Occurrence and Survival of Protozoan Parasites

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Photo Credit: H.D.A. Lindquist, U.S. EPA
Overview

• Study Objectives
• Background Information
  – Organisms of interest
  – Method
• Occurrence Study:
  – Lower Grand River Watershed
  – CSO / Retention Basin Sampling
  – River Raisin Watershed
Experimental Goals

• Occurrence of *Cryptosporidium* spp. and *Giardia* spp. in urban and rural Michigan waters receiving CSO discharges

• Survival of *Cryptosporidium* in natural Michigan waters at temperatures found in environment
Background: Enteric Protozoa

- Cryptosporidium and Giardia
- Single-celled, obligate intracellular parasites
- **Spread by the Fecal-oral route**
  - Protective exterior structure – chlorine resistance
  - Cryptosporidium = oocyst
  - Giardia = cyst
  - Collectively = (oo)cyst
Common Characteristics Affecting Epidemiology

• Shed in the feces of infected animals and humans
• Low infective dose: 1-10 (oo)cysts
• (Oo)cysts immediately infectious
• Environmental dispersal
• (Oo)cysts are stable, survive long periods in environment
• Zoonotic
Occurrence: EPA Method 1623

1. Filtration
Occurrence: EPA Method 1623

2. Elution
Occurrence: EPA Method 1623

3. Centrifugation
Occurrence: EPA Method 1623

1. ImmunoFluroescent Assay (IFA): Separation
Occurrence: EPA Method 1623

1. IFA: Separation
Occurrence: EPA Method 1623

1. IFA: **Separation** – wash step
Occurrence: EPA Method 1623

2. IFA: Dissociation of bead-(oo)cyst complex

3. IFA: Application to slides and staining
Occurrence: EPA Method 1623

4. Enumeration
via fluorescence microscopy & (DIC) microscopy

Stains:
FITC – green
DAPI - blue

Photo Credit: H.D.A. Lindquist, U.S. EPA
Lower Grand River Watershed Sampling Sites

- **Study Period:** April 2005 to August 2006
- **Survey of 17 sites** – Recreational areas
  - 3 chosen for systematic surveillance, all downstream of Grand Rapids
- **Deer Creek Park** – 22 samples
  - Furthest site upstream
- **Riverside Park** - 19 samples
- **North Beach Park** – 19 samples
  - Lake site, North of Grand River mouth
## Market Avenue Retention Basin (MARB)

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Retention Basin</th>
<th>Year Constructed</th>
<th>Basin Capacity (MG)</th>
<th>Covered/Uncovered</th>
<th>Type of Facility</th>
<th>Construction Cost</th>
<th>O &amp; M Cost</th>
<th>Design Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Rapids, MI</td>
<td>Market Avenue RB</td>
<td>June 1992</td>
<td>30.5</td>
<td>offline</td>
<td>$30 million</td>
<td>$40,000</td>
<td>10 yr-1 hr storm</td>
<td></td>
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<tr>
<td></td>
<td>First Compartment</td>
<td></td>
<td>10.68</td>
<td>covered</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Second Compartment</td>
<td></td>
<td>16.68</td>
<td>uncovered</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Third Compartment</td>
<td></td>
<td>3.14</td>
<td>uncovered</td>
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River Raisin Watershed:

- Adrian Water Treatment Plant
- Milk Drain Tile at Forrister Rd
- Wolf Creek at Forrister Rd
- St. Joseph Creek at Beecher Rd
- Main Branch River Raisin at Deerfield Rd
- Stony Creek at Gorman Rd
- Rice Lake Drain at Haley Rd
- Stony Creek at Seneca Rd
- Black Creek at Medina Rd
- Black Creek at Morse Rd
- Deerfield Water Treatment Plant
- Main Branch River Raisin at Crockett Rd
- Blissfield Water Treatment Plant
- Black Creek at Crockett Rd


- 3 surface water treatment plant intakes
- 4 tributary creeks
- 1 field drainage structure

Bean/Tiffin Watershed: 1 tributary creek
Parasite Occurrence

![Graph showing parasite occurrence across different locations. The y-axis represents (Oo)cysts L\(^{-1}\) and the x-axis represents various locations such as North Beach Park, Deer Creek park, Riverside Park, River sites, MARB, River Raisin Total. The graph compares Cryptosporidium (black bars) and Giardia (green bars).]

- Cryptosporidium
- Giardia
### Grand River Seasonal Parasite Occurrence

**Cryptosporidium Occurrence vs Season**

- **Winter**: 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 (Oocysts L$^{-1}$)
- **Spring**: 0.6, 0.5, 0.4, 0.3, 0.2, 0.1
- **Summer**: 0.5, 0.4, 0.3, 0.2, 0.1
- **Autumn**: 0.4, 0.3, 0.2, 0.1

**Giardia Occurrence vs Season**

- **Winter**: 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 (Oocysts L$^{-1}$)
- **Spring**: 0.6, 0.5, 0.4, 0.3, 0.2, 0.1
- **Summer**: 0.5, 0.4, 0.3, 0.2, 0.1
- **Autumn**: 0.4, 0.3, 0.2, 0.1

