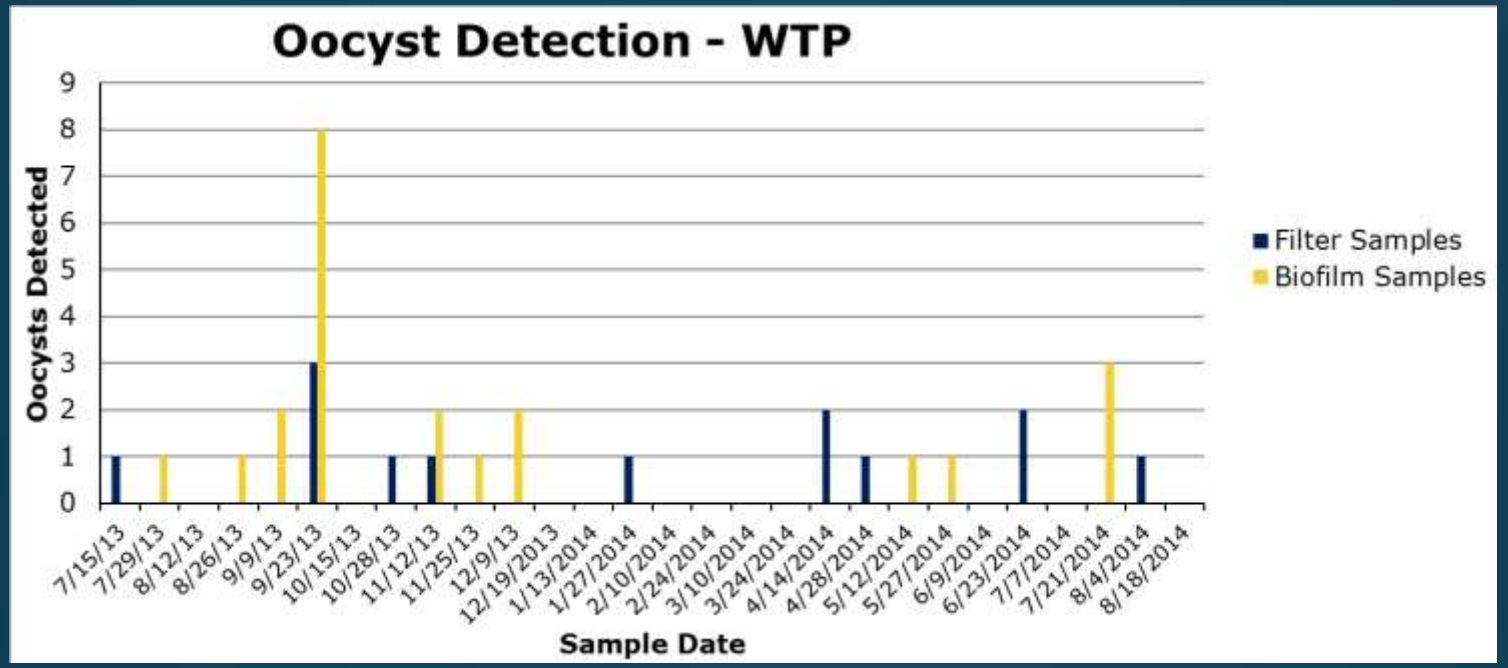
$\bar{x}$=15.1 d
s.d. 3.4 d
range: 10-24 d
n=19

