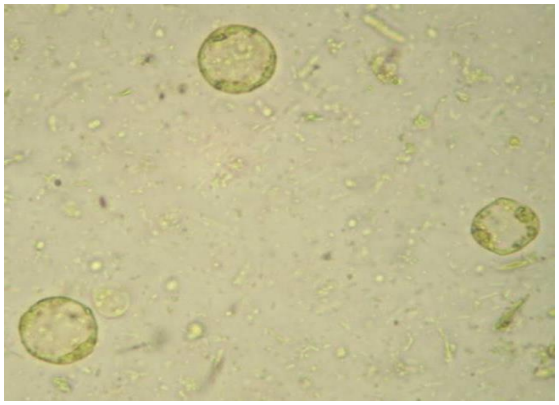
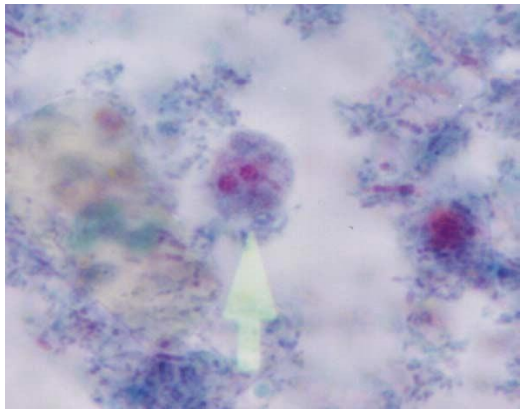




Blastocystis and *Dientamoeba fragilis*: Two Zoonotic Agents or Residents of a Healthy Gut?

Prof. Özgür KURT, M.D. Ph.D.
Acıbadem University School of Medicine
Department of Medical Microbiology,
Ataşehir 34752 – İSTANBUL, TURKEY

CONTENT OF PRESENTATION

- **The aim of this presentation**
- **Basic and latest information about *Dientamoeba fragilis***
- **Basic and latest information about Blastocystis**
- **Discussion:**

  "Projections to future: Friend or foe?"

