Cryptosporidiosis: Human, animal and environmental interface in the Liffey and Lough Gill catchments

Theo de Waal
Outline

- Introduction & Background
- Cryptosporidium in humans
- Cryptosporidium in animals
- Cryptosporidium in surface water
- Conclusion
Cryptosporidium life cycle

- Direct life cycle
- Sporulated oocyst → environment
- Transmission: faecal-oral route
  - Waterborne
  - Foodborne
- Infect microvillus border of GIT – vertebrates
  - 3 spp: Gastric mucosa
  - 1 sp: Respiratory system
Very resistant!
- Oocysts can remain viable in environment & animal liquid waste ~ 1 year
- Resistant to environmental stressors
- Resistant to most chemical disinfectants

http://www.bio-uv.com/fr/site/Piscines-spas-collectifs/Prevention-Cryptosporidium/Prevention-contre-les-pathogenes-parasitaires_129_.html
Cryptosporidium spp: Human

- 17 known Cryptosporidium species
  - 39 Cryptosporidium genotypes
- 9 Cryptosporidium species reported from human cases in England & Wales:
  - C. hominis (50.29%)
  - C. parvum (45.6%)
  - C. meleagridis (0.8%)
- Ireland:
  - C. hominis (20%)
  - C. parvum (80%)
  - C. meleagridis
  - C. deer genotype

Cryptosporidium in Ireland: Human

<table>
<thead>
<tr>
<th></th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008 up to Sept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidiosis¹</td>
<td>431</td>
<td>568</td>
<td>367</td>
<td>609</td>
<td>360</td>
</tr>
</tbody>
</table>

Crude incidence rate:
- 8.7 – 13.4/100,000 annually
- Rural areas reported more cases
- Regional as high as 31.4/100,000 per year

¹Human cryptosporidiosis became a notifiable disease on January 1st 2004
Ireland: Seasonal distribution in humans

Figure 4. Seasonal distribution of cryptosporidiosis notifications 2005 to end quarter 3 2008
Cryptosporidium in Ireland: Animals

- Major cause of enteritis in neonatal animals
- Ireland - very little known
  - Calves
    - 2006: 25.9%\(^1\)
  - Pigs
    - 2005: 15%\(^2\)
  - Sheep/goats
    - ?

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Cryptosporidium in Ireland: Animals

- Major cause of enteritis in neonatal animals
- Ireland - very little known
  - Horses
    - 1991: 29% of diarrhoeic foals
  - Commercial deer herd
    - 2001: Common & asymptomatic

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### Table 2
Mean oocyst counts for fecal samples taken from adult hinds from May 1996 to May 1997

<table>
<thead>
<tr>
<th>Month</th>
<th>n</th>
<th>No. of positive samples</th>
<th>Mean ± S.D. (opg)</th>
<th>Range (opg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>30</td>
<td>0</td>
<td>0.0 ± 0.0</td>
<td>0</td>
</tr>
<tr>
<td>June</td>
<td>30</td>
<td>19</td>
<td>321.4 ± 1030.3</td>
<td>0-4704</td>
</tr>
<tr>
<td>July</td>
<td>30</td>
<td>16</td>
<td>3.8 ± 3.3</td>
<td>0-12</td>
</tr>
<tr>
<td>August</td>
<td>30</td>
<td>14</td>
<td>2.8 ± 3.5</td>
<td>0-12</td>
</tr>
<tr>
<td>October</td>
<td>30</td>
<td>8</td>
<td>3.1 ± 4.0</td>
<td>0-15</td>
</tr>
<tr>
<td>November</td>
<td>30</td>
<td>17</td>
<td>3.8 ± 4.4</td>
<td>0-15</td>
</tr>
<tr>
<td>January</td>
<td>30</td>
<td>6</td>
<td>6.1 ± 27.9</td>
<td>0-148</td>
</tr>
<tr>
<td>February</td>
<td>20</td>
<td>9</td>
<td>3.9 ± 10.1</td>
<td>0-44</td>
</tr>
<tr>
<td>April</td>
<td>30</td>
<td>12</td>
<td>3.7 ± 3.0</td>
<td>0-12</td>
</tr>
<tr>
<td>May</td>
<td>30</td>
<td>13</td>
<td>3796.8 ± 13503.4</td>
<td>0-67590</td>
</tr>
</tbody>
</table>

