Faecal parasitology

Lisette van Lieshout
lvanlieshout@lumc.nl
Leiden Clinical Parasitology Group
Department of Parasitology

Many thanks to the organizers for the invitation

I declare having no conflicts of interest (*)

(*) Department provides the McAb to Rapid Medical Diagnostics for the *Schistosoma mansoni* urine POC-CCA cassette

(*) Member board Parasitology section SKML (Dutch Foundation for Quality Assessment in Medical Laboratories)

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Stool parasite diagnosis

In industrialised settings:
• General trend of reduced prevalence
• Most common diagnosis - pathogens?
  • Giardia; (Enterobius)
  • Outbreaks; e.g. Cryptosporidium, Cyclospora
• How relevant?
  • Dientamoeba fragilis; Blastocystis hominis
  • See a.o.: review Wong et al., (2018); Coyle et al., (2012)
  • Case-control study Bruijnesteijn van Coppenraet et al. (2015)
• Efforts per detected pathogen?
Nucleic Acid Amplification Test (NAAT)

Most common for stool parasites: (multiplex/multi-parallel) real-time PCR (unpreserved faces)

Initiated by *E. histolytica*/*E. dispar* differentiation

Specificity: $\approx 100\%$

Target design, QC-steps in laboratory flow!!!!

Sensitivity: $\approx$ to >>>microscopy

Quality of reference test??

Volume? Distribution of parasite in sample (helminths)?

In house tests: Cq (Ct/Cp) -value => indicates DNA load

Stool samples: negative days/weeks after therapy
**Layout of presentation**

Will NAAT(*) fully replace microscopy for faecal parasites?

1. Should we abandon microscopy as routine diagnostics?

2. What do we learn from multiplex real-time PCRs?

3. What are (or remain) the alternatives, besides NAAT?

4. Trends and challenges; implications for clinicians

(*) NAAT = Nucleic Acid Amplification Test / Most common in parasitology: (multiplex) real-time PCR
Microscopy for faecal parasites – still “the” standard

Van Leeuwenhoek, 1681

(+): very broad!!!!!!!!

(-): observer dependent

(-): not for high throughput

(-): not enough for E. histolytica

Picture of broad microscopy
Parasite-specific procedures – in routine settings

- Specific staining procedures
  - Coccidia, microsporidia, *D. fragilis*
- Lack of sensitivity
  - Concentration procedures
  - Helminths-specific techniques
  - Repeated sampling
# Key studies – in routine use of PCR for stool parasites

<table>
<thead>
<tr>
<th>Source population</th>
<th>N</th>
<th>Study design</th>
<th>Microscopy expertise</th>
<th>PCR targets</th>
<th>Publication</th>
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<td>Prospective survey</td>
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High sensitivity and specificity of PCR for diagnosis of *Giardia lamblia*

Higher DNA loads in microscopy positive cases


Rapid clearance of *Giardia* DNA following treatment

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Cryptosporidiosis
(Cryptosporidium hominis/C. parvum)

Coccidia; epithelial cells
Faecal-oral; food/water => outbreaks; seasonal
Children; self limiting (nitazoxanide); opportunistic

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Routine PCR multiplex including *Cryptosporidium*: earlier detection of outbreaks

Simultaneous increase of *Cryptosporidium* infections in the Netherlands, the United Kingdom and Germany in late summer season, 2012

N Fournet¹,²,³, M P Dege³,⁴,⁵, A T Urbanus¹, G Nichols⁴, B M Rosner⁴, P M Chalmers⁵, R Gorton⁵, K G Pollock⁵, J W B van der Giessen⁵, P C Wever⁶, J W Dorigo-Zetsma⁶, B Mulder⁷, T G Manka⁷, I Overdvest⁷, J G Kusters⁷, W van Peit⁷, L M Kortbeek (Titia.Kortbeek@rivm.nl)¹

MMWR  /  June 28, 2019  /  Vol. 68  /  No. 25

Morbidity and Mortality Weekly Report

Cryptosporidiosis Outbreaks — United States, 2009–2017

Radhika Gharpure, DVM¹,²; Ariana Perez, MPH¹,³; Allison D. Miller, MPH¹,⁴; Mary E. Wikswo, MPH⁵; Rachel Silver, MPH¹,³; Michele C. Hlavsa, MPH¹
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How essential to include *E. histolytica* target in routine multiplex PCR?