ECCMID 2010, Vienna: Workshop on Intestinal Parasites

KURT Ö: **"*Dientamoeba*, an underrecognised intestinal pathogen"**

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

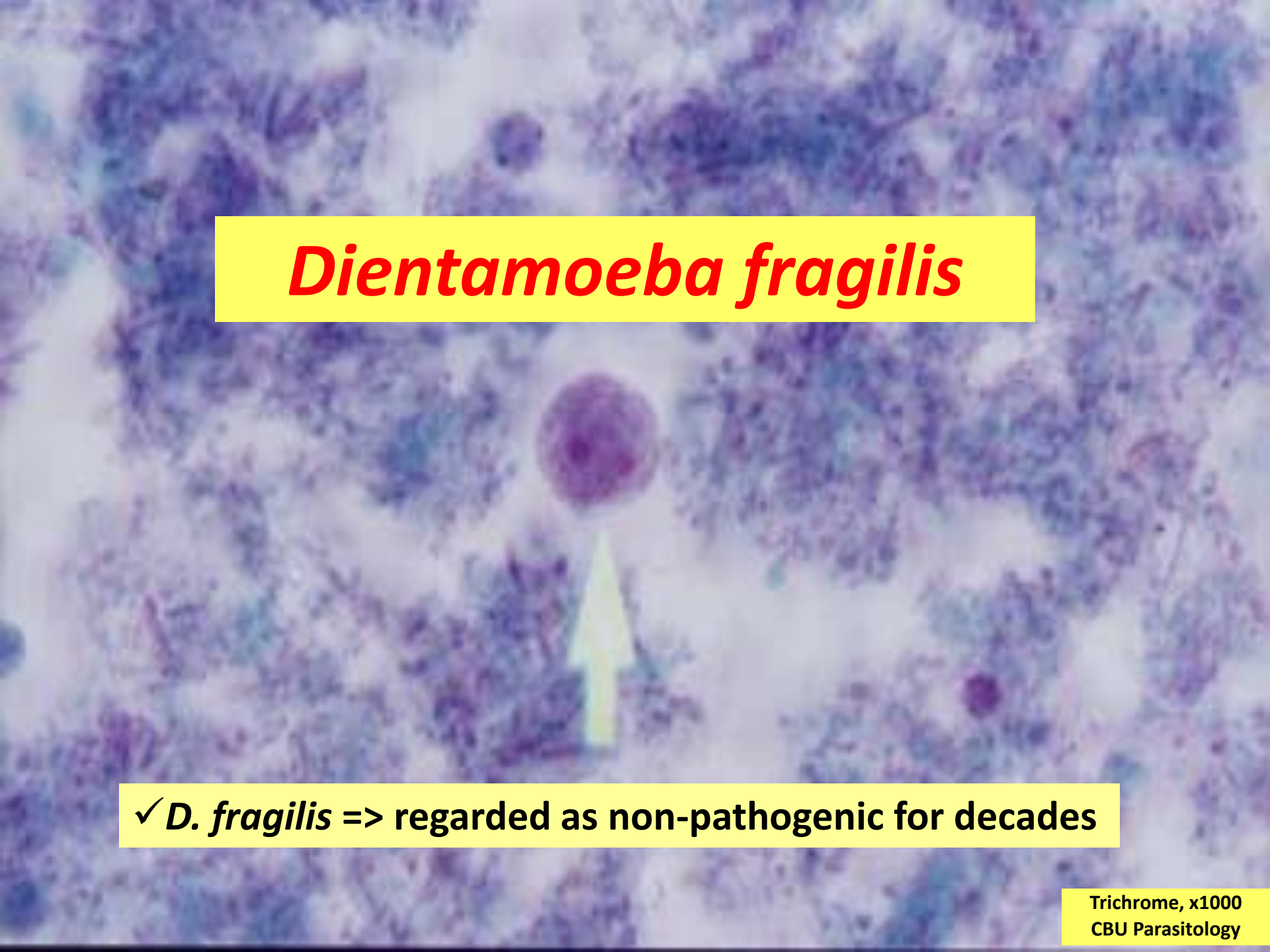
Successive diarrhea-constipation periods

No permanent relief

* Records of Celal Bayar University (CBU) Parasitology Laboratory (2010)

All these cases were found to have only one causative agent common…

Dientamoeba fragilis



✓*D. fragilis* => regarded as non-pathogenic for decades

Trichrome, x1000
CBU Parasitology

Case Report

Blastocystis sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms

Christian Vogelberg [a], Christen Rune Stensvold [b], Stefan Monecke [c], Anette Ditzen [d], Konrad Stopsack [e], Uta Heinrich-Gräfe [f], Christoph Pöhlmann [c,*]

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[b] Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Artillerivej 5, DK-2300 Cope
[c] Institute of Medical Microbiology and Hygiene, Dresden University of Technology, Fetscherstr. 74, 01307 Dre
[d] Department of Internal Medicine I, University Hospital Schleswig-Holstein Campus Kiel, Schittenhelmstr. 12,
[e] Department of Microbiology, Tumor and Cell Biology, Division of Clinical Microbiology, Karolinska Institutet,
[f] Institute of Clinical Pharmacology, Dresden University of Technology, Fiedlerstr. 27, 01307 Dresden, Germany

ARTICLE INFO

Article history:
Received 14 January 2010
Received in revised form 25 March 2010
Accepted 27 March 2010
Available online 2 April 2010

Keywords:
Blastocystis
Urticaria
Subtype 2

ABSTRACT

Blastocystis is a common unicellular intestir
discussion with numerous conflicting rep
heterogeneity among isolates suggests an a
although a clear correlation between sympt
which possibly links *Blastocystis* sp. ST2 infect
and generalized chronic urticaria. Despite re
the gastrointestinal and cutaneous disorders
the parasite and permanent resolution of t
combined application of metronidazole and

Clin Microbiol Infect. 2008 Jun;14(6):601-4. doi: 10.1111/j.1469-0691.2008.02002.x. Epub 2008 Apr 5.