53% positive (10/19)
0-3 oocysts
None viable

Filters → Eluted → IMS → FISH/IFA

$\bar{x}$=75.4 L
s.d. 32.0 L
range: 22-133 L
n=22

45% positive (10/22)
0-4 oocysts
None viable


Oocyst Detection - WTP
Oocysts Detected
0
1
2
3
4
5
6
7
8
9
7/15/13
7/29/13
8/12/13
8/26/13
9/9/13
9/23/13
10/15/13
10/28/13
11/12/13
11/25/13
12/9/13
12/19/2013
1/13/2014
1/27/2014
2/10/2014
2/24/2014
3/10/2014
3/24/2014
4/14/2014
4/28/2014
5/12/2014
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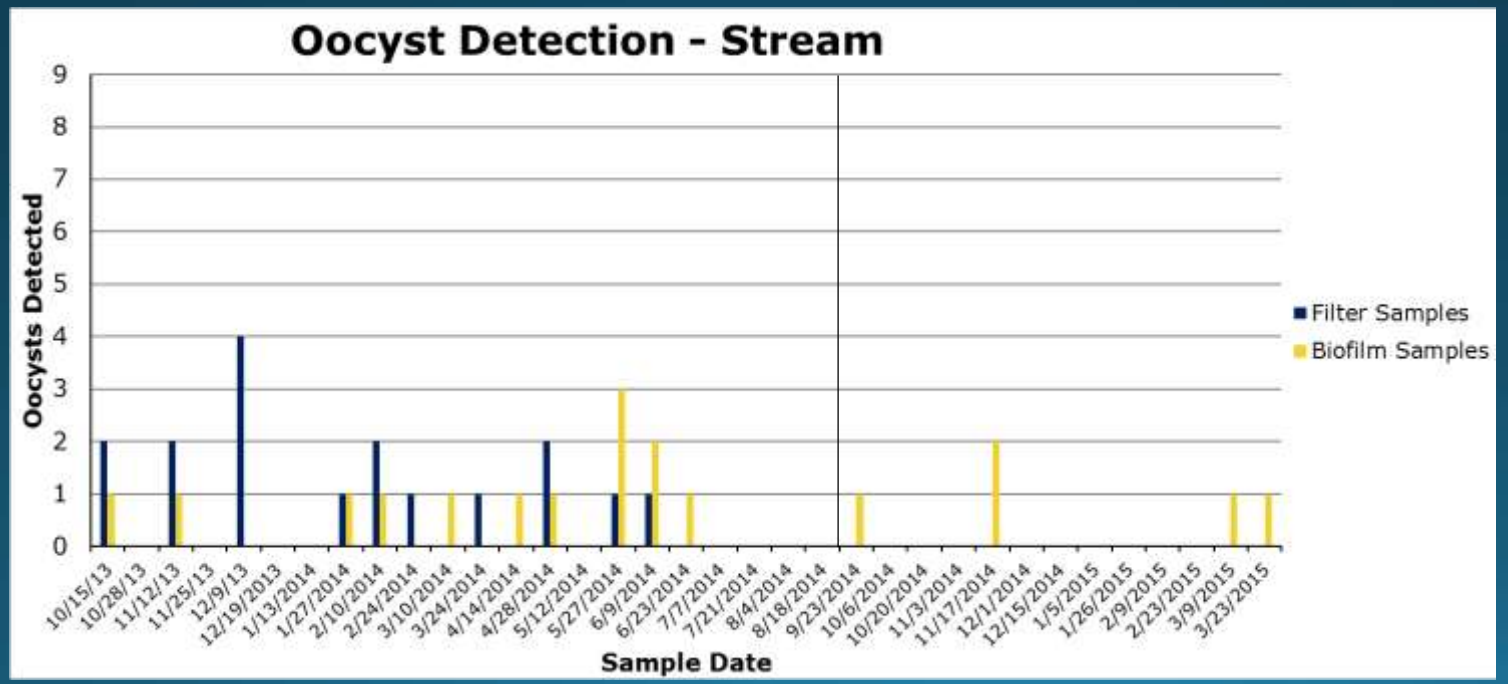
8/4/2014
8/18/2014
Sample Date
Filter Samples
Biofilm Samples


