Educational Workshop

EW03: New insights in frequently observed intestinal parasites
arranged with the ESCMID Study Group for Clinical Parasitology (ESGCP)

Convenor: Tom van Gool (Amsterdam)

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Francis Derouin (Paris, FR)
Loic Favennec (Rouen, FR)
Dientamoeba, an underrecognised intestinal pathogen

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Manisa - TURKEY

Case I:
Male, 6 years
Diarrhea, Non-bloody, 5 / day
Enterobius vermicularis (+)
Lower socioeconomic status

Case II:
Female, 33 years
Intense bloating
Diarrhea (-)
Abdominal pain (-)

Case III:
Male, 71 years
Angioedema
IgE ↑
Eosinophilia
No intestinal symptoms

Case IV:
Female, 47 years
Non-specific itching, discomfort for 6 years
Generally on abdominal area
Successive diarrhea-constipation periods
No permanent relief

* Records of Celal Bayar University (CBU) Parasitology Laboratory

All these cases were found to have only one causative agent common...
Kurt - Dientamoeba, an underrecognized intestinal pathogen

Content of Presentation
- BACKGROUND
- MORPHOLOGY
- EPIDEMIOLOGY
- LIFE CYCLE
- TRANSMISSION
- PATHOGENESIS
- SYMPTOMS
- CLINICAL SIGNIFICANCE
- DIAGNOSIS
- GENETIC DIVERSITY
- TREATMENT

What's it like?
- “An unusual intestinal pathogen.”
- “An enigma shrouded in the mysteries of diagnostic clinical parasitology”
- “A neglected cause of diarrhea”
- “Emerging from obscurity”
BACKGROUND

- 1907, Wenyon
- 1918, Jepps and Dobell
- 1974, Camp et al., electron microscopy
  “Amoeba-like flagellate”
- 1996, Silberman et al., rRNA sequence analysis
  => "a flagellate without an obvious flagella"

Dientamoeba fragilis
MORPHOLOGY

- Trophozoite, no cyst form!
- 5-12 µm
- Often binucleated (60-80%)
- Unique nuclear structure
  - no peripheral chromatin
  - fragmented karyosome
- Leaf-like pseudopodia

EPIDEMIOLOGY

- Cosmopolitan
- Lower socioeconomic status
- Children are at higher risk
- Prevalence rates: 0% - 52.5% (unreliable)

FACTORS AFFECTING THE PREVALENCE RATES
1. Diagnostic method
   Permanent-stained smears, culture, molecular methods
2. Number of stool samples examined in consecutive days
3. Experience of the laboratory staff
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**LIFE CYCLE**

- Fecal - oral route
- To date, the cyst stage has not been identified
- Passage through gastric juice?

Transmission via helminth eggs (e.g., *Enterobius, Ascaris*) may be the explanation!

**TRANSMISSION**

*Role of Enterobius: Two opposite claims*

Prospective Study of the Prevalence, Genotyping, and Clinical Relevance of Dientamoeba fragilis Infections in an Australian Population

*Burrows & Swerdlow (1956)*

Heterakis gallinae egg

Histomonas meleagridis

Transmission of *Histomonas meleagridis* in *Heterakis gallinae* eggs (presence of a model)

- Burrows & Swerdlow (1956)
  - "*D. fragilis*-like bodies in pinworm eggs"

- Menghi (2005) detected *Dientamoeba* DNA on the outer shell of pinworm eggs.
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MANISA

Population: ~ 1.5 Million
Area: ~ 14,000 km²

SARDES: Capital of Lydians (7th Century, BC)

PATHOGENESIS

- Reside in caecum or colonic lumen
- No penetration through intestinal wall
- Mucosal irritation
  - Excess mucus secretion
  - Increased motility
  - Fibrosis

ATLANTIS?
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**SYMPTOMS**

- **No symptoms**
  Mostly adults
- **Mostly GIS-related symptoms**
  25% of patients!
  - Abdominal pain
  - Diarrhea / constipation
  - Nausea
  - Bloating
  - Anorexia
  - Fatigue, weight loss
- **Dermatologic symptoms**
  - Pruritus, urticaria

* Most common symptom in adults in our patient group!

**CLINICAL SIGNIFICANCE**

- Clinical symptoms resolved after treatment
  - Treatment necessary!
  - Symptomatic adult patients
  - Symptomatic children

“It is a significant cause of diarrhea in childhood, especially 4-6 years”
(Millet et al., 1983)

Asymptomatic carriers do not require treatment!

**CLINICAL SIGNIFICANCE**

Issues raised related to Dientamoebiasis

- Association with IBS (Irritable Bowel Syndrome)
  - Borody et al, 2002 => 21 IBS patients with *D. fragilis*
    - Abdominal cramping, bloating, constipation, diarrhea
    - 14 relieved after effective treatment
  - *D. fragilis* cause IBS-like symptoms and chronic infection
  - Relationship doubtful!
    (Windsor J, McFarlane L, 2005; Stark D, et al., 2007)
**CLINICAL SIGNIFICANCE**

**Issues raised related to Dientamoebiasis**

- Association with dermatologic complaints (Urticaria, pruritus)
  
  Yang & Scholten, 1977
  Spencer et al., 1982
  Kurt et al, unpublished data

Large-scale studies required!

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**DIAGNOSIS**

- **Microscopy**
  
  Permanent-stained smears
  (Trichrome, Hematoxylene-Eosine, Chlorazol black)

- **Culture**
  
  Robinson’s medium, Dobell’s medium

- **Molecular methods**
  
  Diagnosis and genotyping (PCR, RT-PCR, RFLP)

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**DIAGNOSIS**

**Microscopy**

Important issues:

1. Fresh or preserved stool sample
   (SAF, PVA, Schaudinn’s fixatives)

2. Examination of multiple stool samples
   “Intermittent shedding”

3. Experience of laboratory staff
   Reading of stained smears
   Differential diagnosis of *D. fragilis*
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Examination of permanent-stained smears are necessary for microscopic diagnosis

D. fragilis, Lugol's solution x400
D. fragilis, 0.9% saline x400
D. fragilis, Trichrome x1000

D. fragilis, SAF, Trichrome x1000
D. fragilis, SAF, Iron hematoxyline x1000

Differential diagnosis (x1000, Trichrome)

Iodamoeba bûtschlii  Dientamoeba fragilis  Endolimax nana
**Kurt - Dientamoeba, an underrecognized intestinal pathogen**

**DIAGNOSIS - Culture**

- "D. fragilis is one of the easiest of the human intestinal amoeba to isolate and grow in vitro."  
  C. Dobell, 1940

- "Culture is more sensitive and easier compared to microscopy”  
  Windsor et al., 2003

- Robinson’s medium!
- Boeck - Drbohlav’s, Dobell’s

**APPEARANCE IN CULTURE**

- Rice-starch ingested, refractile trophozoites,
- 6-40 µm
- Small, irregular pseudopodia
- Confirmed by trichrome-stained smears

**Drawbacks of Culture Method**

- Requires time & staff
- Expensive compared to microscopy
- Less sensitive than PCR and Real-Time PCR
  => not for routine diagnosis!

