

Detection and genetic characterization of *Cryptosporidium* spp. among patients infected with HIV and renal transplant recipients, by conventional and molecular techniques

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Background

Cryptosporidium

- Obligate, intracellular, Coccidian, protozoan parasite.
- Infects microvilli of intestinal epithelial cells
- Transmission via contaminated water, food, human-to-human and animal-to-human.
- Important cause of diarrhea in immunocompromised patients such as patients with Human immunodeficiency virus (HIV) and renal transplant (RT) recipients.
- Till date around fifteen species of have been known to infect humans:
C. hominis, *C. parvum*, *C. ubiquitum*, *C. viatorum*, *C. meleagridis*, *C. canis*, *C. felis*, *C. wrairi*, *C. baileyi*, *C. muris*, *C. andersoni*, *C. serpentis*, *C. fayeri*, *C. suis*, *C. bovis*

C. parvum and *C. hominis* are the commonest ones.

- However, there is scanty data on cryptosporidiosis and genotyping of *Cryptosporidium* among RT recipients.
- Genetic characterization of *Cryptosporidium* is essential to investigate the genetic diversity and might aid in tracing source of infection, thus helping to control its spread.
- Conventional microscopy can not differentiate the different species of *Cryptosporidium*.
- Molecular techniques such as polymerase chain reaction (PCR)/ Restriction fragment length polymorphism (RFLP) and real-time PCR (qPCR)/ Melting curve analysis (MCA) are required for genetic characterization.

Aim: This study was designed for detection of *Cryptosporidium* by microscopy/ PCR and genetic characterization by PCR- RFLP and qPCR/ MCA among patients with HIV infection and RT recipients; also to compare conventional (microscopy) with molecular techniques.

Patients and methods

550 immunocompromised patients
(360 RT and 190 HIV)

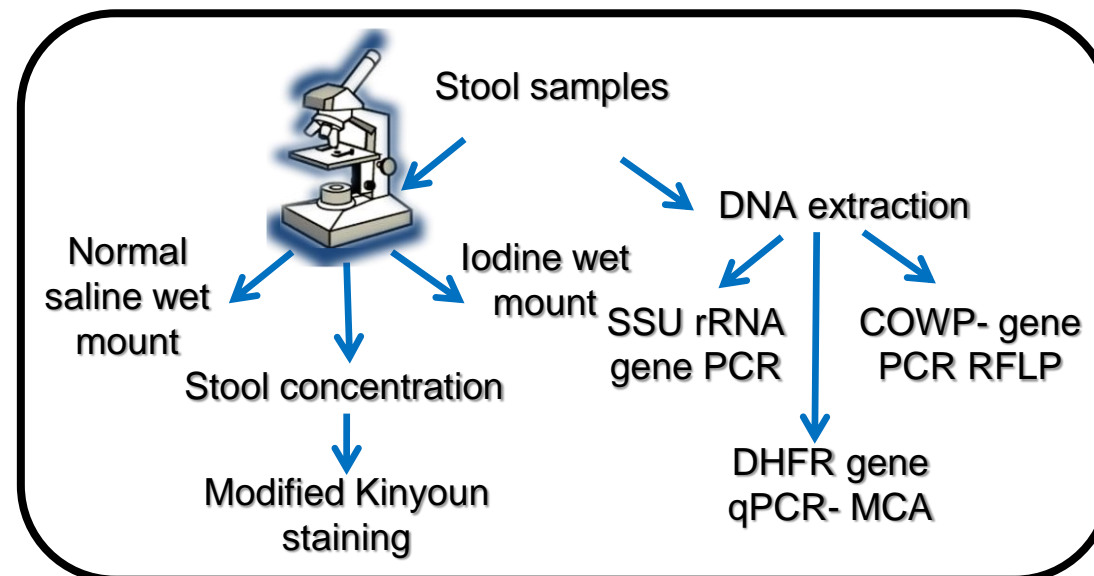
Stool x 3

- Microscopy
- SSU PCR
- COWP PCR- RFLP
- DHFR- qPCR MCA

Cryptosporidium Positive cases (RT + HIV)