Oocyst Detection - Stream
Oocysts Detected
0
1
2
3
4
5
6
7
8
9
10/15/13
10/28/13
11/12/13
11/25/13
12/9/13
12/19/2013
1/13/2014
1/27/2014
2/10/2014
2/24/2014
3/10/2014
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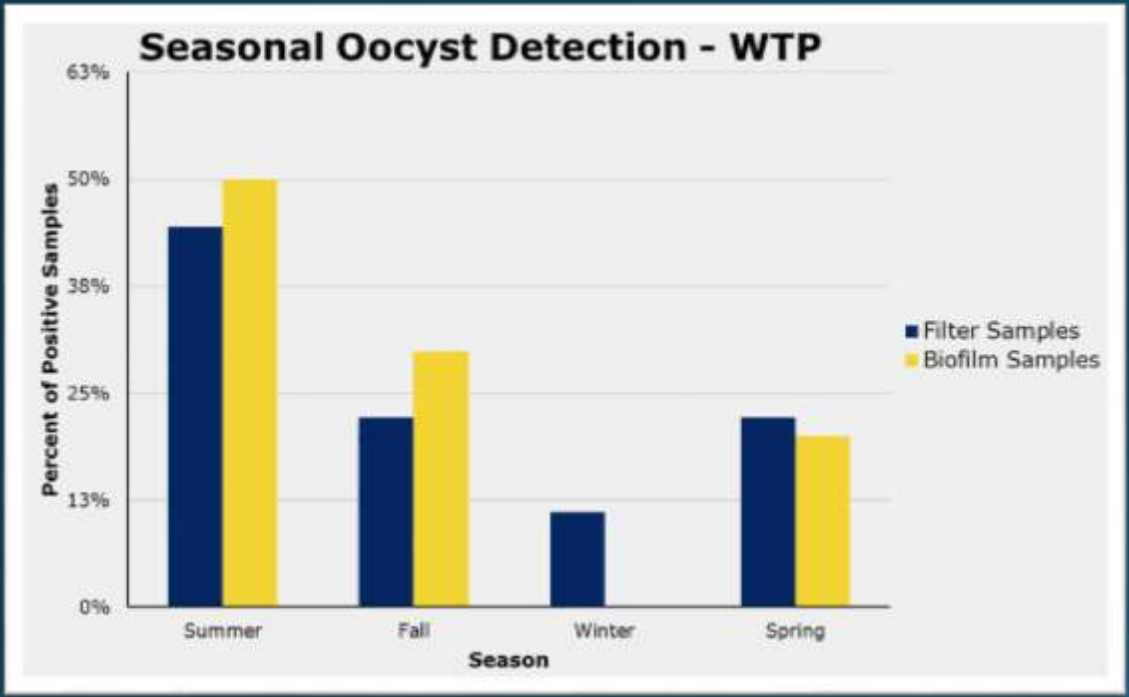
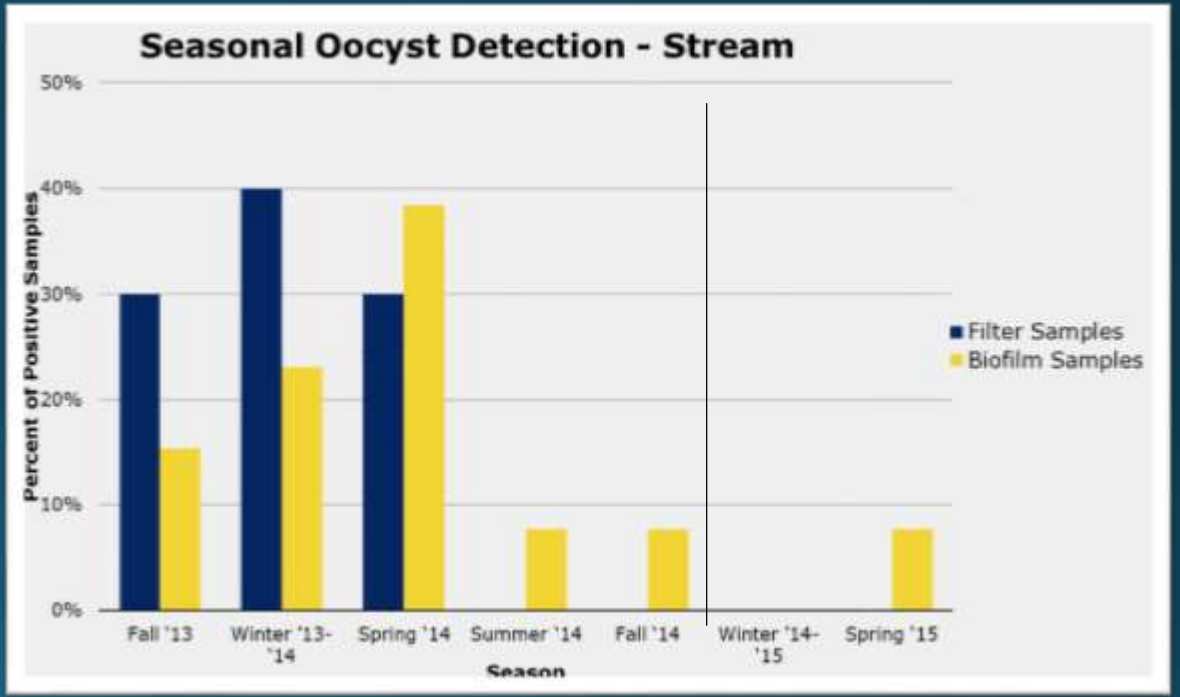
10/20/2014
11/3/2014
11/17/2014
12/1/2014
12/15/2014
1/5/2015
1/26/2015
2/9/2015
2/23/2015
3/9/2015
3/23/2015
Sample Date
Filter Samples
Biofilm Samples