No axenic culture of *D. fragilis*!

=> effective doses of available drugs?
=> drug against the parasite directly?
**DIAGNOSIS – Molecular Methods**

- **Peek et al. (2004)**
  PCR after DNA isolation directly from stool

- **Stark et al. (2006)**
  Real-Time PCR
  Sensitivity & Specificity % 100

**Which method is the best?**

Stark et al., 2010

Comparison of microscopy, two culture techniques, conventional and real-time PCR for the detection of Dientamoeba fragilis in clinical stool samples

- **RT – PCR is the gold standard!**
Genetic Diversity of *D. fragilis*

- Johnson and Clark (2000) => SSU rRNA
  - Genotype 1 (n=11), Genotype 2 (n=1)
  - Peek et al. (2004)
  - Windsor et al. (2004)
  - Stark et al. (2005) \{No Genotype 2!\}

- Genetic diversity in SSU rRNA is low!

New targets needed!

- Bart et al. (2008):
  - "Genotyping according to differences of cytosine locations on ITS-1 region (C-profiling) is succesful"

  - ITS-1 region: "Effective epidemiologic marker"

Hussein et al. (2009):

- High Resolution Melting curve (HRM) analysis after PCR
- "HRM analysis is an effective epidemiologic marker!"
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**TREATMENT**

- Treatment of patients
- Drug of choice

No consensus!

Many reports indicating the relief of symptoms and eradication of *D. fragilis* after proper treatment.

**Necessary for symptomatics!**

**Large-scale, controlled studies urgently needed!**

**TREATMENT**

**Therapeutic options in the literature**

- Diphetarson (Keystone JS, et al., 1983)
- Carbarsone (Dardick K., 1983)
- Tetracycline (Kean, BH, Malloch CL, 1966; Dardick K., 1983)
- Iodoquinol (Spenser MJ et al, 1982)
- Erythromycin (Preiss U et al., 1991)
- Paromomycin (Vandenberg et al, 2007).
- Metronidazole (Spenser MJ et al, 1979; Cuffari et al, 1998)
- Secnidazole (Girginkardeşler N, et al, 2003)
- Ornidazole (Kurt Ö, et al, 2008)

**A comparison of metronidazole and single-dose emidazole for the treatment of dientamoebiasis**

Ö. Kurt, N. Girginkardeşler, F. C. Delgatlı, A. Ocaköz and U. Z. ÖK

Department of Pathology, School of Medicine, Celal Bayar University, Manisa, Turkey

**ABSTRACT**

Recent reports of the pathologic potential of *Dientamoeba fragilis* have underlined the need for an effective treatment against this colon-dwelling parasite. Metronidazole is a well-known and commonly used anti-parasitic agent, but another 5-nitroimidazole derivative, emidazole, may be preferable, where available, because of its lower half-life and fewer side-effects. This study compared the efficacies of metronidazole and emidazole in a group of 152 patients with dientamoebiasis. Patients were randomly assigned two treatment groups: group 1 received metronidazole for 3 days, 20 mg/kg/day for children and 500 mg/day for adults, in three equal doses, while group 2 received a single oral dose of emidazole, 300 mg/kg/day for children and 2 g for adults. Stool samples were examined for metronidazole and emidazole by using thin-layer chromatography, and clinical cure was defined as complete resolution of symptoms. Both treatments were equally effective in eradicating metronidazole and emidazole, with both groups showing 92.8% vs. 84.6%, p = 0.039 and 91.5% vs. 96.5%, p = 0.003, respectively. Patients in the emidazole group reported fewer side-effects than patients in the metronidazole group, none of whom required termination of treatment. These results suggest that single-dose emidazole may be an important alternative agent for the treatment of dientamoebiasis.

**Keywords:** Antiprotozoal agents, *Dientamoeba fragilis*, dientamoebiasis, metronidazole, emidazole, treatment

Kurt - Dientamoeba, an underrecognized intestinal pathogen

TREATMENT ALTERNATIVES

CHILDREN

<table>
<thead>
<tr>
<th>AGENT</th>
<th>DOSE</th>
<th>Efficacy (%)</th>
<th>SIDE EFFECTS</th>
<th>REFERENCE</th>
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</thead>
<tbody>
<tr>
<td>MIONDAZOLE</td>
<td>3 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
</tr>
<tr>
<td>MIONDAZOLE</td>
<td>3 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
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<tr>
<td>PAROMOMYLINE</td>
<td>55 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
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<tr>
<td>METRONIDAZOLE</td>
<td>500 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
</tr>
<tr>
<td>KETOCONAZOLE</td>
<td>200 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
</tr>
</tbody>
</table>

TREATMENT ALTERNATIVES

ADULTS

<table>
<thead>
<tr>
<th>AGENT</th>
<th>DOSE</th>
<th>Efficacy (%)</th>
<th>SIDE EFFECTS</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
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<td>PAROMOMYLINE</td>
<td>75 mg/day</td>
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<tr>
<td>METRONIDAZOLE</td>
<td>500 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
</tr>
<tr>
<td>KETOCONAZOLE</td>
<td>200 mg/kg</td>
<td>90.0</td>
<td>None</td>
<td>Vanderslim 2007</td>
</tr>
</tbody>
</table>

TREATMENT OF DIENTAMOEBIA FRAGILIS INFECTION WITH PAROMOMYLINE

Paromomycin (95% anthraquinone, 96% bioassay weight) was administered in 15 children with Dientamoeba fragilis infection by oral administration at the dosages of 55 mg/kg of body weight once daily for 7 days.