A comparison of metronidazole and single-dose ornidazole for the treatment of dientamoebiasis.

Kurt O[1], Girginkardeşler N, Balcioğlu IC, Ozbilgin A, Ok UZ.

Author information

enic potential of Dientamoeba fragilis have underlined the need for an effective
dwelling protozoan. Metronidazole is a well-known and commonly used anti-
5-nitroimidazole derivative, ornidazole, may be preferable, where available,
and fewer side-effects. This study compared the efficacies of metronidazole and
patients with dientamoebiasis. Patients were randomised into two treatment groups:
tronidazole for 5 days, 20 mg/kg/day for children and 1.5 g/day for adults, in three
= 56) received a single oral dose of ornidazole, 30 mg/kg for children and 2 g for
xamined on the seventh and 14th days after treatment, and clinical symptoms were
acy of treatment. A statistically significant difference was recorded between the
metronidazole, both parasitologically (92.9% vs. 69.6%, p 0.001) and clinically
Patients in the metronidazole group reported more side-effects than patients in the
om required termination of treatment. These results suggest that single-dose
ant alternative agent for the treatment of dientamoebiasis.

469-0691.2008.02002.x

Free full text

Then, many studies and case reports aroused in late 90's indicating Blastocystis and D. fragilis as causes of gastrointestinal and dermatological clinical cases.

Eur J Clin Microbiol Infect Dis. 2015 Oct;34(10):1995-8. doi: 10.1007/s10096-015-2442-6. Epub 2015 Jul 15.

Dientamoeba fragilis prevalence coincides with gastrointestinal symptoms in children less than 11 years old in Sweden.

Ögren J[1], Dienus O[1], Löfgren S[1], Einemo IM[2], Iveroth P[2], Matussek A[3].

Author information

Abstract

Dientamoeba fragilis is a protozoan with a debated role in gastrointestinal (GI) disease. Although correlated to GI symptoms, no virulence factors have been described. In this study, we evaluated the cause of GI symptoms in children at two schools, with children aged 1 to 10 years, in the county of Jönköping, Sweden. D. fragilis infection correlated to GI symptoms in children and Enterobius vermicularis correlated to D. fragilis infection.

PMID: 26173693 PMCID: PMC4565872 DOI: 10.1007/s10096-015-2442-6

[PubMed - indexed for MEDLINE]

Parasitol Res (2011) 108:553–560
DOI 10.1007/s00436-010-2097-2

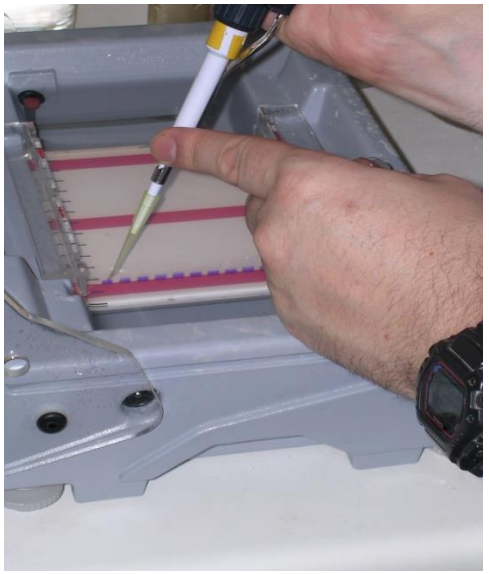
ORIGINAL PAPER

Association of *Blastocystis hominis* genetic subtypes with urticaria

Dina M. Abdel Hameed · Omayma M. Hassanin · Nehal Mohamed Zuel-Fakkar

Received: 4 September 2010 / Accepted: 16 September 2010 / Published online: 5 October 2010

Then, something start to change in our point view!