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Cryptosporidium in Ireland: Environment

- Contamination of catchments used for drinking water abstraction has resulted in outbreaks of cryptosporidiosis worldwide
- Several Irish studies have detected *Cryptosporidium* species in Irish river basins\(^1,\)\(^2\)
- 2005 EPA risk assessment - Irish public water supply
  - 8% high risk
  - 13% very high risk
- Recent outbreaks in Ireland
  - Galway – 2007
    - \~240 confirmed cases

Research need

- Research should be undertaken to: Elucidate the prevalence, epidemiology and mode of transmission of Cryptosporidium in the Irish context*

*Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee (2004)*
Project objectives

- to identify the chief source(s) of *Cryptosporidium* oocysts in the environment during the spring peak
- to compile a database of *Cryptosporidium* species and subtypes that occur in livestock, wildlife, and the environment in 2 model water reservoir systems in the east and the west of the country.
- to identify species and subspecies that occur in the human population
Study area 1: Hydrometric Area 09

- Eastern River Basin District
- HA09 (The “Liffey Catchment Area”) - most densely populated hydrometric areas in Ireland
Study area 1: Hydrometric Area 09

- **Land use**
  - *Urban = 21%*
  - *Agricultural land = 61%*
    - Pastures = 46%;
    - Arable land and crop cultivation = 12%;
    - Managed forests = 3%
  - cattle in the middle catchment
  - sheep/forestry in the upper catchment
Study area 1: Hydrometric Area 09

- The Liffey Catchment area (HA09)
  - 676 km²
  - 503.7 km of river channels

- 6 significant abstractions from surface waters
  - Poulaphouca Reservoir
    - 252,000 m³/day
  - Leixlip Reservoir
    - 148,000 m³/day
Study area 2: Lough Gill

- Western River Basin District
- HA35 (The Lough Gill catchment)

- Relatively sparsely populated
- < 0.5 mill people
- Urban infrastructure
  - ~ 0.03% of the basin area
Study area 2: Lough Gill

- Lough Gill 2 km east of Sligo town
- 10th largest lake in RI
  - 8 km long x 3.5 km wide
    - steep limestone shores and underwater cliffs
  - over 20m deep in places
  - surface area of 14km²
- Catchment area 400km²
  - Cattle & sheep farming & deer population
- Main water supply for Sligo town
  - Two water treatment plants - Cairn’s Hill and Foxes Den
- Water supply for North Co. Leitrim
Project Team

- **University College Dublin**
  - Dr Theo de Waal
  - Dr Annetta Zintl, Ms Carolyn Read, Prof Grace Mulcahy
  - PhD student: Ms Marzieh Mirhashemi

- **Institute of Technology, Sligo**
  - Dr Frances Lucy
  - Technician: Mr Declan Feeney

- **USA: Johns Hopkins Bloomberg School of Public Health**
  - Prof Thaddeus Graczyk
  - Technician: Ms Leena Tamang

- Teagasc: Animal Production Research Centre
  - Dr Barbara Good

- **UK: Cryptosporidium Reference Unit**
  - Dr Rachel Chalmers

- **Fingal County Council**
  - Mr George Sharpson
Analysis of human Cryptosporidium isolates

- *Cryptosporidium* species and subspecies present in human cryptosporidiosis cases
  - 186 *Cryptosporidium*-positive human stool samples collected from Irish patients between 2000 and 2007
  - 95 stool samples submitted to the UK *Cryptosporidium* reference lab in 2008
Typing to species …

- Oocyst concentration
- DNA extraction
- PCR-RFLP analysis of the SSU rRNA (Xiao et al. 2001) and/or COWP loci (Spano et al. 1997; Pedraza-Diaz et al. 2001)

sequence analysis of the gp60 region (Peng et al 2001; Alves et al 2003)

>DQ192508.gp60-IIa allele

...CAGCCGTTCCACTCAGAGGAACTTTAAAGGATGTTCTCTGTTGAGGGC
TCA TCA TCG TCA TCG TCA TCG TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA TCA
ACATCAACCGTCGCACCAGCAAATAAAGGCAAGAACTGGAGAAGAA…

‘IIaA18G3R1’