15 children were treated with paromomycin at 75 mg/kg of body weight once daily for 5 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 7 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 14 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 28 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 56 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 84 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 112 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 140 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 168 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 200 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 250 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 300 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 350 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 400 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 450 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 500 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 550 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 600 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 650 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 700 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 750 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 800 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 850 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 900 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 950 days.

15 children were treated with paromomycin at 55 mg/kg of body weight once daily for 1000 days.
Kurt - Dientamoeba, an underrecognized intestinal pathogen

**IMPORTANT ISSUES CONCERNING TREATMENT**

- Patients should also be assessed for the pinworm (*E. vermicularis*) coinfection!
- Efficacy of treatment is assessed at least two times, with permanent smears.

**Briefly,**

*D. fragilis* should not be neglected!

- Prevalent…
- Significant cause of diarrhea in children…
- Symptomatic cases need treatment!

**THANK YOU...**

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Van Gool - Blastocystis, new insights in a frequently observed intestinal parasite

**Blastocystis spp. infection**

….an intriguing “new” field of interest….

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**Intestinal protozoa**

- E. histolytica
- Giardia lamblia
- Cryptosporidium
- Cyclospora
- Isospora
- D. fragilis

**Intestinal helminths**

- Ascaris
- Hookworm
- Taenia
- Strongyloides

**Intestinal protozoan parasites observed in routine clinical practice**

- Entamoeba histolytica
- Giardia lamblia
- Dientamoeba fragilis
- Cryptosporidium spp.
- Isospora belli
- Cyclospora cayetanensis
- Microsporidia spp

**Blastocystis hominis**

- Entamoeba dispers
- Entamoeba coli
- Entamoeba hartmanni
- Iodamoeba butschlii
- Endolimax nana
- Chilomastix mesnili

pathogens
non- pathogens
Van Gool - Blastocystis, new insights in a frequently observed intestinal parasite

**Diagnosis of intestinal parasites: microscopy still useful**

![](image1)

**Combination of use of a fixative and multiple stool testing increases sensitivity of microscopy**

![](image2)

**Intestinal protozoa observed with Triple Feces Test (TFT) in routine practice (n: 462) AMC**

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Diagnostic yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia intestinalis</td>
<td>24 (5,2)</td>
</tr>
<tr>
<td>Entamoeba histolytica/dispar</td>
<td>18 (3,9)</td>
</tr>
<tr>
<td>Dientamoeba fragilis</td>
<td>45 (9,7)</td>
</tr>
<tr>
<td><strong>Blastocystis “hominis”</strong></td>
<td><strong>124 (26,8)</strong></td>
</tr>
<tr>
<td>Entamoeba coli</td>
<td>65 (14,0)</td>
</tr>
<tr>
<td>Entamoeba hartmanni</td>
<td>23 (5,0)</td>
</tr>
<tr>
<td>Endolimax nana</td>
<td>47 (10,2)</td>
</tr>
<tr>
<td>Chilomastix mesnii</td>
<td>10 (2,2)</td>
</tr>
<tr>
<td>Iodamoeba butschli</td>
<td>12 (2,6)</td>
</tr>
</tbody>
</table>

*Most common protozoan parasite!*
Blastocystis infection in humans: the “common perception”….

*Blastocystis hominis* is a non-pathogen:
because of:
lack of difference in prevalence of infection in symptomatic and asymptomatic patients…

But….
many patients with complaints of abdominal pain, diarrhea, bloating and / or flatulence……
and only *Blastocystis* in stools……

→ Clinicians often are not convinced about non-pathogenicity and ask for treatment options!

Clinical symptomatology assumed, from (many) case reports, to be related to *Blastocystis* infection

Abdominal pain, acute and chronic diarrhea, bloating and flatulence

allergic cutaneous lesions, urticaria
(parasite molecules activation i.e. IL3, IL4 secreting Th2 cells mediating IgE responses)
Blastocystis hominis: until recently a total mystery!

- Taxonomic status unknown…
- Mode of transmission unknown….
- Clinical significance debated….
- Best diagnostic procedure undetermined….
- Effective therapy unknown…..

Where belongs Blastocystis taxonomically?

Is it a yeast, a fungus, a protozoan….?
Van Gool - Blastocystis, new insights in a frequently observed intestinal parasite

**Stages in lifecycle of Blastocystis spp.**

*a polymorphic protozoan:

1. Trophozoite form
2. Cyst form

**known stage in routine clinical practice:**

*Blastocystis “hominis”* in lugol’s iodine stain

**Morphological features**

- *Vacuolar form, 3-120 um*
- *Granular form*
- *Amoeboid form, 10-15 um*
- *Cyst form (2-5 um, survival 1 month 25°, 2 months 4°)*
Before availability of advanced molecular studies little knowledge and relative little scientific activity…

But, Blastocystis proved to have extensive genetic Diversity and the infection also proved common in many animals!

With a variety of molecular methods in the recent past many different "clades", "subtypes", "ribodemes", "subgroups" and "clusters" were described, resulting in increasing interest and…..much confusion…..

New nomenclature:
Blastocystis sp. with 9 subtypes in humans!

9 subtypes (species!)
Van Gool - Blastocystis, new insights in a frequently observed intestinal parasite

**Different “subtypes” = genetically different “species”**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>Subtype 1</td>
<td></td>
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<tr>
<td>Subtype 2</td>
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<tr>
<td>Subtype 3</td>
<td>14.2%</td>
<td>15.0%</td>
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<td></td>
</tr>
<tr>
<td>Subtype 4</td>
<td>12.3%</td>
<td>11.7%</td>
<td>11.5%</td>
<td></td>
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</tr>
<tr>
<td>Subtype 5</td>
<td>14.0%</td>
<td>14.2%</td>
<td>12.1%</td>
<td>12.3%</td>
<td></td>
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<tr>
<td>Subtype 6</td>
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<td>14.8%</td>
<td>16.1%</td>
<td>14.8%</td>
<td>14.8%</td>
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<td></td>
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<tr>
<td>Subtype 7</td>
<td>15.4%</td>
<td>15.7%</td>
<td>14.8%</td>
<td>14.0%</td>
<td>14.0%</td>
<td>14.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtype 8</td>
<td>12.1%</td>
<td>12.7%</td>
<td>10.9%</td>
<td>6.7%</td>
<td>12.5%</td>
<td>15.7%</td>
<td>13.4%</td>
<td></td>
</tr>
<tr>
<td>Subtype 9</td>
<td>13.4%</td>
<td>13.8%</td>
<td>14.8%</td>
<td>15.0%</td>
<td>13.4%</td>
<td>5.0%</td>
<td>14.4%</td>
<td>15.2%</td>
</tr>
</tbody>
</table>

**Comparison:**
- for 615 nucleotides of the same locus, difference
  - Entamoeba histolytica/E. dispar 1.1% an E. histolytica and E. hartmanni 11.2%.
- for 2245 nucleotides of the same locus, difference Plasmodium vivax and
  - P. malariae 7.7% and P. vivax and P. falciparum is 11.7%

**Blastocystis subtypes are as different as different Plasmodium species**

Different subtypes may indicate different epidemiological characteristics, i.e. reservoirs and ways of transmission

Probably humans carry its “own” Blasto-subtype(s) but also zoonotic Blasto spp.

