Diagnostic tests for Cryptosporidium

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What is Cryptosporidium?

- Protozoan, monoexenous, obligate parasite
- The oocyst stage is shed mainly in faeces
  - Robust, transmissive stage
  - Contains 4 infective sporozoites, each capable of invading a host cell
  - Multiplies by asexual reproduction and cycling AND by sexual reproduction with autoinfection within the host
  - Massive numbers of oocysts can be shed in faeces and some hosts can be chronically infected
  - Infectious dose is plausibly 1 oocyst
What is cryptosporidiosis?

“An illness caused by Cryptosporidium and characterized by diarrhoea, abdominal cramps, loss of appetite, low-grade fever, nausea, and vomiting”.

The disease can be prolonged and life-threatening in severely immunocompromised persons.
Complications: pancreatitis, sclerosing cholangitis, biliary cirrhosis.

No licensed treatment in UK; management of high risk patients is especially difficult

• 427 lab confirmed immune-competent cases with diarrhoea (Hunter et al., Emerging Infectious Diseases 2004):
  – 96% abdominal pain
  – 65% vomiting
  – 59% fever
  – Mean duration 12.7 days
  – 14% hospitalised for 1 to 9 d (mean 3 d)
Cryptosporidium and the immunocompromised patient

1996 – HAART introduced: controls problems of cryptosporidiosis in AIDS patients in developed world

Cryptosporidiosis is now increasingly recognised in other T-cell immunodeficiencies (esp. haematological and T-cell primary)

Has a devastating effect where treatment is not available (lack of HAART, fake drugs, undefined treatment modalities)

Treatment modalities:
2002 – FDA approved nitazoxanide in children
2005 – FDA approved nitazoxanide in adults
Trials still underway in immunocompromised patients
Long term sequelae

In developing countries:
children exhibit poor growth, depressed cognitive function

Generally:
possible links to reactive arthritis and irritable bowel syndrome
suggested relapse in inflammatory bowel disease
The problems with *Cryptosporidium*

- High potential for contamination/transmission from infected hosts
- Multiple sources; multiple transmission routes
- Robust oocyst stage
- Survives in environment, waste water treatment, water treatment
- Resistance to common disinfection (e.g. chlorine)
- Causes outbreaks
- Some patients highly vulnerable
- Long term sequelae
- Limited treatment options
A neglected pathogen


- These infections occur “in developing countries where climate, poverty and lack of access to services influence outcomes” and “exhibit a considerable and increasing global burden, and impair the ability of those infected to achieve their full potential, both developmentally and socio-economically”.

Cryptosporidiosis in the UK

- Up to 6000 reported cases p.a.
- Incidence about 10 per 100 000 population
- Most cases are *C. parvum* or *C. hominis*, but unusual infections and outbreaks occur (e.g. *C. cuniculus*)
- BUT under-ascertainment of up to 7.4 cases in the community for every case reported to disease surveillance (IID study 1)
Clinical laboratory practices for the detection and reporting of *Cryptosporidium* in community cases of diarrhoea in the UK.

Rachel Chalmers, Brian Campbell, Nigel Crouch, Angharad Davies.

Eurosurveillance, in press. (25th Nov 2010)
Survey of 200 diagnostic labs in UK in 2008

- 100% response rate
- 145 (73%) tested all stool samples for *Cryptosporidium*
- 55 (27%) applied selection criteria
- 190 (95%) report cases (surveillance and public health).
<table>
<thead>
<tr>
<th>Country / Government Office Region</th>
<th>Laboratories testing all stools / number of laboratories (%)</th>
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</thead>
<tbody>
<tr>
<td>Wales</td>
<td>14/ 14 (100%)</td>
</tr>
<tr>
<td>Scotland</td>
<td>26/ 26 (100%)</td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>5/ 6 (83%)</td>
</tr>
<tr>
<td>England</td>
<td>100/154 (65%)</td>
</tr>
<tr>
<td>East Midlands</td>
<td>7/8 (88%)</td>
</tr>
<tr>
<td>East</td>
<td>12/18 (67%)</td>
</tr>
<tr>
<td>London</td>
<td>7/23 (30%)</td>
</tr>
<tr>
<td>North East</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>North West</td>
<td>24/27 (89%)</td>
</tr>
<tr>
<td>South East</td>
<td>11/22 (50%)</td>
</tr>
<tr>
<td>South West</td>
<td>10/15 (67%)</td>
</tr>
<tr>
<td>West Midlands</td>
<td>14/17 (82%)</td>
</tr>
<tr>
<td>Yorkshire &amp; the Humber</td>
<td>12/15 (80%)</td>
</tr>
</tbody>
</table>
Selection criteria used by 55 labs

- age (n=38)
- immune status (n=36)
- stool consistency (n=37)
- duration of diarrhoea (n=3)
- overseas travel (n=22)
- farm visit or animal contact (n=17)
- clinician’s request (n=13)
- during an outbreak (n=3).

- The 38 laboratories that selected specimens according to age used the following upper limits:
  2 years (n=1), 5 years (n=3), 6 years (n=1), 8 years (n=1), 9 years (n=1), 10 years (n=4), 11 years (n=1), 12 years (n=1), 14 years (n=2), 15 years (n=6), 16 years (n=9), 45 years (n=5), 50 years (n=1), 60 years (n=2)
Can selection criteria be justified?

• Stool consistency – No!

• Age – depends!

• <45 yrs
Regional differences in the proportion of laboratories selectively testing stools were significantly correlated with *Cryptosporidium* report rates to national surveillance (rs=0.61, df=11, p=0.03).
The methods used to diagnose cryptosporidiosis

- AP stain (76%)
- mZN stain (22%)
- EIA (2%)
conclusions

• Differences in laboratory practice in testing for Cryptosporidium contribute to local and regional variation in reported cases.

