Update on Clinical Parasitology Developments

Dr. Claire Alexander
Consultant Clinical Scientist
Scottish Parasite Diagnostic and Reference Laboratory
Stobhill Hospital
Cryptosporidium

- Infective oocysts in faeces

Transmission routes:
- Water/food to human
- Human to human
- Animal to human

- Can infect over 150 species of mammals

- 11 Cryptosporidium species known to infect humans
Cryptosporidium Species

- suis
- parvum
- canis
- hominis
- meleagridis
- andersoni
- tyzzeri
- muris
- cuniculus
- ubiquitum
- felis
Cryptosporidium genotypes (60+)

- Pig
- Horse
- Sheep
- Cattle
- Marsupial
- Opossum
- Skunk
- Monkey
- Ferret
- Fox
- Muskrat
- Squirrel
- Chipmunk
- Bear
- Deer
- Mouse
Clinical Symptoms

- Diarrhoea (98%)
- Abdominal pain (95%)
- Low grade fever (59%)
- Nausea & vomiting (65%)

- Incubation period is 2-14 days
- Length of symptoms is 3-21 days
- Need as few as 9 oocysts for infection
- Single bowel movement can contain 10000000000 oocysts!!
What happens when a Cryptosporidium positive stool arrives at SPDRL?

1. DNA Extraction + Internal Control DNA
2. Multiplex PCR
   - C. hominis
   - C. parvum
3. ssrRNA PCR-RFLP
4. Sequencing
5. Subtyping Assay
Subtyping of Cryptosporidium

1) Observe oocysts
2) Speciate
3) GP60 Gene Amplification

- Families - Extensive sequence differences in the non-repeat regions
  \(C.\text{hominis}\) families = Ia, Ib, Id-Ig
  \(C.\text{parvum}\) families = IIa-IIii, IIk, IIIl

- Tandem repeats - trinucleotides TCA, TCG or TCT at the 5'end of the gene
  tcatcatcatcatcatcatcgtcatcatcatcatcatcat

- Subtypes differ in the number of tandem repeats
Reporting of Cryptosporidium Subtypes

The subtype begins with the family, followed by the number of trinucleotide repeats

e.g. *C. hominis* IbA10G2

Subtype family Ib: 10 copies of the TCA repeat; 2 copies of the TCG repeat

*C parvum* has repeat sequences (usually one or two, ACATCA) after the serine repeats labelled R;
e.g. *C. parvum* IIaA15G2R1

Subtype family IIa; 15 copies of TCA; 2 copy of TCG; 1 copy of ACATCA Repeat
Scottish Crypto Ref Lab Data April 2012 – present
2012 – present 563 Scottish cases

176 samples referred to SPDRL

C. parvum n=114 (110 between April - July 96%)

C. hominis n=50 (37 between Aug-Nov 74%)
Mixed (C. parvum & C. hominis) n=1

Others (C. ubiquitum n=5 (Scottish Water & Grampian; C. meleagridis n=1 (Glasgow); C. cuniculus n=1 Lanarkshire)

Insufficient material n=3; Unamplifiable sample n=1
5 potential outbreaks;
2 Lothian (?petting farm, predominant subtype \textit{C.parvum} IIaA17G1R1; ?swimming pool \textit{C.hominis} IbA10G2)

1 Tayside (?swimming pool \textit{C.parvum} IIaA15G2R1; IIaA19G1R1)

1 UK wide (?foodborne \textit{C.parvum} IIaA15G2R1)

1 Borders (All \textit{C.hominis} but 2 groups - IbA10G2; IaA14R3).

Less common subtypes isolated;
\textit{C.parvum} IIgA9R1 (Glasgow) - rare
\textit{C.parvum} IIdA16G1 (Fife)
\textit{C.parvum} IIcA5G3 (Lothian)
Future Challenges

Prevailing *Cryptosporidium* subtypes in Scottish human cases?

Prevailing *Cryptosporidium* subtypes in farm animals in Scotland - Moredun Institute

Optimum procedures for water sampling - Scottish Water, Drinking Water Inspectorate

*Cryptosporidium* whole genome sequencing - Sanger Institute

Metabolomics
**Other Developments**

*Entamoeba* species;  
- *E. histolytica*  
- *E. dispar*  
- *E. moshkovskii*

PCR assays for detection in stools & liver abscesses; real-time multiplex; PCR-RFLP approach

Molecular sequencing-based assays for detection of common and unusual malaria species

Molecular testing of *Ancathamoeba* species

Rapid antigen detection of *Schistosome* species
Thanks!

[Image of a group of five people standing together]