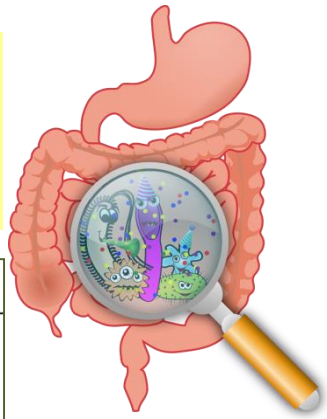
1. Development of specific PCR protocols

D. fragilis & Blastocystis => very common in communities!

(Denmark: 43% / The Netherlands: 22% / Senegal: 100%)

Pathogens? Regular residents of gut?

2. Introduction of NGS => better identification of the microorganisms in *"Gut Microbiota"*



Eur J Clin Microbiol Infect Dis (2015) 34:1039–1044
DOI 10.1007/s10096-015-2312-2

ARTICLE

Low prevalence of *Blastocystis* sp. in active ulcerative colitis patients

N. G. Rossen · A. Bart · N. Verhaar · E. van Nood · R. Kootte · P. F. de Groot · G. R. D'Haens · C. Y. Ponsioen · T. van Gool

PLOS | PATHOGENS

PEARLS

Are Human Intestinal Eukaryotes Beneficial or Commensals?

Julius Lukeš[1,2,3]*, **Christen Rune Stensvold**[4], **Kateřina Jirků-Pomajbíková**[1], **Laura Wegener Parfrey**[3,5]*

1 Institute of Parasitology, Biology Centre, České Budějovice, Czech Republic, 2 Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic, 3 Canadian Institute for Advanced Research, Toronto, Canada, 4 Statens Serum Institut, Copenhagen, Denmark, 5 Departments of Botany and Zoology, University of British Columbia, Vancouver, Canada

OPEN ACCESS Freely available online

PLOS | ONE

Blastocystis Is Associated with Decrease of Fecal Microbiota Protective Bacteria: Comparative Analysis between Patients with Irritable Bowel Syndrome and Control Subjects

Céline Nourrisson[1,2,3], **Julien Scanzi**[4,5], **Bruno Pereira**[6], **Christina NkoudMongo**[1], **Ivan Wawrzyniak**[2,3], **Amandine Cian**[7], **Eric Viscogliosi**[7], **Valérie Livrelli**[1,8], **Frédéric Delbac**[2,3], **Michel Dapoigny**[4,5], **Philippe Poirier**[1,2,3]*

1 CHU Clermont-Ferrand, Centre de Biologie, Laboratoire de Parasitologie-Mycologie, Hôpital G. Montpied, Clermont-Ferrand, France, 2 Clermont Université, Université Blaise Pascal, Laboratoire Microorganismes: Génome et Environnement, BP 10448, Clermont-Ferrand, France, 3 CNRS, UMR 6023, LMGE, Aubière, France, 4 CHU Clermont-Ferrand, Service de Médecine digestive et hépatobiliaire, Hôpital Estaing, Clermont-Ferrand, France, 5 Clermont Université, Université d'Auvergne, UMR 1107 INSERM, Neuro-Dol, Clermont-Ferrand, France, 6 CHU Clermont-Ferrand, DRCI, 'Délégation Recherche Clinique et Innovation', Clermont-Ferrand, France, 7 Université Lille Nord de France, INSERM U1019, CNRS UMR 8204, Centre d'Infection et d'Immunité de Lille (CIIL), Institut Pasteur, Biologie et Diversité des Pathogènes Eucaryotes Emergents, Lille, France, 8 Clermont Université, Université d'Auvergne, Centre de Recherche en Nutrition Humaine Auvergne, M2iSH, 'Microbes, Intestin, Inflammation et Susceptibilité de l'Hôte UMR INSERM/Université d'Auvergne U1071 USC-INRA 2018, BP 10448, Clermont-Ferrand, France

Scand J Gastroenterol. 2013 May;48(5):638-9. doi: 10.3109/00365521.2013.780094. Epub 2013 Mar 25.