Transmission of *Blastocystis*:
- human-to-human, animal-to-human
- animal-to-animal, human-to-animal routes
Transmission of Blastocystis subtypes between human and animals

Suggestion of transmission of different subtypes (Tan et al. 2008)

Mode of infection: prevalence of human Blastocystis in four different epidemiological settings in China (no. 2316, 192 isol.)

M: no. 407/75 isol. (18 %) Y: no. 170/10 (6 %)
E: no. 239/78 isol. (33 %) S: no. 1500/29 (2 %)

Age-prevalence: peak in younger age groups in highly endemic settings, in low endemic set in higher age groups

Mehai: also high prevalences of intestinal helminths
Lan Huai Li, Parasitological Research 2007

Blastocystis is extremely ubiquitous parasite with a worldwide distribution

In general higher prevalences of Blastocystis infection in developing countries which is related to poor hygiene, exposure to animals and consumption of contaminated food and water

Travellers from western countries increased risk of infection in tropical countries?
Proper diagnosis of *Blastocystis* spp.:
a necessity for any further study
but ......still no clarity which technique is best!

<table>
<thead>
<tr>
<th>Diagnostic methods for <em>Blastocystis</em> from stools</th>
<th>Assumed sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy: Ridley concentration from stool</td>
<td>poor</td>
</tr>
<tr>
<td>Microscopy: permanent stains from fixed stool(s)</td>
<td>good (?)</td>
</tr>
<tr>
<td>Culture in i.e Jones’ medium</td>
<td>best (?)</td>
</tr>
<tr>
<td>Molecular diagnosis from culture</td>
<td>best (?)</td>
</tr>
<tr>
<td>Molecular diagnosis direct from stools</td>
<td>best (?)</td>
</tr>
</tbody>
</table>

Diagnosis and subtyping of *Blastocystis* spp.
in Academic Medical Center, Amsterdam

- Comparison diagnostic sensitivity and specificity
  - Microscopic examination with Triple Feces Test
  - Sequence confirmed PCR direct from stools
- Which subtypes / species of *Blastocystis* are present in patient population in AMC?
Methods

**Triple Feces Test**: microscopic examination of direct iodine smear on SAF fixed samples no. 1 and 3

- TFT package for patients
- Examination of two samples of each patient

**Vacuolar stage of Blastocystis**

Molecular diagnosis

DNA isolation directly from feces sample 2

- PCR of small subunit ribosomal RNA gene (SSU rRNA) with F1 and BHCRseq3 primers
- Agarose gel electrophoresis of ~550 bp product
- Unidirectional sequencing of PCR product
- Comparison to reference sequences using CodonCode and MEGA

subtype 1
subtype 2
subtype 3
subtype 4

Results:

442 consecutive patients. 102 both negative with microscopy and PCR and 335 positive with both tests. Only 5 discrepancies.

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>Sequence confirmed PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>102</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>335</td>
</tr>
</tbody>
</table>
Van Gool - Blastocystis, new insights in a frequently observed intestinal parasite

Prevalence and subtyping of Blastocystis spp. in Academic Medical Center, Amsterdam

Overall prevalence 22.3%

Blastocystis typing: different species within families

Family A1 infected with one identical species

- ST2

Family B2 infected with different species or not infected

- ST3

- ST1

Studies with similar molecular method: Amsterdam and Brussels

prevvalence of Blastocystis subtypes

Academic Medical Center, Amsterdam (n=442)

Hôpital St Pierre, Brussels (n=400)
Different pathogenic potential with different subtypes/ species?


Pathophysiological variability of different genotypes of human Blastocystis hominis Egyptian isolates in experimentally infected rats:
- Isolate from symptomatic patients: severe abnormalities in rats
- Isolate from asymptomatic patients: mild pathology

Subtype 1 pathogenic, variants among 3 and 4

Intra-subtype variation of Blastocystis subtypes

Importance: pathogenicity, treatment follow-up

Blastocystis

- Subtype 1
  - 1a
  - 1b
- Subtype 2
  - 2a
  - 2b
- Subtype 3
  - 3a
  - 3b
- Subtype 4
- Subtype 5
- Subtype 6
- Subtype 7
- Plasmodium
  - falciparum
  - vivax
  - knowlesi
  - malariae
  - Variant type ovale
  - Classic type ovale

NJ-tree based on alignment of 432-544 nucleotides of SSU rRNA gene with pairwise gap deletion

Therapy of Blastocystis spp.

a clear matter?
Several options for therapy: according to literature several quite succesfull!

<table>
<thead>
<tr>
<th>Study type</th>
<th>Drug administered</th>
<th>Number of patients</th>
<th>No. of patients</th>
<th>No. of days</th>
<th>Description of treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Metronidazole</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>Given for 10 days</td>
<td>90%</td>
</tr>
<tr>
<td>Adults</td>
<td>Tinidazole</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>Given for 15 days</td>
<td>95%</td>
</tr>
</tbody>
</table>

However….treatment failure not uncommon: diagnostic problems, resistance, other subtypes?

<table>
<thead>
<tr>
<th>Study type</th>
<th>Drug administered</th>
<th>Number of patients</th>
<th>No. of patients</th>
<th>No. of days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Metronidazole</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Adults</td>
<td>Tinidazole</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

Current therapy of Blastocystis spp. (Stensvold, JCG 2010)

<table>
<thead>
<tr>
<th>Study type</th>
<th>Drug administered</th>
<th>Number of patients</th>
<th>No. of patients</th>
<th>No. of days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Metronidazole</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Adults</td>
<td>Tinidazole</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>
More research needed to identify new and effective drugs for Blastocystis spp. infection!