140 Cryptosporidium Negative Controls (80 RT + 60 HIV)

200 Healthy controls



Microscopy was done on smears of concentrated fecal samples, subject to modified Kinyoun's staining and counterstained by malachite green (1)

PCR of the following genes were performed:

- SSU rRNA gene: Small subunit rRNA gene (1)
- COWP- gene: *Cryptosporidium* oocyst wall protein gene. The enzyme used for RFLP after COWP PCR was Rsa I (1)
- qPCR of DHFR gene: Dihydrofolate reductase gene (2)

References:

1. Ujjala Ghoshal, Asmita Dey, Prabhat Ranjan, Sonali Khanduja, Vikas Agarwal, Uday Chand Ghoshal Identification of opportunistic enteric parasites among immunocompetent patients with diarrhoea from northern India and genetic characterisation of *Cryptosporidium* and *Microsporidia*. Indian Journal of Medical Microbiology. 34: 60-6. 2016
2. Asmita Dey, Ujjala Ghoshal, Vikas Agarwal, Uday Chand Ghoshal. Genotyping of *Cryptosporidium* species and its clinical manifestations in patients with renal transplantation and human immunodeficiency virus infection. Journal of Pathogens. Volume 2016, Article ID 2623602, 9 pages.

Results

Cryptosporidium spp. was detected in:

- 74/ 550 (13.5%) patients by microscopy
- 74/ 550 (13.5%) patients by SSU- PCR
- 66/ 550 (12%) patients by COWP PCR
- 75/ 550 (13.6%) patients by DHFR qPCR
- The parasite was not detected among controls
- *Cryptosporidium* spp. was more frequent in immunocompromised patients than HC (75/ 550, 13.6% vs. 0/ 200, 0%, p<0.001)
- 71 patients (28 HIV patients [28/ 190, 14.7%], and 43 RT recipients [43/360, 11.9%]) showed mono-infection with *Cryptosporidium* spp.
- 2 had mixed infection with *Cystoisospora*, 1 with *Cyclospora* and 1 with *Strongyloides*.

Comparative analysis of the diagnostic tools:

| | | Microscopy | |
|---------|-----|------------|-----|
| | | (+) | (-) |
| SSU PCR | (+) | 74 | 0 |
| | (-) | 0 | 476 |

Sensitivity: 100%, Specificity: 100%
PPV: 100%, NPV: 100%

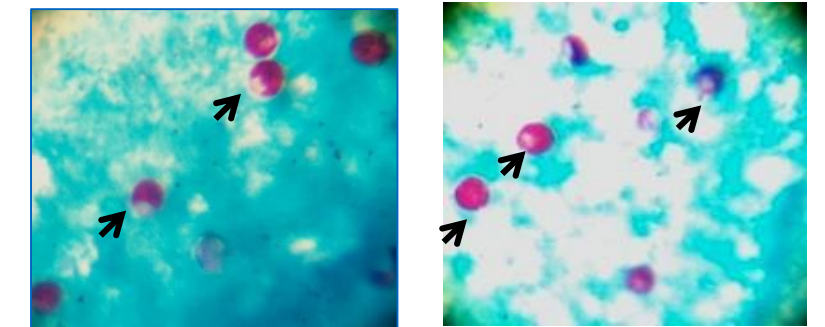
| | | Microscopy | |
|----------|-----|------------|-----|
| | | (+) | (-) |
| COWP PCR | (+) | 66 | 0 |
| | (-) | 8 | 476 |

Sensitivity: 89.2%, Specificity: 100%
PPV: 100%, NPV: 98.35%

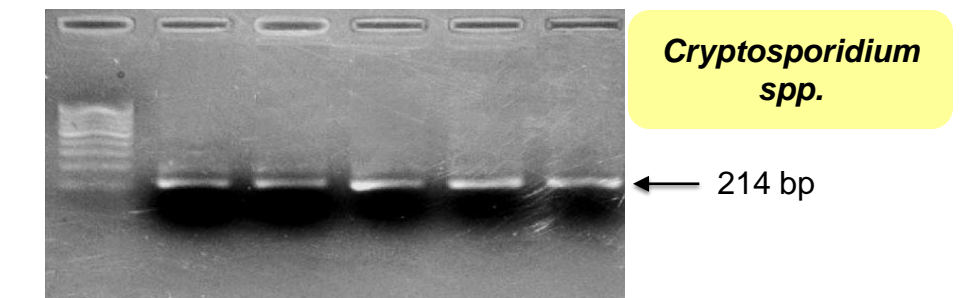
| | | Microscopy | |
|-----------|-----|------------|-----|
| | | (+) | (-) |
| DHFR qPCR | (+) | 74 | 1 |
| | (-) | 0 | 475 |

