Cryptosporidium Risk

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Cryptosporidiosis Outbreak

- Östersund (Sweden) (2010-2011)
- 27,000 affected
- Boil water notice lasting 84 days
- Sewage Cross connection
- Cost of $31 million
Cryptosporidium is still problematic for the water industry!

- Ubiquitous in source and waste-waters
- Resistance to chlorine disinfection
- Absence of more easily measured surrogates
- Ever-present risk of Cryptosporidium oocysts often entails the implementation of multiple barrier approaches to ensure risk is minimised
Significant capital expenditure for water utilities

- Removal of Cryptosporidium in treatment processes can be highly variable.

- Under highly adverse conditions oocyst challenges may be large enough to challenge the ability of existing barriers to satisfy water quality guidelines.

- The reduction of this risk may result in significant capital expenditure for water utilities.

- But what is Cryptosporidium RISK?
To understand *Cryptosporidium* risk, what do we need to know?

- Oocyst Density
- Species (genotype) (*C. hominis*, *C. parvum* or others)
- Infectivity
Development of a *Cryptosporidium* Toolbox

**Total Count**
Presumptive & Confirmed IMS/Antibody

**Species/Genotype**
FISH probes, PCR markers, Sequencing and Phylogenetic Analysis

**Infectivity**
Cell Culture/Rapid RT-Real time PCR

**Single Format Assay**
What we can now offer in this spot?

- A new Single Format Assay that enables from an individual grab sample
  - Oocyst density (number) and recovery rate = oocysts/10L
  - Infectivity = % of oocysts which are infective (infectious fraction)
  - Species (genotype) (C. hominis, C. parvum or others)

DNA extraction

DNA amplification (PCR)

DNA sequence analysis
Cryptosporidium Investigations

• Smart Water/WaterRA sponsored Wastewater Project
  – 3 Victorian WWTPs
  – 2 SA WWTPs

• Sydney Catchment Authority Consultancy
  – >12 STPs as well as upstream & downstream of discharges
  – Event

• SA Water Catchment Work
  – Kersbrook
  – Clarendon

• WaterRA Storm Water Re-use Project
  – Barker Inlet
  – Adelaide Airport
Inactivation of *Cryptosporidium* across the wastewater treatment train - Phase 2

• Quantify not only the removal of oocysts from various stages of the wastewater treatment train but also their inactivation

• Thereby accurately quantifying the “true effect” of the treatment train on oocyst risk reduction
Inactivation of Cryptosporidium across the wastewater treatment train - Phase 2
Infectivity of *Cryptosporidium*

<table>
<thead>
<tr>
<th>WWTP</th>
<th>33% ± 9</th>
<th>40% ± 11</th>
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</thead>
<tbody>
<tr>
<td>Western Treatment Plant</td>
<td>33% ± 7</td>
<td>28% ± 20</td>
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<tr>
<td>Altona WWTP</td>
<td></td>
<td></td>
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<tr>
<td>Mt Martha WWTP</td>
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<td>Aldinga</td>
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<td>Glenelg</td>
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</table>
Substantial inactivation can occur at a WWTP!
Comparison of the LRVs for **Total oocysts**, **Infectious oocysts only**, and **Differential Risk** across the Western Treatment Plant WWTP 55E Lagoon System between the raw sewage sampling point through to Lagoon 2.

<table>
<thead>
<tr>
<th>Sampling Round</th>
<th>Round 1 15th Jan</th>
<th>Round 2♀ 5th March</th>
<th>Round 3 7th May</th>
<th>Round 4 2nd July</th>
<th>Round 5 27th Aug</th>
<th>Round 6 22nd Oct</th>
<th>Round 7 26th Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRV (log10) Total oocysts</td>
<td>1.38</td>
<td>0.81</td>
<td>0.31</td>
<td>0.31</td>
<td>-0.14</td>
<td>-0.09</td>
<td>0.48</td>
</tr>
</tbody>
</table>

♀ Indicates a spike in oocyst density during the round and a significant increase in the oocyst challenge on the WWTP.

n.a. data not available

LRV (Log10) for Total oocysts was calculated on the total number of oocysts/10L in the raw sewage and the total number of oocysts/10L at the final point of analysis.
Barriers are not 100%

- A Victorian WWTP Plant with UV disinfection after batch reactor system
- 2 large challenges during cryptosporidiosis outbreak
Comparison of the LRVs for **Total oocysts**, **Infectious oocysts only**, and **Differential Risk** across a treatment train between the raw sewage sampling through to post UV.

<table>
<thead>
<tr>
<th>Sampling Round</th>
<th>Round 1 22(^{\text{nd}}) Jan</th>
<th>Round 2 19(^{\text{th}}) March</th>
<th>Round 3 21(^{\text{st}}) May</th>
<th>Round 4 18(^{\text{th}}) July</th>
<th>Round 5 24(^{\text{th}}) Sept</th>
<th>Round 6 19(^{\text{th}}) Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRV (log(_{10})) Total oocysts</td>
<td>1.33</td>
<td>1.12</td>
<td>0.98</td>
<td>0.86</td>
<td>0.49</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*Indicates a spike in oocyst density during the round and a significant increase in the oocyst challenge on the WWTP.