Why not only for those patients exposed to tropical region?

**Illustrative case:**

A&E: 56-year-old healthy Dutch male

Travels regularly to Berlin, no travel outside EU

10 days watery diarrhoea, bloody & mild fever & **extreme** rectal pain

- Bacteriology culture of blood, urine, stool: **no abnormalities**
- Virology & Bacteriology stool PCRs: **no abnormalities**
- Parasitology stool microscopy: **no abnormalities**
- Parasitology: routine PCR for *E. histolytica/ Giardia / Cryptosporidium*
  - *E.histolytica*: Ct= 25.2  - Serology confirmation
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Cyclosporiasis
*Cyclospora cayetanensis*

Coccidia; epithelial cells; travel and food related: (sub) tropics (Central America, Nepal, Indonesia)

Periods (intermittent) of:
- explosive watery diarrhoea
- vomiting
- severe weight loss
- (abdominal pain, myalgias, low-grade fever, and fatigue)

Self limiting in healthy cases (10-12 weeks); severe in immuno-compromised

Treatable with trimethoprim-sulfamethoxazole (Cotrim)
Increasing awareness of cyclosporidiasis

Repeated outbreaks US, Canada related to imported food


Outbreaks UK related to holidays in Mexico

### Table 1  Selected commercialized nucleic acid amplification tests for enteric parasitic infections

<table>
<thead>
<tr>
<th>NAAT</th>
<th>Parasites detected</th>
<th>Separate DNA extraction step required?</th>
<th>Platform used</th>
<th>Specimens per run</th>
</tr>
</thead>
</table>
| xTAG GPP (Luminex, Austin, TX)            | *Cryptosporidium* sp.  
*Giardia* sp.  
*Entamoeba histolytica*                                                          | Yes                                    | Luminex only               | 96                                                     |
| RIDA®GENE Parasitic Stool Panel           | *Cryptosporidium* sp.  
*Giardia* sp.  
*Entamoeba histolytica*  
*Dientamoeba fragilis*                                                               | Yes                                    | Several                    | Typically up to 96, depending on platform used         |
| (R-Biopharm AG, Darmstadt, Germany)       |                                                                                      |                                        |                             |                                                        |
| FilmArray Gl                              | *Cryptosporidium* sp.  
*Giardia* sp.  
*Entamoeba histolytica*  
*Cyclospora cayetanensis*                                                            | No                                     | BioFire only                | 1                                                      |
| (BioFire Diagnostics, Salt Lake City, UT) |                                                                                      |                                        |                             |                                                        |
| FTD Stool parasites                       | *Cryptosporidium* sp.  
*Giardia* sp.  
*Entamoeba histolytica*                                                               | Yes                                    | Several                    | Typically up to 96, depending on platform used         |
| (Fast-track Diagnostics, Sliema, Malta)   |                                                                                      |                                        |                             |                                                        |
| Gastroenteritis/parasite panel            | *Cryptosporidium* sp.  
*Giardia* sp.  
*Entamoeba histolytica*                                                               | Yes                                    | Several                    | Typically up to 96, depending on platform used         |
| (Diagenode, Liege, Belgium)               |                                                                                      |                                        |                             |                                                        |

**NAAT** nucleic acid amplification tests
Which protozoa next to be included in PCR panels?

**Microsporidia**
Severe diarrhoea, mainly in immunocompromised (IC) patients
90% *Enterocytozoön bieneusi*
*Encephalitozoön intestinalis*; treatment by albendazole

LUMC: standard screening for IC patients;
2018 3% positive; (19/658 samples)