**Number observations:**

<table>
<thead>
<tr>
<th></th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
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</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>8</td>
<td>18</td>
<td>27</td>
<td>7</td>
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<tr>
<td><strong>River</strong></td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td><strong>Beach</strong></td>
<td>2</td>
<td>6</td>
<td>9</td>
<td>2</td>
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</tbody>
</table>
Methods: Survival

- Microcosms of waters from Deer Creek Park
- Microcosms seeded with *Cryptosporidium* (Sterling Parasitology Laboratory, Az)
  - Final concentration ~$10^6$ oocyst/ml
- 2 microcosms at 25°C, 2 at 4°C, aliquots in microcentrifuge tubes at -8°C
- Positive control: *Cryptosporidium* seeded into sterile distilled water held at 4°C. Final concentration ~$2 \times 10^7$ oocyst/ml
Methods: Survival

- Bleach treatment of aliquot from microcosms
- Dilution series used to infect HCT-8 (human endothelial adenocarcinoma) cell culture in 8-chambered well slides
- Staining of slides
- Most Probable Number (MPN) Analysis
## Survival Results

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Sample Seeded with <em>Cryptosporidium</em></th>
<th>Infectivity Assessment (Days)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>-8</td>
<td>Deer Creek Sample</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Deer Creek Sample</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>Deer Creek Sample</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Positive Control</td>
<td>+</td>
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</table>
Grand River Watershed Observations

• Occurrence
  – Cryptosporidium & Giardia detected most often in Riverside Park.
  – Cryptosporidium detected in >60% of Grand River and MARB samples - usually at low levels
  – Compared to river:
    • MARB Cryptosporidium concentrations higher by 1 order of magnitude
    • MARB Giardia concentrations higher by 3 orders of magnitude
  – Cryptosporidium concentrations: Spring - variable, summer consistently 0.1 – 0.2 L⁻¹

• Survival
  – Infectivity of samples at -8°C fell below detectable levels after 14 days
  – Infectivity of samples at 25°C fell below detectable levels after 35 days
  – Samples at 4°C still infectious by 71 days.
Conclusions

• Low Health Risks at Beach site:
  • 26.3% parasite occurrence at levels between 0.1-0.2 L\(^{-1}\)
• The Grand River receives parasites from normal sewage discharges and on occasion receives high levels from CSOs.
• February – August, 2008:
  – The MARB prevented \(5.19 \times 10^9\) Cryptosporidium oocysts and \(6.92 \times 10^{11}\) Giardia cysts from entering the river
Acknowledgements

• Joan B. Rose
• Rose Lab Personnel
• City of Grand Rapids
• NOAA
• MDEQ
Questions?
## Occurrence Results

<table>
<thead>
<tr>
<th>Site</th>
<th>N (sites or events)</th>
<th>% Cryptosporidium (+)</th>
<th>% Giardia (+)</th>
<th>% Cryptosporidium &amp; Giardia (+)</th>
<th>% Either Cryptosporidium or Giardia (+)</th>
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</thead>
<tbody>
<tr>
<td>Deer Creek Park</td>
<td>22</td>
<td>63.60</td>
<td>40.90</td>
<td>18.20</td>
<td>81.80</td>
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<tr>
<td>Riverside Park</td>
<td>19</td>
<td>73.68</td>
<td>89.47</td>
<td>73.68</td>
<td>94.74</td>
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<tr>
<td>North Beach Park</td>
<td>19</td>
<td>26.32</td>
<td>26.32</td>
<td>10.53</td>
<td>42.11</td>
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<tr>
<td>Other Grand River sites</td>
<td>23</td>
<td>26.00</td>
<td>8.70</td>
<td>8.70</td>
<td>26.00</td>
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<tr>
<td>Total Grand River sites</td>
<td>83</td>
<td>47.00</td>
<td>40.00</td>
<td>26.50</td>
<td>60.20</td>
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<td>River Raisin Watershed (Non-CSO)</td>
<td>28</td>
<td>46.4</td>
<td>32.1</td>
<td>25</td>
<td>53.6</td>
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<td>River Raisin Watershed (CSO)</td>
<td>14</td>
<td>50</td>
<td>14.3</td>
<td>7.1</td>
<td>57.1</td>
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<td>Total River Raisin Watershed sites</td>
<td>45</td>
<td>46.67</td>
<td>22.22</td>
<td>15.56</td>
<td>53.33</td>
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<tr>
<td>MARB</td>
<td>9</td>
<td>66.7</td>
<td>100</td>
<td>66.7</td>
<td>100</td>
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## Occurrence Results

<table>
<thead>
<tr>
<th>Site</th>
<th><strong>Cryptosporidium range</strong></th>
<th><strong>Giardia range</strong></th>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>min</td>
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<tr>
<td>North Beach Park</td>
<td>0</td>
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<tr>
<td>Deer Creek Park</td>
<td>0.165</td>
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<td>Riverside Park</td>
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<td>0</td>
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<tr>
<td>River Raisin Watershed - Non CSO</td>
<td>0.0785</td>
<td>0</td>
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<tr>
<td>River Raisin Watershed - CSO receiving sites</td>
<td>0</td>
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<td>MARB</td>
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