• Effect of Notifiable status (Schedule 2 organism) yet to be seen.
Comparison of the diagnostic sensitivity and specificity of seven Cryptosporidium assays in use in the UK.

Rachel Chalmers, Brian Campbell, Nigel Crouch, Andre Charlett, Angharad Davies.

Submitted to Journal of Clinical Microbiology

Thanks to Catherine Shepherd, Sharon Poynton and the staff of the Enteric Laboratory in Public Health Wales, Microbiology ABM for facilitating this study…..

and the companies for supplying the test kits and technical support for the study.
Which methods?

1. mZN staining and examination of 50 fields at x400 magnification (NSM)

2. AP staining and examination of 50 fields at x200 magnification (NSM)

3. IFM (Crypto-Cel, CelLabs, Buckingham UK) and examination of entire 9mm diam well at x200 magnification


5. IVD Research Inc Giardia/Crypto Combo, Launch Diagnostics

6. Techlab Giardia/Cryptosporidium CHEK®, Inverness Medical

7. Immunochromatographic lateral flow (ICLF) Cryptosporidium assay (RIDAQUICK® Cryptosporidium cassette, R Biopharm Rhone, UK).
EIAs conducted using an automated processing system, Dynex DS2™, Inverness Medical.

Positive EIA results in Giardia/Cryptosporidium combo assays differentiated using IFM (Crypto-Cel and Giardia-Cel, CelLabs, Buckingham UK).
Diagnostic sensitivity and specificity assessment

- 152 true positives
- 107 true negatives

- Defined by IMS-IFM and PCR results

- Testing at least 250 samples ensured that differences in sensitivities and specificities in excess of 10% had an 80% chance of being detected at a 5% significance level
<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Sensitivity (95% CI)</th>
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<tbody>
<tr>
<td>mZN microscopy</td>
<td>75.7% (68.3% to 81.8%)</td>
</tr>
<tr>
<td>RIDAQUICK® Cryptosporidium ICLF</td>
<td>84.9% (78.3% to 89.7%)</td>
</tr>
<tr>
<td>Remel ProSpecT™ G/C Microplate EIA</td>
<td>91.4% (85.9% to 94.9%)</td>
</tr>
<tr>
<td>AP Microscopy</td>
<td>92.1% (86.7% to 95.4%)</td>
</tr>
<tr>
<td>IVD Research Inc G/C Combo EIA</td>
<td>92.8% (87.5% to 95.9%)</td>
</tr>
<tr>
<td>Techlab G/C CHEK® EIA</td>
<td>93.4% (88.3% to 96.4%)</td>
</tr>
<tr>
<td>Crypto-Cel IF microscopy</td>
<td>97.4% (93.4% to 99.0%)</td>
</tr>
</tbody>
</table>
Specificity

- 100% (96.5% to 100%)
- The positive predictive value of the tests would be high.
- Specificity also depends on microscopy skill and choice of confirmation method.
Giardia

- Confirmed in one or two samples positive by each of the EIA kits where no positives were identified by the NSM.

- Percent agreement appeared high (>98%) but the number of positive samples was small.

- Substantial improvement in the diagnosis of Giardia by EIA compared to the NSM has been reported (Ellam et al., Eurosurv 2008).
conclusions

• Technical problems reported with first generation Cryptosporidium EIAs leading to false-positive and false-negative results appear to have been overcome by improved reagents and automated systems for plate washing and objective results reading.

• The requirement to confirm and resolve the genus in EIA-positive samples in combination assays adds assurance, and single assays should be confirmed likewise. This is especially important to eliminate false-positives.

• The EIAs tested here offer an alternative to traditional diagnostic tests and may enable continued testing of the high proportion of acute diarrhoea cases for Cryptosporidium and expand patient testing for Giardia, often restricted to patients with foreign travel history.
What about PCR?

- PCR detects more positives than microscopy
- Disruption procedure required
- DNA extraction options
- PCR primer selection
- PCR platforms
Comparative extraction of *Cryptosporidium* DNA from human faeces

Kristin Elwin, Guy Robinson, Brian Campbell, Hannah Fairclough, Rachel Chalmers

In preparation

Thanks to SfAM for partial support through the “Students in to Work Grant” awarded to Hannah Fairclough, Aberystwyth University, summer 2010
Phase 1: Performance of DNA extraction methods measured by conventional SSU rRNA gene PCR

<table>
<thead>
<tr>
<th>Method</th>
<th>200 opg faeces (100 per extraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt flotation, heat disruption and QIAamp DNA Mini kit</td>
<td>25/25</td>
</tr>
<tr>
<td>QIAamp DNA stool kit</td>
<td>1/25</td>
</tr>
</tbody>
</table>
Phase 2: comparison of cycle threshold ($C_T$) values from 2 extraction methods using a TaqMan real-time SSU rRNA gene PCR

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Number positive stools (N=46)</th>
<th>Mean $C_T$ values</th>
<th>Paired T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt float, boil, Qiagen</td>
<td>46</td>
<td>28.54</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Adapted Boom (guanidinium thiocyanate &amp; bead beating)</td>
<td>43</td>
<td>34.93</td>
<td></td>
</tr>
</tbody>
</table>
Collaborative work with HPA

• 2012 Olympics resilience planning: PCR for investigation of outbreaks of gi disease

• Can a single extraction method be used for bacteria, viruses and parasites? No!

• Adapted, automated Boom method being optimised for DNA extraction in the development of a multiplex real-time PCR protocol for Cryptosporidium, Giardia and Entamoeba histolytica
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