Effective drug treatment also of importance to get insight in presumed pathogenicity:

….true clinical significance of Giardia for humans only recognized when drugs were available to eliminate the parasite…..

Conclusion

△ Blastocystis a most common occurring intestinal parasite in humans
△ Asymptomatic carriage of Blastocystis spp. is most common. In some patients infection can be associated with symptomatology
△ Humans are not infected with one Blastocystis species ("hominis") but with many (up to 9) different subtypes or species
△ Subtypes 1, 2, 3 and 4 are most common among humans
△ Relation between subtypes / species and pathology is not (yet) established, many studies are ongoing
△ Microscopic examination of fixed stool samples and seq. confirmed PCR direct from stools are reliable for diagnosis and subtyping.
△ Proper therapy is not known. Metronidazole, Co-trimoxazol and Nitazoxanide can be options.

Acknowledgements

Aldert Bart, Section Parasitology, AMC
Olivier Vandenberg, Saint Pierre Ziekenhuis, Brussels
Technicians, AMC and Hospital Saint Pierre
Van Gool - Blastocystis, new insights in a frequently observed intestinal parasite

Blastocystis research: a booming field!
Cryptosporidium, Isospora and Cyclospora: Clinical Importance and Outbreak Management

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Several common features between Cryptosporidium, Cyclospora and Isospora

- Protozoa, Apicomplexa, Coccidia
- Sexual and asexual replication in the intestinal mucosa
- Shedding of oocysts in stools
- Resistance of oocysts in the environment

In humans
- Oral contamination
- Responsible for outbreaks (Cryptosporidium, Cyclospora)
- Disease: diarrhea, opportunistic infections (Cryptosporidium, Isospora)
- Diagnosis in fecal samples

Specificities of intestinal life cycles

Cryptosporidium
Shedding of sporulated (infective) oocysts

Intracellular but extracytoplasmic replication

Source: Chen et al. NEJM. 2002
Source: ANOFEL CD Rom 3
Specificities of intestinal life cycles

**Isospora, Cyclospora (e.g.)**
- Shedding of unsporulated oocysts
- Intracytoplasmic replication

Source: Ortega & Sanchez CML, 2010

Cryptosporidium spp and cryptosporidiosis

Cryptosporidium species infecting humans

<table>
<thead>
<tr>
<th>Species</th>
<th>Major hosts</th>
<th>Minor hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. hominis</td>
<td>Humans</td>
<td>Humans, rock hyrax, mountain goat</td>
</tr>
<tr>
<td>C. parvum</td>
<td>Cattle, livestock,</td>
<td>Deer, mice, pigs</td>
</tr>
<tr>
<td></td>
<td>humans</td>
<td></td>
</tr>
<tr>
<td>C. muris</td>
<td>Rodents</td>
<td>Humans, deer, cattle</td>
</tr>
<tr>
<td>C. suis</td>
<td>Pigs</td>
<td>Humans</td>
</tr>
<tr>
<td>C. felis</td>
<td>Cats</td>
<td>Humans, cattle</td>
</tr>
<tr>
<td>C. canis</td>
<td>Dogs</td>
<td>Humans</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Deer, cattle</td>
<td>Humans</td>
</tr>
<tr>
<td>cervine genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. meleagridis</td>
<td>Turkeys</td>
<td>Humans, parrot</td>
</tr>
</tbody>
</table>

= zoonosis

Derouin - Cryptosporidium, Isospora and Cyclospora: clinical importance and outbreak management

**Distribution of Cryptosporidium spp. in immunocompetent individuals (%)**

- **Peru (533 children)**
  - 16: C. hominis
  - 9.6: C. parvum
  - 2.1: C. meleagridis
  - 4.3: C. canis
  - 2.1: C. felis

  - 9.6: C. hominis
  - 0.5: C. parvum
  - 3.5: C. meleagridis
  - 49.2: C. canis

**Clinical symptoms**

1. Immuneocompetent individuals

   **Incubation time:** 7 +/- 2 days

   **Signs:** Non-bloody watery diarrhea, abdominal pain, asthenia, nausea, vomiting. Moderate fever in 40-60% of cases

   **Weight loss:** (mean 4.5 kg), 50-75% of cases

   No difference for intestinal symptoms between C. hominis and C. parvum cases

   **Non gastrointestinal symptoms** (joint pain, eye pain, recurrent headaches, dizzy spells, fatigue)

   Significantly more frequent in patients infected with C. hominis compared to C. parvum

   **Duration of symptoms:** 12 +/- 6 days (up to 40 days)

   Transient relapses (2 days) may occur in up to 30% of cases.

   **Sequela:** intestinal, non gastrointestinal, impact on nutritional status and growth.

   **Oocyst shedding:** 3-8 days (up to 90 days)

2. HIV infected patients

   **Intestinal symptoms**

   Severe and persistent diarrhea leading to chronic malabsorption

   Abdominal pain, vomiting

   Weight loss +++

   Severity and duration of symptoms: increase with CD4 counts decrease.

   **Biliary involvement**

   In up to 30% of cryptosporidiosis cases

   Papillary stenosis, sclerosing cholangitis, acalculous cholecystitis.

   Gastric involvement may present as nausea and vomiting, with or without diarrhea.

   **Cryptosporidial pneumonitis**

   Occasionally reported: probable aspiration of organisms from the GI tract.

   **Outcome:**

   In patients who fail to respond to therapy, death usually occurs in 3-6 months.

Sources: Cama et al. EID 2008, Chalmers et al. Eurosurveillance 2009
Geographical distribution of cryptosporidiosis

### Worldwide distribution
Up to 10% in developing countries with poor sanitary conditions
- 0.5 to 2% in developed countries
- HIV infected patients
  - Before HAART: 3 to 25% according countries
  - 50-90% reduction of incidence after HAART

### In Europe:
- Collected and recorded by health agencies in several European countries
- Confirmed cases from 16 countries are reported to the European Basic Surveillance Network (BSN)
- No "official" report from many European countries
- Information lacking for HIV-infected patients

<table>
<thead>
<tr>
<th>Country</th>
<th>Case</th>
<th>HIV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>27</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Ireland</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>


### Age distribution
- **England and Wales**
  - *C. parvum*: mean age 15 years, median: 8 years
  - *C. hominis*: mean age 17, median 9 years

- **Ireland**
  - Large predominance of cases in children

- **France**
  - Majority of cases in adults. Peak in children <4 years of age. No significant difference between *C. hominis* and *C. parvum*

- **Spain**
  - 40-90% <15 years


### Seasonal variations
- **England and Wales**
  - Spring peaks due to *C. parvum*, *C. hominis* more prevalent during the late summer and early autumn and in patients reporting recent foreign travel.