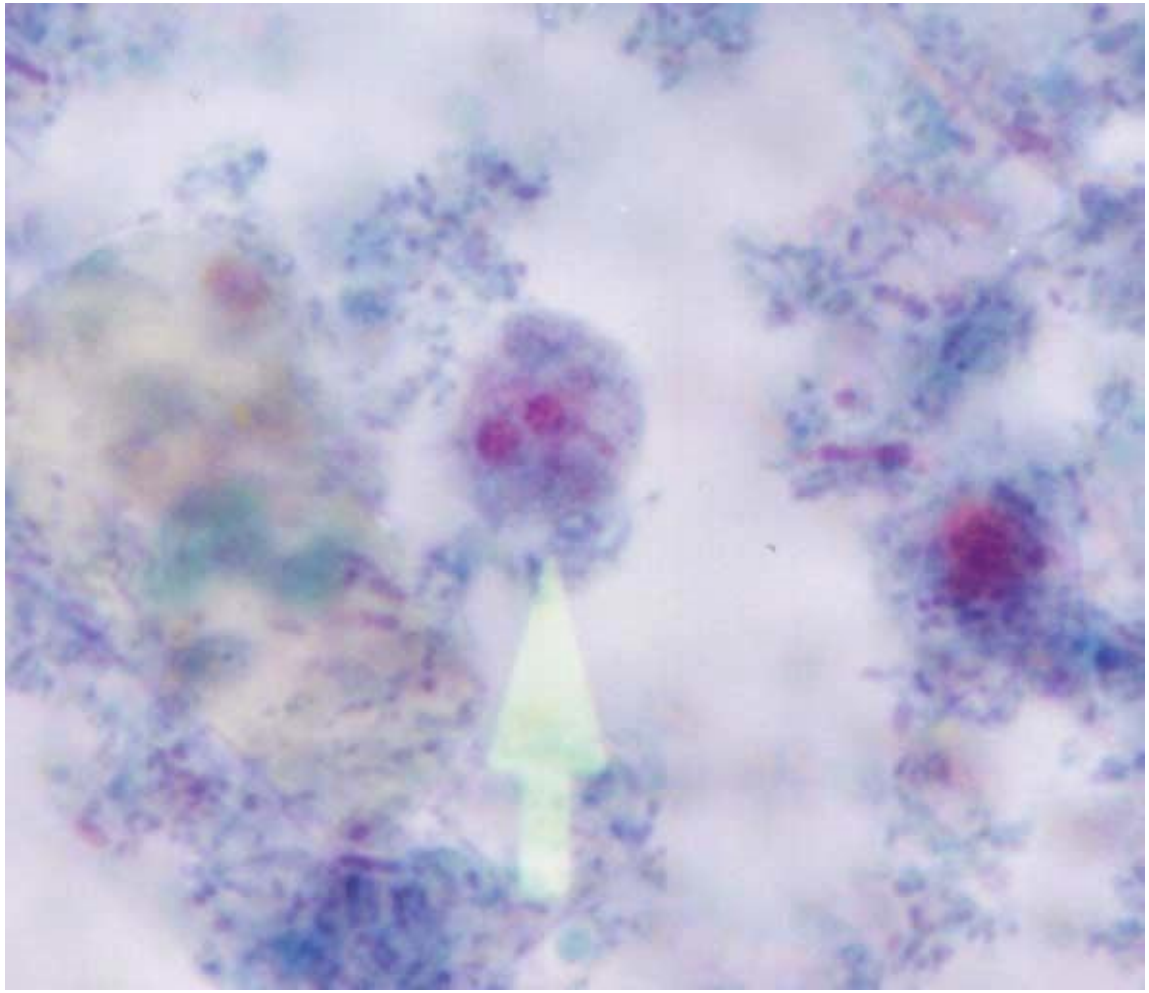
Active ulcerative colitis associated with low prevalence of Blastocystis and Dientamoeba fragilis infection.