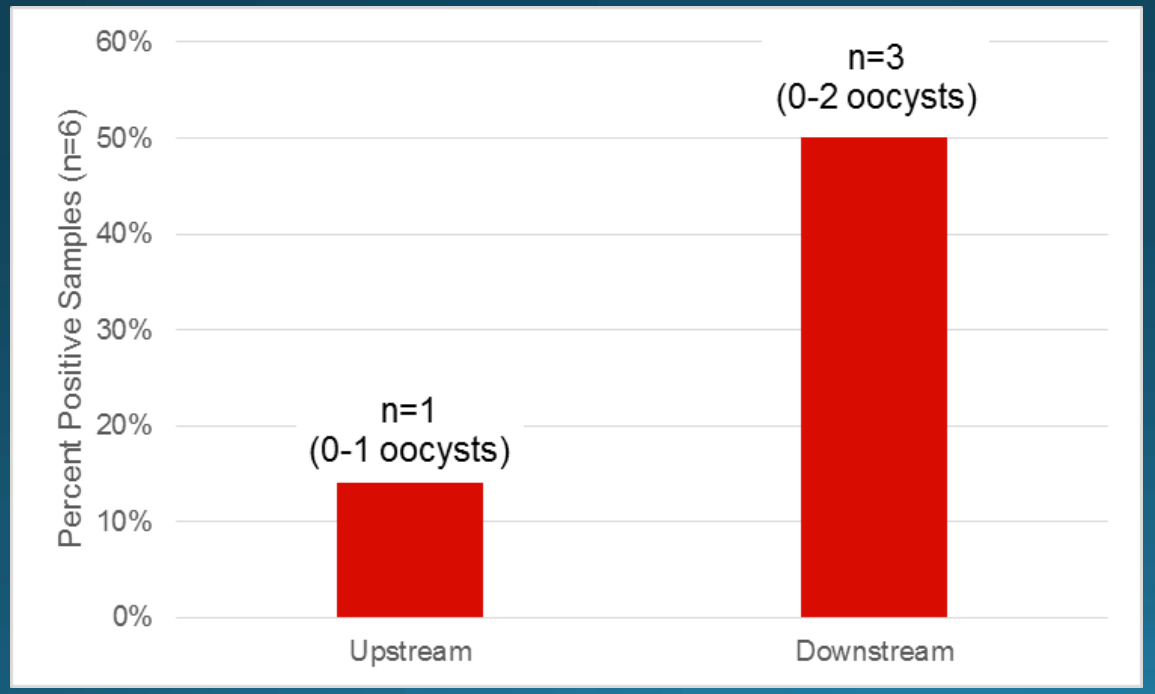
Seasonal Oocyst Detection (Filters vs Biofilms)