- **Ireland**: spring peak only

- **France**: 2 peaks with no significant difference in species distribution

- **Spain**: seasonal peak in June

- **Germany, Sweden**: seasonal peak in autumn

### Risk factors

**Strongly associated with illness (general population)**:
- Travel outside the United Kingdom (C. hominis)
- Contact with another person with diarrhea.
- Helping a child <5 years of age to use the toilet (C. hominis)
- Number of glasses of tap water drunk at home each day

**Associated with illness in HIV infected patients**:
- Increased susceptibility according to CD4 counts:
  - x 2 for CD4 counts of 500-1000/mm³
  - x 3.6 for CD4 of 100-200/mm³
  - x 6 for CD4 counts <100/mm³

**Other susceptible immunocompromised patients**:
- Transplant patients: case reports in renal, liver, intestinal and SCT recipients but no prospective study on prevalence
- Primary immunodeficiencies: X-linked Hyper IgM syndrome with CD40 ligand deficiency; higher susceptibility to Cryptosporidium infection


### Outbreaks of cryptosporidiosis in Europe

- Large variation according to the nature of the surveillance systems and to the quality of the public water supply.
- Probable underestimation in some countries
- Major outbreaks: contaminated drinking water
- Others causes: fecal contamination of swimming pools or recreational areas, foods, person to person transmission (child care facilities)

**England and Wales**:
- 149 cryptosporidiosis outbreaks between 1983 and 2005*
  - 55 linked to municipal drinking water supply
  - 6 to private water supplies
  - 43 to swimming pools
  - 16 to contact with animals


### Outbreaks of cryptosporidiosis associated with water. USA 1984-2007 (n=147)

![Graph showing outbreaks associated with water](image)

Source: Yoder Exp. Parasitol. 2010
Isospora belli and Cyclospora cayetanensis

Clinical symptoms

- **Incubation time:** estimated at 1 week for Isospora
- **Immunocompetent**
  Acute, watery diarrhea, abdominal pain and cramps. Low grade fever.
  More severe in children and in elderly patients.
  Duration of symptoms: Usually self limited within 2-3 weeks; oocyst shedding may persist for 2 to 3 weeks after the patient recovers.
- **Immunocompromised**
  Isospora: Profuse diarrhea. Long persistence of symptoms; several months or more.
  Malabsorption, weight loss, dehydration (possible life-threatening).
  Alithiasic cholangitis and cholecystitis, reactive arthritis, and rare disseminated infection. High rate of recurrences (50%).
  Cyclospora: More severe symptoms, higher weight loss and longer duration than in immunocompetent patients. Cases report of biliary involvement (acalculous cholecystitis).

Epidemiology

- **Geographical distribution**
  Worldwide, but largely predominant in tropical and subtropical countries.
  High prevalence in Haiti, Guatemala, Peru, Nepal, Southeast Asia.
  Identified risk factors in endemic countries: consumption of untreated water, lack of adequate sanitation.
  In Europe: mainly observed in travelers returning from endemic countries and AIDS patients. Isosporiasis more frequent than Cyclosporiasis. Rare Cyclospora outbreaks.

- **Outbreaks**
  Isospora: 3 limited waterborne outbreaks
  Cyclospora: 32 reported outbreaks between 1990 and 2009 (12 to 1475 patients)
  Most are classified as foodborne outbreaks (raspberries, berries, salad, fruits, fresh basil). Probably related to contamination of field watering systems and parasite retention on the fruit/leaf.

Laboratory diagnosis and treatment

Diagnosis: demonstration of oocysts in fecal samples

<table>
<thead>
<tr>
<th></th>
<th>Cryptosporidium</th>
<th>Isospora</th>
<th>Cyclospora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>4.5-6.5 μm, spherical</td>
<td>23-36x12-17 μm, ovoid</td>
<td>8-10 μm, spherical</td>
</tr>
<tr>
<td>Modified Ziehl-Neelsen acid fast</td>
<td>Acid fast, safranin, auramine, immunofluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstained, light microscopy</td>
<td>Alternative: MZN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autofluorescence</td>
<td>Alternative: Phase contrast microscopy, MZN, safranin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen</td>
<td>Immuno chromatography, 70-90% sensitivity for C. parvum, C. hominis infections, 20-30% for other species</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>PCR</td>
<td>Yes, Well developed but not commercialized, RT-PCR, genotyping</td>
<td>Yes (specialized laboratories)</td>
<td>Yes (specialized laboratories)</td>
</tr>
</tbody>
</table>

* ANOFEL cryptosporidium network, unpublished

Treatment

Cryptosporidiosis
Few drugs have some activity and none is curative.
Nitazoxanide: Reduction of symptoms and parasitic load in immunocompetent individuals
No evidence for a significant reduction in the duration or frequency of diarrhea in patients with AIDS (only used as compassionate treatment)
Paromomycin: Anticytosporidial activity in vitro and in animal models
Reduce clinical symptoms without eradicating the parasites in immunocompetent patients. In AIDS patients, not more effective than placebo

Isosporiasis, cyclosporiasis
Cotrimoxazole:
Trimethoprim-sulfamethoxazole (TMP-SMX): first line treatment: 960mg twice daily for 7 days
Ciprofloxacin
Ciprofloxacin is an acceptable alternative if TMP-SMX is contra-indicated: 500mg twice daily for 7 days
High rate of relapses in HIV-infected patients: need for secondary prophylaxis as CD count remain low
Other alternative
Pyrimethamine (50mg-75mg per day)

Prevention and control

Prevention

Preventing contamination:
- Preventing contamination of water resources and foods
- Individual recommendations (especially for immunocompromised patients):
  - Food and hand hygiene +++
  - Avoid contact with human feces (diaper changing), infected animals, farm animals (for Cryptosporidium)
  - Avoid drinking untreated raw water
  - Be aware that bathing in lakes, rivers, outdoor recreational areas, swimming pools might be contaminated
  - Precautions with drinking tap, ice, fruits, fresh fruit juice. Preferential consumption of bottled water and pasteurized juices
  - Reinforced recommendations when traveling (especially in countries with low sanitary conditions)