Petersen AM, Stensvold CR, Mirsepasi H, Engberg J, Friis-Møller A, Porsbo LJ, Hammerum AM, Nordgaard-Lassen I, Nielsen HV, Krogfelt KA.

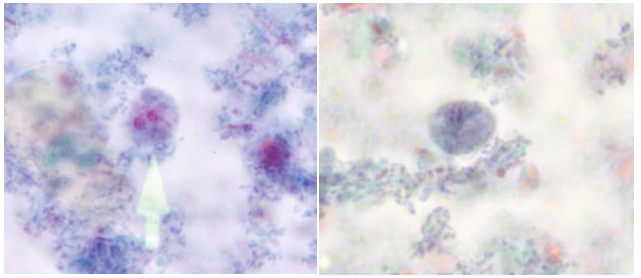
PMID: 23528075 [PubMed - indexed for MEDLINE]

Friends or Foes:
What is the future of the studies on these protists?

Dientamoeba fragilis



Dientamoeba fragilis: What's it like?



- **"An enigma shrouded in the mysteries of diagnostic clinical parasitology"**

Windsor JJ and Johnson EH. *Br J Biomed Sci*, 1999.

- **"A neglected cause of diarrhea"**

Girginkardeşler N et al. *Clin Microbiol Infect*, 2003.

- **"Emerging from obscurity"**

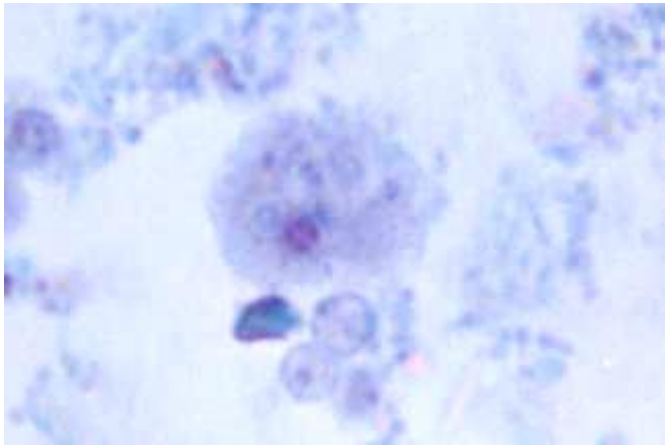
Johnson EH et al. *Clin Microbiol Rev*, 2004.

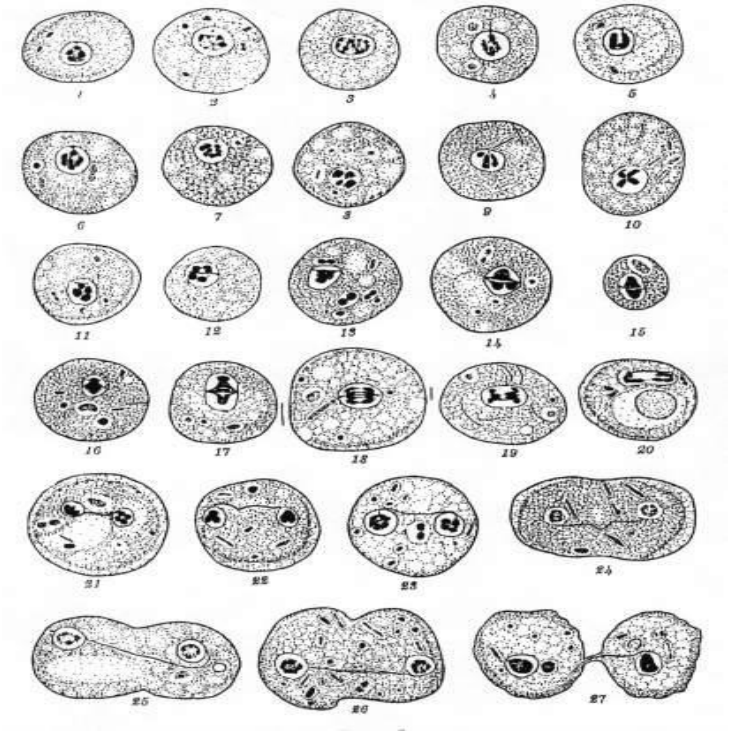
- **"*Dientamoeba fragilis*, the neglected Trichomonad of the Human Bowel"**

Stark D, Barratt J, Chan D, Ellis JT. *Clin Microbiol Rev, 2016*.