**Cysto-isospora belli**
Severe diarrhoea, mainly in immunocompromised (IC) patients
More related to HIV+, Africa
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Essential helminths not to miss

Illustrative case (1):

Male, 61 year, born in Surinam, 30 years in NL

History of intestinal complaints, frequent nausea, lack of appetite, cough

• No eosinophilia (so lack of diagnostic trigger)
• Vasculitis, prednisone
• Situation deteriorates
• Stool samples microscopy negative => parasitology reference lab (LUMC)
Essential helminths not to miss

Stool PCR positive *Strongyloides stercoralis*
Microscopy finally confirmed
Stored serum (> 3 years): highly positive
Potentially life threatening; treatment: Ivermectin

Serology
Different formats; in-house, commercial
Sensitivity vs specificity

Combination PCR & serology: any tropical exposure
Essential helminths not to miss

Illustrative case (2):
Dutch male, 27 years, 4 months internship in Malawi
Including a weekend in CapeMcclear at month 2

Urticaria < 3 days; >3 weeks: fever, malaise, sweating, diarrhoea
At week 4: haematuria; praziquantel 40 mg/kgbw

In following 9 months:
Change in semen: lumpy texture, watery, yellow
• Visit to LUMC, because 3 weeks haematospermia, rusty
Essential helminths not to miss

Serology - *Schistosoma*

=> strongly positive

Active infection?

- Urine: microscopy negative
- Urine: PCR weakly positive (Ct 39.1)
- Semen: PCR strongly positive (Ct 25.1)
- Semen: sporadic eggs of *S. haematobium*

Symptoms disappear after retreatment with praziquantel

PCR semen and urine negative in follow-up
Male/Female Genital Schistosomiasis (MGS/FGS) PCR can facilitate the diagnosis

http://www.njmonline.nl/getpdf.php?id=565
What are the alternatives to NAAT?

**Antibody detection (serology)**
- Amoebiasis
- Strongyloidiasis
- Schistosomiasis

**Antigen detection**
- Protozoa coproantigen tests?
- *E. histolytica, Giardia, Cryptosporidium* => PCR
- Schistosoma (blood, urine)
  - POC-CCA urine test strip for *S. mansoni*
  - Ultra-sensitive CAA test still experimental

Non-microscopy
Post-travel screening on parasitic infections?

Routine PCR stool screening (HGC + Ss)

- N=556 asymptomatic Dutch travellers to LMIC (>1 month); PT-stool
- 2 weeks after return (HGC-PCR)
- 12 weeks after return (HGC + Ss)

*Giardia* N=29 (5%); 6 before travel

*Cryptosporidium* N=4 / *E. histolytica* N=0 / *Strongyloides* N=1

*Schistosoma* serology in 145/200 to SSA
N=9 seroconverted, 7/9 asymptomatic

**Conclusion:**

- Screening of asymptomatic travellers by stool PCR HGC + Ss: not efficient
- Serology *Schistosoma*: yes, if exposed (SSA)
NAAT will replace microscopy as a routine diagnostic test

I think it is a mammoth

Cartoon – early diagnosis

Mmmmm...Let’s first see our PCR result

Importance of EQAS in NAAT
Helminths: Cool et al., (manuscript in prep)
Take home’s; Trends in stool parasite diagnostics:

- Reduction of observer-dependent techniques
- Majority of negatives => drives high-throughput, multiplex approach
- Less by discipline => syndromic based diagnostic approach

Parasite PCR in routine setting: need for standardization, QC, EQAS

- Centralisation of diagnostic expertise; for complicated cases
  - Full microscopy (specific symptoms; exposure in tropics, immune compromised)
  - Serology & specific PCR for rare parasites

Clinicians be aware:
Which targets are fully covered in your diagnostic setting?
Thank you!

Research = Teamwork

Besides acknowledgements to the funders; many thanks to colleagues, and (international) collaborators

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Part of the LUMC Center of Infectious Diseases Schisto-CoHSI team