Sensitivity: 100%, Specificity: 99.7%
PPV: 98.6%, NPV: 100%

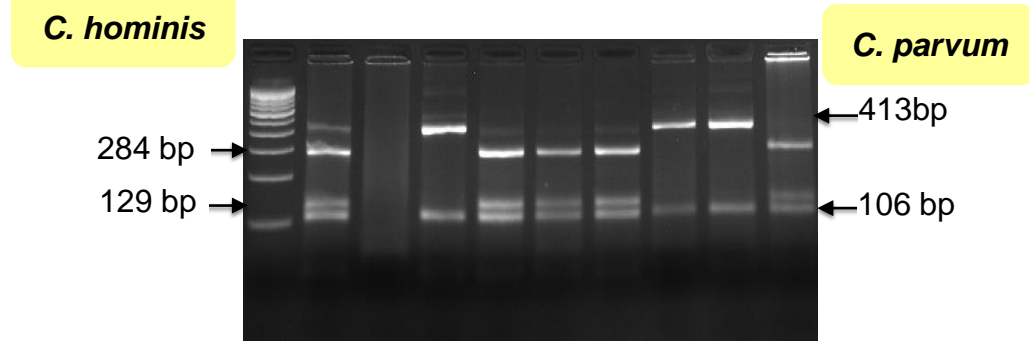
Representative pictures of microscopy and PCR/ RFLP



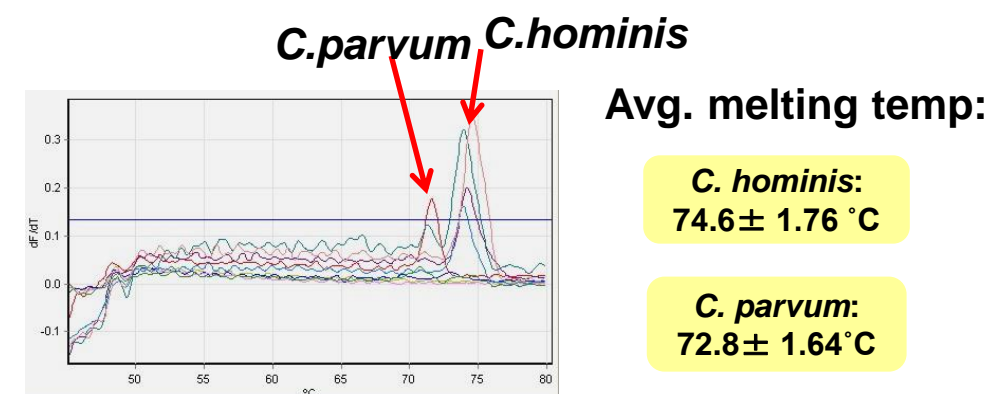
Partially pink oocysts stained by Modified (cold) Kinyoun's stain



2nd round of SSU- PCR amplicons (214 bp): *Cryptosporidium* spp.



COWP PCR- RFLP bands: *C. hominis* and *C. parvum*



DHFR qPCR- MCA: Melt curve peaks of *C. hominis* and *C. parvum*

| <i>Cryptosporidium</i> spp. detected by 2 techniques | qPCR- MCA | COWP- PCR- RFLP |
|--|----------------|-----------------|
| <i>Cryptosporidium hominis</i> | 52/75 (69.33%) | 48/66 (72.7%) |
| <i>Cryptosporidium parvum</i> | 21/75 (28%) | 16/66 (24.2%) |
| Mixed infection | 2/75 (2.6%) | 2/66 (3.03%) |