Dientamoeba fragilis
MORPHOLOGY

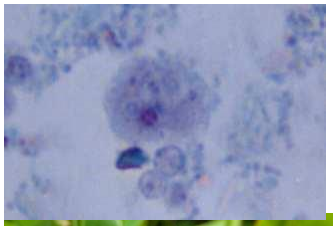
- Unique nuclear structure
  - no peripheral chromatin
  - fragmented karyosome
  - *Leaf-like pseudopodia in culture*
- Trophozoite; 5-12 µm
- Cyst and precystic forms recently described! *(Munasinghe et.al., 2013)*
- Often binucleated (60-80%)





***D. fragilis* morphology, Wenrich DH, 1937**

D. fragilis as a zoonotic agent



Detection of Dientamoeba fragilis in animal faeces using species specific real time PCR assay.

Chan D[1], Barratt J[2], Roberts T[3], Phillips O[3], Šlapeta J[4], Ryan U[5], Marriott D[3], Harkness J[3], Ellis J[6], Stark D[7].

Author information

Abstract

Dientamoeba fragilis is a potentially pathogenic, enteric, protozoan parasite with a worldwide distribution. While clinical case reports and prevalence studies appear regularly in the scientific literature, little attention has been paid to this parasite's biology, life cycle, host range, and possible transmission routes. Overall, these aspects of Dientamoeba biology remain poorly understood at best. In this study, a total of 420 animal samples, collected from Australia, were surveyed for the presence of Dientamoeba fragilis using PCR. Several PCR assays were evaluated for sensitivity and specificity. Two previously published PCR methods demonstrated cross reactivity with other trichomonads commonly found in animal samples. Only one assay exhibited excellent specificity. Using this assay D. fragilis was detected from one dog and one cat sample. This is the first report of D. fragilis from these animals and highlights the role companion animals may play in D. fragilis transmission. This study demonstrated that some published D. fragilis molecular assays cross react with other closely related trichomonads and consequently are not suitable for animal prevalence studies.

KEYWORDS: Animal reservoirs; Dientamoeba fragilis; real-time PCR

Vet Parasitol. 2008 Jan 25;151(1):21-6. Epub 2007 Oct 9.

Gorillas are a host for Dientamoeba fragilis: an update on the life cycle and host distribution.

Stark D[1], Phillips O, Peckett D, Munro U, Marriott D, Harkness J, Ellis J.

Author information

Abstract

Dientamoeba fragilis is a gastrointestinal protozoan that has a worldwide distribution and is emergeing as a common cause of diarrhea. As D. fragilis has a propensity to cause chronic illness with symptoms similar to irritable bowel syndrome (IBS) it is not surprising that some patients with D. fragilis are misdiagnosed as having IBS. In contrast to most other pathogenic protozoa very little is known about its life cycle, epidemiology and mode of transmission. What role animal reservoirs play in the transmission of this parasite is unknown. Consequently we undertook a prospective study to determine the host distribution of D. fragilis. Over a 2-year-period, 608 faecal samples from a wide range of animal and bird species, including pigs and other food species, were screened using permanent stained smears for the presence of D. fragilis. Trophozoites of D. fragilis were only detected in Western lowland gorillas (3/10) (Gorilla g. gorilla) and confirmed by PCR targeting the SSU rRNA gene. The limited host range detected suggests human infection may not involve transmission from other animal species. In addition, we provide an update on the limited knowledge about the life cycle of this parasite and its host distribution.