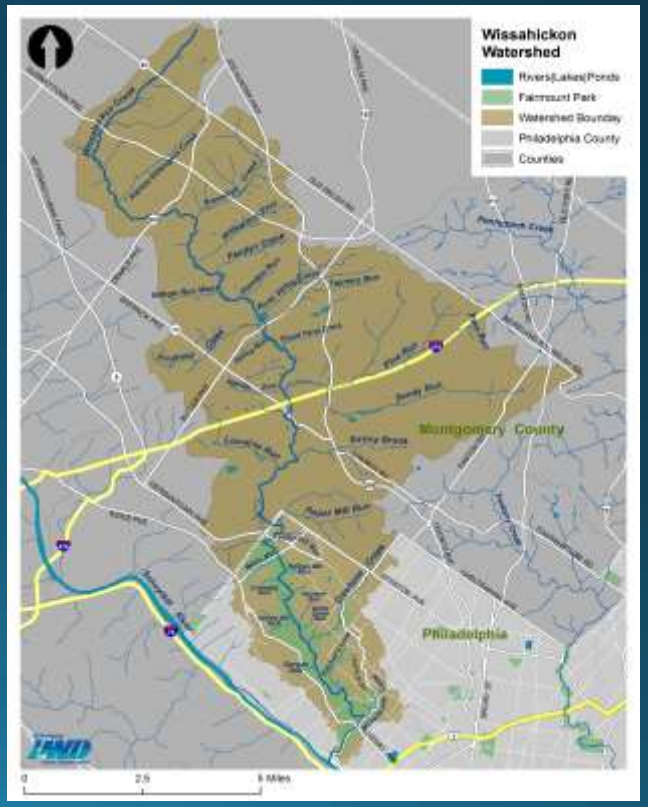
Benthic Rock Biofilms

- Biofilms sampled from benthic rocks collected upstream and downstream of a defective sewer lateral
  - 6 samples (1 per month, October - March)



Conclusions

- Benthic rock biofilm sampling may identify point sources of oocysts along the length of a waterway
  - May provide historic look at water quality conditions
- Oocyst detection in slide biofilms is comparable to oocyst detection in filtered water samples
  - Frequency, oocyst numbers



Long-term Significance

Biofilm monitoring is much less expensive than filtration

- Filters $120 each; Slides $3 per set; Rocks $0

For a utility monitoring 3 WTP intakes, 2x/month:

$$filters: \frac{2\ sample\ dates}{month} \cdot \frac{3\ locations}{sample\ date} \cdot \frac{filter}{location} \cdot \frac{\$120}{filter} \cdot \frac{12\ months}{year} = \mathbf{\$8,640\ per\ year}$$

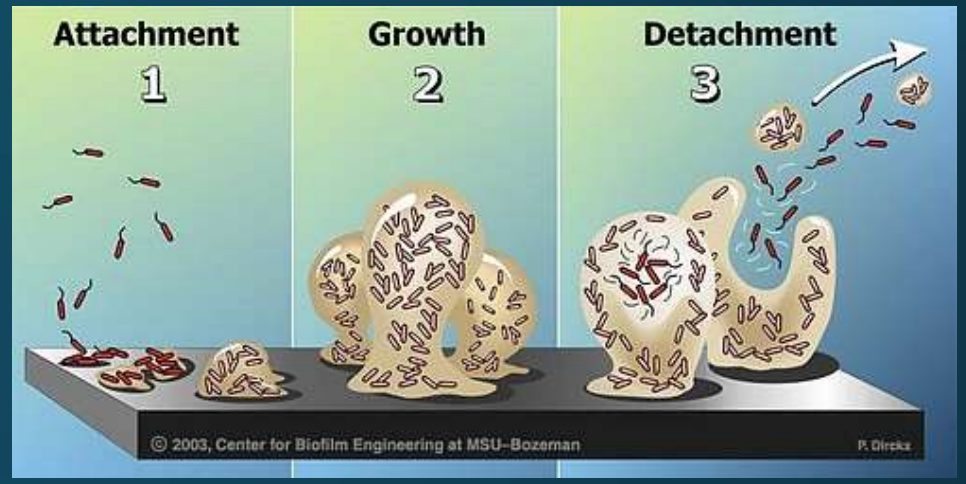
$$slides: \frac{2\ sample\ dates}{month} \cdot \frac{3\ locations}{sample\ date} \cdot \frac{slide\ set}{location} \cdot \frac{\$3}{slide\ set} \cdot \frac{12\ months}{year} = \mathbf{\$216\ per\ year}$$