Chimioprophylaxis: Not recommended

In immunocompromised patient: immune reconstitution has resulted in a marked decrease of prevalence in HIV-infected patients
= probable best preventive measure

Impact of Water Regulation (UK)

Drinking Water Inspectorate:
Section 70 of the 1991 Act makes it a criminal offence for a water company to supply water that is unfit for human consumption.
It is a regulatory duty on water companies to notify DHM of any event which has the potential to give rise to a significant risk to public health

Total number of case patients associated with waterborne outbreaks of infectious intestinal disease.
- Public water supplies
- Private water supplies
- Other water supplies
- Swimming pools

Source: Smith et al. EID 2006
## Impact of efficient water treatment processes

Cases of primary cryptosporidiosis per 100,000 person-years before and after membrane filtration introduced into public water supplies (England)

<table>
<thead>
<tr>
<th>Rate</th>
<th>Mar 1, 1986-Feb 29, 2000</th>
<th>Mar 1, 2000 - Aug 31, 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Goh et al EID 2005

## Key points for investigation and management of a Cryptosporidium waterborne outbreak (drinking water)

- Confirm the Cryptosporidium origin of the outbreak and estimate its extent
- Epidemiological investigation: random digit telephone surveys and/or case-control studies
- Case record and notification to public health authorities
- Confirm diagnosis: local or regional expert labs
- Test tap water and resources for Cryptosporidium (US-EPA 1622, NF T90-455.)
- Keep the isolates for further genotyping in reference laboratories
- Mobilization and information
  - Mobilize community partners (local health departments, laboratories, daycare centers,)
  - Provide basic hygiene messages for the public, including immunocompromised patients (hot-line)
  - Warning recommendations (UK)
    - Do not use for drinking and food preparation
    - Do not use for drinking or cooking
    - Do not use for drinking, cooking or washing: limited to major risks
  - Recommendations to prevent person to person transmission
- Engage the media to help disseminate these public health messages
- Provide safe water
  - Tanks, other water resource (≥10L/person/day), bottle water
- Consider adding supplemental disinfection systems to the water treatment unit (UV, filtration)
- Identify the possible origin and technical causes of contamination and improve water quality


## Conclusions

- Probable underreporting of cryptosporidiosis, isosporiasis, cyclosporiasis and consequences of infection.
  - Improve diagnostic practice in laboratories
  - Develop educational information for the population and for practitioners (misknowledge diseases)
  - Investigate long term consequences of infection: sequelae, nutritional status, link with inflammatory diseases (cancer?)
- Risk assessment of environmental and food related risks
  - Need for cost-effective methods to identify oocysts in water and in foods
  - Case reporting at a national /European level
- Prevention and treatment
  - Education of risk factors and hygiene
  - Promote research on anti-Cryptosporidium drugs and disinfectants

40
Treatment failure of intestinal protozoal infection

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Parasitological and/or clinical treatment failure?
Parasitological treatment failure: persistence of intestinal parasites 5-7 days after end of first line therapy (microscopy/PCR)
– « True » drug resistance (documented mechanisms): giardiasis, intestinal amoebiasis
– Limited effect of pharmacological compounds: cryptosporidiosis

Clinical treatment failure
– Reinfection
– Post-infectious syndromes

Major human intestinal protozoal pathogens
• Giardia duodenalis
• Entamoeba histolytica
• Cryptosporidium sp.
• Isospora belli
• Cyclospora cayetanensis
• Dientamoeba fragilis
Treatment failure of *Entamoeba histolytica* infection: context

- **Current chemotherapeutic: association (adults):**
  - nitro-5 imidazoles: Metronidazole 500mg to 750mg tid for 7d or tinidazole 800mg tid for 5d
  - lumen-acting drugs: diloxanide furoate (500mg t.i.d. for 10d) or tiliquinol (100mg b.i.d. for 10d) or paromomycin (10mg t.i.d. for 7d)

- **Efficacy:** 90%-100%; treatment failures are only occasionally observed

Treatment failure of *Entamoeba histolytica* infection: mechanisms

(Wassmann et al., J Biol Chem, 1999)

- Metronidazole resistance
- Detoxification
Favennec - Treatment failure of intestinal protozoal infections

Treatment failure of *Giardia duodenalis* infection

*Giardia duodenalis* genotypes from human isolates

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Major host(s)</th>
<th>Minor hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>A I</td>
<td>human, livestock,</td>
<td>dog, cat, gorilla, guinea pig</td>
</tr>
<tr>
<td></td>
<td>beaver</td>
<td></td>
</tr>
<tr>
<td>A II</td>
<td>human</td>
<td></td>
</tr>
<tr>
<td>B III</td>
<td>human, beaver</td>
<td>dog, musked rat, chinchilla</td>
</tr>
<tr>
<td>B IV</td>
<td>human</td>
<td></td>
</tr>
</tbody>
</table>

Epidemiology of human giardiasis

Risk factors for of human 
giardiasis

- Nappy changing (Hoque et al., Epidemiol. Infect., 2003)
- Travelling abroad (Espelage et al., BMC Public Health, 2010)
- Consuming daily green salad (Espelage et al., BMC Public Health, 2010)
- Immunocompromise (Espelage et al., BMC Public Health, 2010)
- Drinking raw water (Hoque et al., Epidemiol. Infect., 2003)
- Oro-anal sex (Sandberg et al., AIDS Educ Prev, 2005)
- Regular use of swimming pool (at least once a week) (Hoque et al., Epidemiol. Infect., 2003)

Biological diagnosis of human 
*Giardia duodenalis* infection

- Conventional methods (flotation or sedimentation techniques): repeated tests are necessary (Goka et al., Trans R Soc Trop Med Hyg, 1990)
- Immunofluorescent assays appear more sensitive than conventional methods or antigen detection (ELISA or immunochromatographic assay) (Selim et al., J Egypt Soc Parasitol, 2009)
- Real-time PCR appears to be a « gold standard » method (Calderaro et al., Diag. Microb. Infect. Dis., 2009)

Current therapies for *Giardia duodenalis* infection: nitro-5 imidazoles

**Efficacy of nitro-5 imidazoles in human giardiasis (randomized control clinical trials)** (Gussi et al., Biologics, 2004)