**** Not calculated as absence of infective oocysts in the raw sewage

LRV (Log\(_{10}\)) for Total oocysts was calculated on the total number of oocysts/10L in the raw sewage and the total number of oocysts/10L at the final point of analysis.

> LRV (Log\(_{10}\)) calculated on the conservative assumption if 1 infectious oocyst had been detected in each replicate sample.
Seasonality and Oocyst Removal

LRV v Temperature for 55EP6

R² = 0.878

Temperature (°C)

LRV
Take-home Messages & Questions from “Phase 2”

• Captured data from a large and widespread cryptosporidiosis outbreak within Victoria

• Removals at WWTPs are seasonal

• Is the oocyst challenge seasonal for WWTPs?

• Not all oocysts in raw sewage are infective

• Oocyst infectivity in the raw sewage is not seasonal

• Importance of lagooning for both removal and inactivation was established

• Why is the infectious fraction of oocysts out of the clarifier effluent for a number of WWTPs increased compared to the raw sewage?

• By including infectivity into LRV calculations the risk may be significantly reduced at some WWTPs, compared to considering only total oocyst numbers
Sydney Catchment Authority Consultancy  (Lisa Hamilton, SCA)

- Analysed samples from 12 STPs over 9 rounds (raw, pre-UV and post UV)
  - Oocyst Loads
  - Raw infectivity & Pre-UV infectivity ranged from 30% to as high 70%
  - Post UV infectivity = No infectious oocysts detected

- Analysed a number of samples upstream & downstream of STPs
  - Total oocyst numbers = 146
  - Infectious oocysts found = 12
  - All infectious oocysts identified *C. hominis* Ib
Sydney Catchment Event/Incident 2013 (Andrew Ball, SCA)

- Large rain fall event & Storm water infiltration into sewers
- Sewer bypasses occurred
- At same time a slug of dirty water high in oocysts numbers (100 oocysts/10L) headed towards off-take in Warragamba
- Oocysts in bypass highly infective
- Oocysts in slug not infective (wildlife source from runoff in catchment, not from the bypass event)
C8078 – Kersbrook Creek Pathogen Reduction Project

- Can watercourse fencing reduce *Cryptosporidium* export?
Kersbrook Pathogen Reduction Project (capital project C8078)
(Brooke Swaffer, Environment Services at SA Water)

**WaterShed 2**

- **3 infectious foci**
- *C. ryanae*
- *C. bovis*
First Flush Diversion (Clarendon) and the Risk posed by *Cryptosporidium*

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Samples</th>
<th>Oocysts Inoculated</th>
<th>Infectious Foci</th>
<th>Cryptosporidium Species/Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single Format Assay</strong></td>
<td>71</td>
<td>383</td>
<td>12</td>
<td><em>C. cuniculus</em> subtype Vb (5)</td>
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<td><em>C. fayeri</em> (3)</td>
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<td><em>C. meleagridis</em> (2)</td>
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<td></td>
<td><em>C. sp. mouse genotype II</em> (1)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1 unable to be identified</td>
</tr>
<tr>
<td><strong>Direct Genotyping Assay</strong></td>
<td>41</td>
<td></td>
<td></td>
<td><em>C. cuniculus</em> subtype Vb (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. fayeri</em> (2)</td>
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<td></td>
<td></td>
<td><em>C. sp. mouse genotype II</em> (1)</td>
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<td></td>
<td></td>
<td><em>C. sp. (novel)</em> (2)</td>
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<td></td>
<td></td>
<td><em>C. canis</em> (1)</td>
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<td><em>C. muris</em> (20)</td>
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<td></td>
<td><em>C. bovis</em> (7)</td>
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<td></td>
<td></td>
<td><em>C. ryanae</em> (6)</td>
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<td></td>
<td></td>
<td><em>C. tyzzeri</em> (3)</td>
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<td></td>
<td></td>
<td></td>
<td><em>C. sp. (rat/sheep)</em> (3)</td>
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<td></td>
<td></td>
<td><em>C. parvum</em> (3)</td>
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</tbody>
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Water RA Storm Water Re-use Project

• Inlets of two storm water harvesting sites
  – Barker Inlet (predominantly residential & industrial catchment)
  – Adelaide Airport (inputs from rural and non-urbanised reserve)

• Oocyst Densities
  – Barker Inlet (N.D. – 263 oocysts/10L)
  – Adelaide Airport (14-184 oocysts/10L)

• Infectious fraction (majority of rounds had no detects)
  – Barker Inlet (2.2%) (C. parvum)
  – Adelaide Airport (1.4%) (C. parvum)
Conclusions and Questions Raised

• Assays that enable comprehensive risk assessment
  — undertaking this on both source and wastewaters

• Does it make us re-think *Cryptosporidium* Risk, where does the greatest risk lie?

• Are there WWTPs streams that can contribute to source water?

• Does this information have the potential to impact treatment?

• Are new assets or interventions to address *Cryptosporidium* risk?

• How do you sit in relation to Health Based Targets?