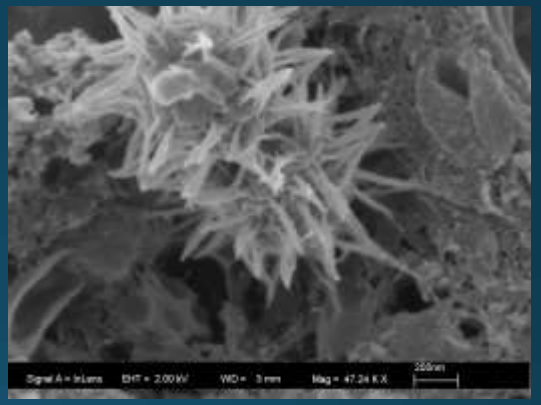
$$rocks: \mathbf{\$0\ per\ year}$$

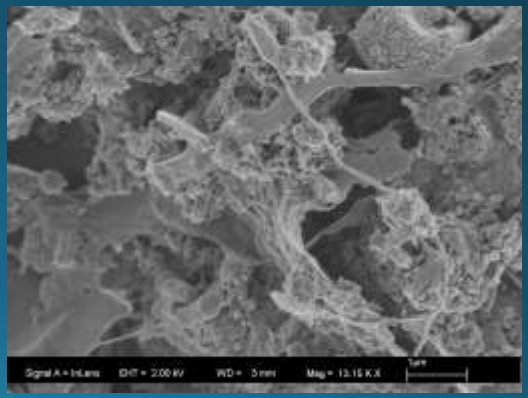
Biofilm monitoring could permit more frequent monitoring at more locations in a watershed; may be used to identify oocyst point sources