<table>
<thead>
<tr>
<th>Agents</th>
<th>Dose (adults)</th>
<th>Cure rate: mean ± SD (range, number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>metronidazole</td>
<td>250mg t.i.d.x 7d</td>
<td>81.5 ± 18.6 (71.0 - 92.0, n=21)</td>
</tr>
<tr>
<td>tinidazole</td>
<td>2g s.d.x 1</td>
<td>91.1 ± 6.3 (87.2 - 95.0, n=10)</td>
</tr>
<tr>
<td>ornidazole</td>
<td>2g s.d.x 1</td>
<td>97.6 ±2.5 (95.4-99.8, n=3)</td>
</tr>
<tr>
<td>secnidazole</td>
<td>2g s.d.x 1</td>
<td>79.4</td>
</tr>
</tbody>
</table>

Side effects:
- more pronounced for metronidazole
- abdominal pain, nausea, diarrhea, modification of taste
Current therapies for *Giardia duodenalis* infection: benzimidazoles

Efficacy of benzimidazoles in human giardiasis (randomized control clinical trials) (Busatti et al., Biologics, 2009)

<table>
<thead>
<tr>
<th>Agents</th>
<th>Dose (adults)</th>
<th>Cure rate: mean ± SD (range, number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>albendazole</td>
<td>400 mg - 5 days</td>
<td>73.4 ± 19.8 (58.7 – 88.1, n=9)</td>
</tr>
<tr>
<td>mebendazole</td>
<td>200 mg t.i.d. - 5 days</td>
<td>65.6 ± 17.3 (50.4 – 80.8, n=8)</td>
</tr>
</tbody>
</table>

No significant side effect

Current therapies for *Giardia duodenalis* infection: thiazolides

Efficacy of thiazolides in human giardiasis (randomized control clinical trials) (Busatti et al., Biologics, 2009)

<table>
<thead>
<tr>
<th>Agents</th>
<th>Dose (adults)</th>
<th>Cure rate: mean ± SD (range, number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitazoxanide</td>
<td>500mg b.i.d. x 3d</td>
<td>79.7 ± 1.8 (77.2 – 82.2, n=3)</td>
</tr>
</tbody>
</table>

No significant side effect

Other therapies for *Giardia duodenalis* infection

- furazolidone: 100 mg q.i.d. x 10d
  Side effects: gastrointestinal disturbance

- quinacrine: 100 mg t.i.d. x 5d
  Side effects: hepatitis, anemia

- paromomycin: 500 mg t.i.d. x 10d
  Side effects: nausea, abdominal cramps, diarrhea
Mechanisms of nitro compound *Giardia duodenalis* resistance

- Metronidazole resistance
- Furazolidone and quinacrine resistance

Mechanisms of benzimidazole and nitazoxanide *Giardia duodenalis* resistance

- **Benzimidazole:**
  - Changes in cytoskeletal structure (Upcroft et al., Microb Drug Resist, 1996)
  - Changes in the expression of enzymes involved in energy metabolism and ATP production (Arguello-garcia et al., Infect. Gen. Evol., 2009)

- **Nitazoxanide**
  - Overexpression of the therapeutic target: protein phosphatase isomerases 2 and 4 (Muller et al., JAC, 2008)

**In vitro* Giardia duodenalis* chemosensitivity assays**

- **Methods**
  - Axenic cultures in tubes or microwells (Boeren et al., JAC, 1994)
  - Adherence to fibers (Crouch et al., Trans R Soc Trop Med Hyg, 1986)
  - Adherence to enterocytic cell culture (Favennec et al., Parasitol Res, 1992)

- **Limitations**
  - Need for previous excystation
  - Usually proposed for the study of new compounds
  - General limitations of *in vitro* methods
**In vivo evaluation of resistance in Giardia duodenalis**

- Animal model: neonatal mice (Lemée et al., JAC, 2000)
  - association between treatment failure and high ID$_{50}$ for:
    - metronidazole: ID$_{50}$ >120mg/kg
    - albendazole: ID$_{50}$ >40mg/kg
  - Limitation: need of previous excystation

**Why it is important to give correct treatment: PI IBS**

- Post-infectious irritable bowel syndrome (PI-IBS) is a common disorder wherein symptoms of IBS begin after an episode of acute gastroenteritis.
- Reported incidence: 5% to 32%
- Mechanisms not fully understood (sub clinical gut inflammation, changes in intestinal permeability, aeration of gut flora)
- First described with bacterial pathogen (Salmonella, Shigella, Campylobacter)
- Recently, post giardiasis IBS (mainly diarrheic patients)
- Occurrence in 4% of patients (Robertson et al., Trends Parasitol, 2010)
- Post giardiasis symptoms include abdominal symptoms and chronic fatigue syndrome (March et al, BMJ Infect Dis, 2009)

**Management of persisting clinical symptoms after initial giardiasis therapy**

- Three coprological examinations within one week 8 days after the end of therapy

  - No parasite shedding: consideration of nitroimidazole side effects or post infectious symptoms.
  - Yes: second line one day therapy with nitro-5 imidazole agent (secnidazole, tinidazole or ornidazole)
  - Parasite shedding: Is it due to bad drug compliance?
  - No: are there risk factors for reinfection?
  - Yes: reduction of risk factors and/or treatment of children/relatives
  - No: resistance?
Management of *Giardia duodenalis* resistance to therapy

- **Is the patient immunocompromised?**
  - Yes: double dose therapy
    - **Success**
    - Change therapy: Switch to benzimidazoles
  - **Failure**
    - Failure: combined therapy: metronidazole + albendazole
      (Cacopardo et al., Clin. Ther., 1995)
    - Success
  - **Failure**
    - Failure: nitazoxanide (Abboud et al., CID, 2001) or quinacrine
      (Brasseur and Favennec, Parasite, 1996) or paromomycin therapy

Key points for investigation and management of treatment failure of human giardiasis

- Confirm that clinical treatment failure is possibly related to drug resistance:
- Check the persistence of parasite in the stool after treatment to eliminate post infectious symptoms
- Look for risk factors for reinfection and/or immunocompromise
- Management of *Giardia duodenalis* resistance to therapy
  - Switch to benzimidazoles
  - Combine therapy: metronidazole and albendazole
  - Use other therapies: nitazoxanide, quinacrine, paromomycin

Conclusion

- The frequency of human giardiasis treatment failure associated with drug resistance remains unknown
- Until now, the mice model remains the only one that may confirm « true » drug resistance
- The limited number of anti-giardial compounds available in Europe underlines the need of research in this field.