Prevalence and seasonal distribution of Cryptosporidium spp

<table>
<thead>
<tr>
<th></th>
<th>2000</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. parvum</td>
<td>48</td>
<td>12</td>
<td>46</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>C. hominis</td>
<td>2</td>
<td>13</td>
<td>6</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>C. meleagrisidis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cervine gt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>mixed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Seasonal distribution of Cryptosporidium spp (2005-2008) and average number of reported cases (__, right hand y axis)

*C. ryanae*
14 different gp60 subtypes identified

IIaA18G3R1 type most prevalent

Annual distribution of C. parvum gp60 subtypes

Annual distribution of Gp60 subtypes (2000-2008)
C. hominis gp60 subtypes


- 2008 (n=15): Detection of ‘new’ subtypes IbA9G3R1 (n=1) and IdA2 (n=2)
Geographical distribution of Cryptosporidium spp (2005-2008)
Cryptosporidium species and subspecies in livestock and wildlife spp.

- Lough Gill catchment
  - 4 Cattle, 3 Sheep and 1 Cattle/Sheep farms
- Liffey catchments
  - 3 Cattle, 3 Sheep, 3 Horse farms

<table>
<thead>
<tr>
<th>Month</th>
<th>Liffey catchments</th>
<th>Lough Gill catchment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Sheep</td>
</tr>
<tr>
<td>March</td>
<td>13</td>
<td>48</td>
</tr>
<tr>
<td>April</td>
<td>13</td>
<td>48</td>
</tr>
<tr>
<td>May</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>June</td>
<td>22</td>
<td>30</td>
</tr>
</tbody>
</table>
Sample processing

Faecal sample

- ELISA
- Sugar Flotation

Smear preparation

DNA extraction

- 18S rRNA - Nested PCR

IFAT

Kinyon's stain
Comparison of different techniques on cattle samples

Comparison of different techniques on sheep fecal samples
Environmental sampling 2009

- Sampling
  - Lough Gill & Liffey Catchment
    - Winter - January
    - Spring/Summer – March, April, May, June, July
    - Autumn – October

- Biomonitors
Biomonitors

**Asellus aquaticus - water hoglouse**
- 30 minute sample
- Four sites on the River Liffey
  - Sites downstream of storm-water sewage overflows

**Dreissena polymorpha, zebra mussels**
- 300g
- Four sites at Lough Gill
- Analysis by IFA, FISH\(^1\) and nested PCR\(^2\)

\(^1\)Graczyk, et al., 2006. *Applied Environmental Microbiology*; 72: 3390-3395
Biomonitoring Results – River Liffey

_Cryptosporidium oocysts in Asellus_ collected from River Liffey

<table>
<thead>
<tr>
<th>Location</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sallins</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Clane</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Abbeycourt</td>
<td>3</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Castletown</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

_C. parvum_
Lough Gill – Zebra Mussel Samples
**Biomonitoring Results – Lough Gill**

*Cryptosporidium* oocysts in *zebra mussel* collected from Lough Gill lake.

<table>
<thead>
<tr>
<th>Location</th>
<th>January</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazelwood</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Parkes Castle</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Inishfree</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Whitewood</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sligo Intake</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Holywell</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Drinking Water Plant</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*C. parvum*
Humans

- *C. parvum* predominant, spring peak
- *C. hominis*: weak bi-modal pattern
- *C. meleagris*, *C. ryanae* first reported in 2008
- gp60 subtypes
  - *C. parvum* - IIaA18G3R1 predominant in all parts of the country
  - *C. hominis* - IbA10G2R1 predominates
Summary & Conclusions

- **Animals**
  - IFAT useful screening techniques
  - ~28% samples PCR+

- **Environment**
  - Hoglouse & Zebra mussels good biomonitors
  - *Cryptosporidium* detected in all sites
Acknowledgements

- Human study
  - Annetta Zintl

- Animal study
  - Marzieh Mirhashemi

- Environmental study
  - Frances Lucy, Declan Feeney
  - Thaddeus Graczyk, Leena Tamang

- Funding
  - EPA – STRIVE programme
"RAW SEWAGE IN THE CORRIB? NO-ONE KNEW?
GO BACK TO SLEEP,
GALWAY, GO BACK TO SLEEP."

Woohoo!
It's over!
No more brushing
my teeth
with beer.