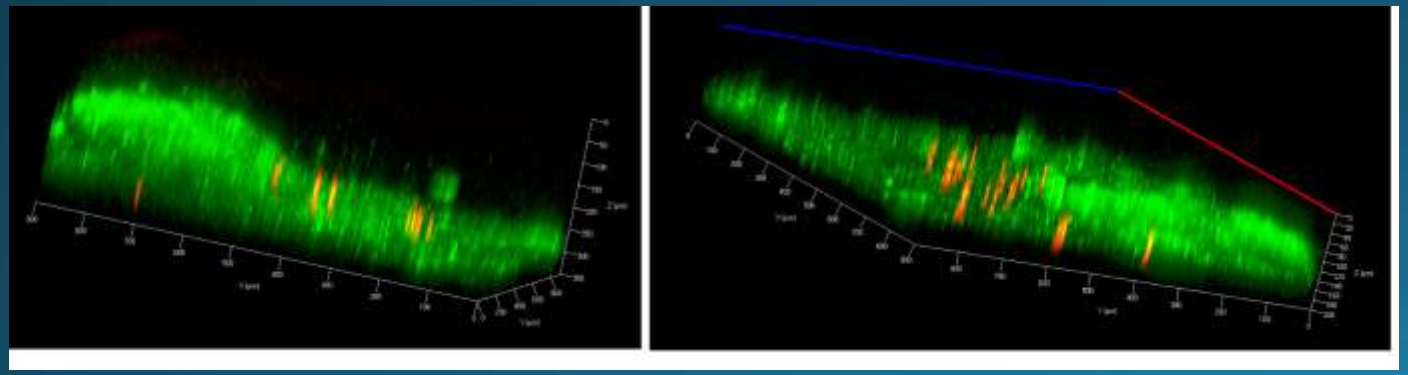
Limitations

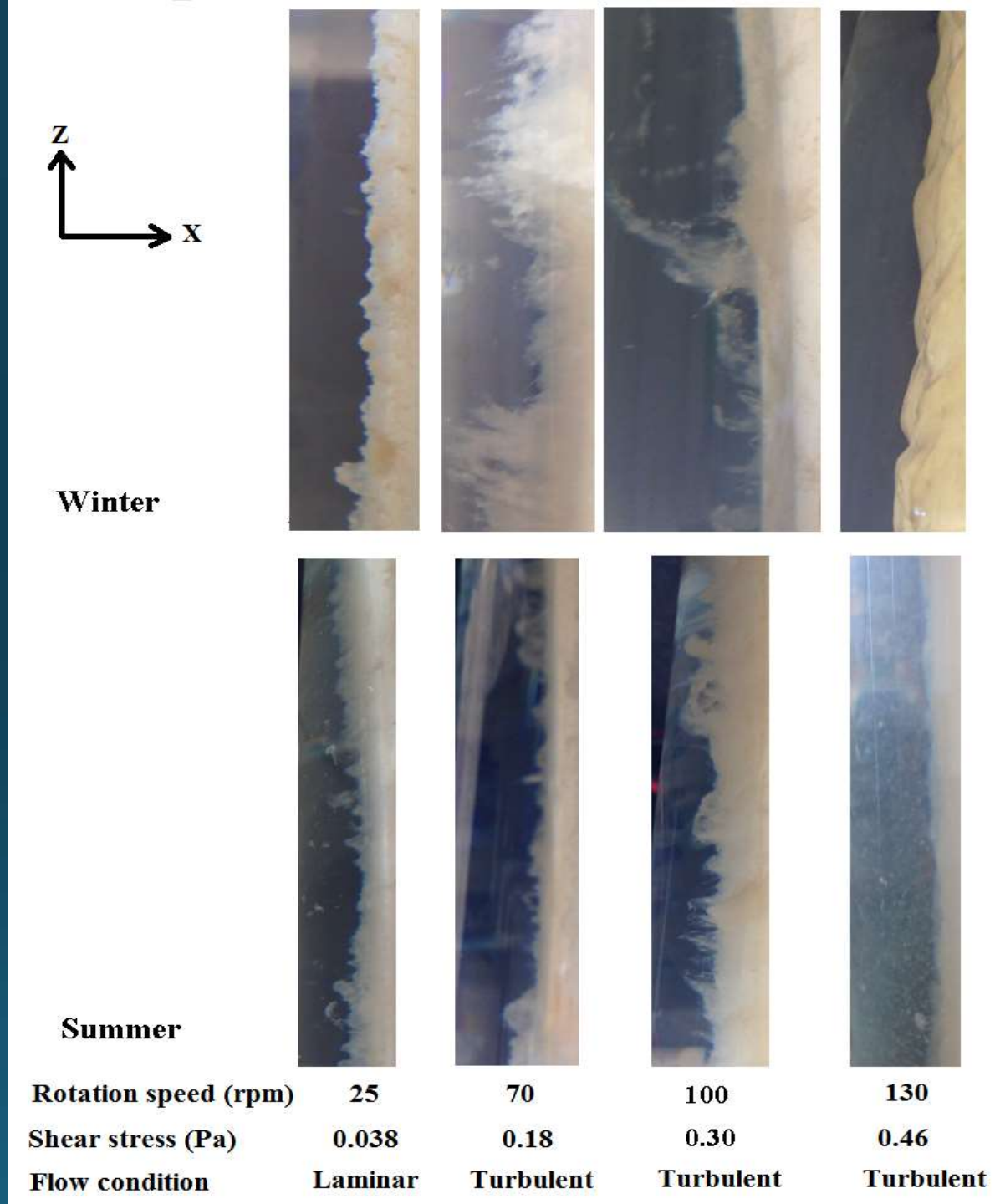
- Determination of oocyst concentration depends on **stream velocity** at biofilm surface, **time** of sampling, and oocyst **attachment efficiency**
- Oocyst attachment efficiency may not be constant
  - Variability in biofilm composition and architecture











Mature biofilm at different shear

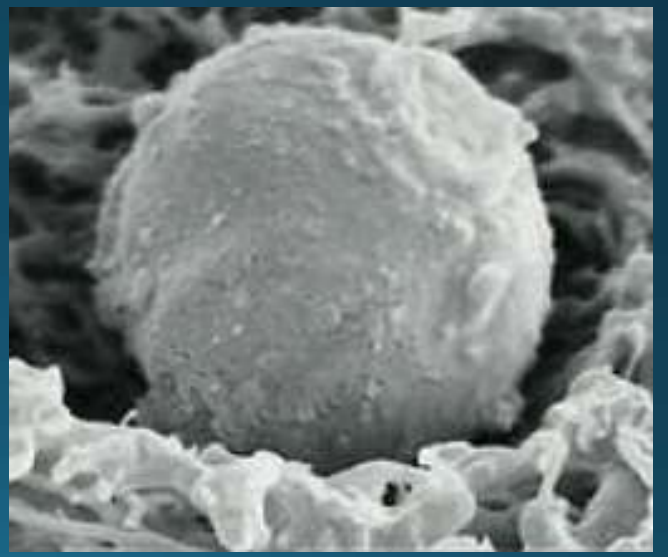
Mature biofilm by season

Ongoing Work

What are the mechanisms and associated kinetics of oocyst attachment to biofilms? Can an engineered surface be designed for improved oocyst detection?

Goal: development of a biomimetic surface for standardized oocyst detection

- Antimicrobial (prevent biofilm formation)
- Inexpensive (sell for <$5)
- Constant oocyst attachment efficiency over 3-day deployment



http://www.marvistavet.com/assets/images/single_cryptosporidium_oocyst.gif

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