Emerg Infect Dis. 2012 May;18(5):838-41. doi: 10.3201/eid1805.111093.

Pigs as natural hosts of Dientamoeba fragilis genotypes found in humans.

Cacciò SM[1], Sannella AR, Manuali E, Tosini F, Sensi M, Crotti D, Pozio E.

Author information

Abstract

Dientamoeba fragilis is a common intestinal parasite in humans. Transmission routes and natural host range are unknown. To determine whether pigs are hosts, we analyzed 152 fecal samples by microscopy and molecular methods. We confirmed that pigs are a natural host and harbor genotypes found in humans, suggesting zoonotic potential.

PMID: 22515838 PMCID: PMC3358053 DOI: 10.3201/eid1805.111093

[PubMed - indexed for MEDLINE] Free PMC Article

SYMPTOMS

- **No symptoms**

  Mostly adults

- **<u>Mostly GIS-related symptoms</u>**

  25% of patients!

  Abdominal pain

  Diarrhea / constipation

  Nausea

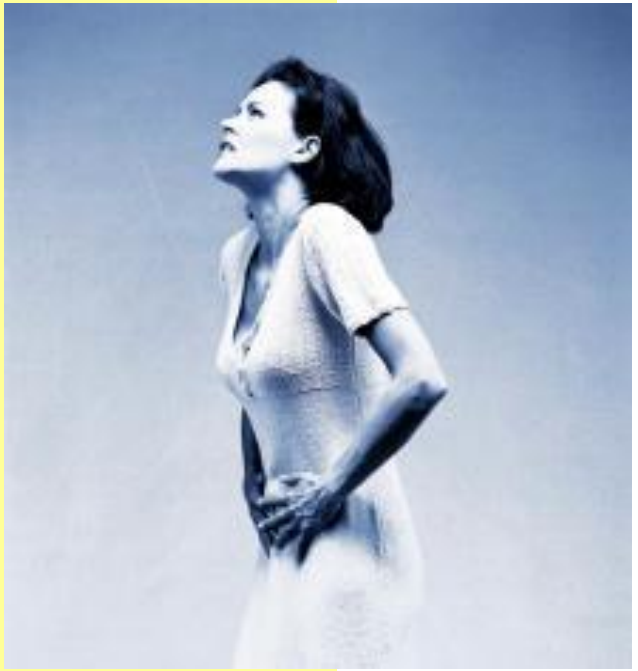
  Bloating xxxx

  Anorexia

  Fatigue, weight loss

- **<u>Dermatologic symptoms</u>**

  Pruritus, urticaria



Published Clinical Reports on *D. fragilis*

Kafkas Univ Vet Fak Derg
18 (Suppl-A): A145-A149, 2012
DOI:10.9775/kvfd.2012.6080

RESEARCH ARTICLE

Dientamoeba fragilis ile Enfekte Hastaların Genel Özellikleri: Manisa İlinden Yüz Hastalık Kohortun İnceleme Sonuçları

Özgür KURT * Ülgen Z. OK **

* Celal Bayar Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, TR-45140 Muradiye, Manisa - TÜRKİYE
** Celal Bayar Üniversitesi, Tıp Fakültesi, Parazitoloji Anabilim Dalı, TR-45030 Manisa - TÜRKİYE

Makale Kodu (Article Code): KVFD-2012-6080

General Features of Patients Infected with *Dientamoeba fragilis*: Assessment of a Cohort of One Hundred Patients from Manisa Province

Summary

Dientamoeba fragilis is a flagellated protozoon dwelling in the colonic lumen. It was shown that it may cause clinical manifestations which required effective anti-microbial therapy. General features of 100 *D. fragilis*-infected patients were assessed in a study conducted in the Parasitology Laboratory of Celal Bayar University Hospital. Patients were mostly females, between 18 and 50 years, and almost two-thirds of them were admitted with gastrointestinal complaints such as bloating, abdominal pain, diarrhea and fatigue. Thirty-four of 100 patients were coinfected with *Blastocystis* sp., while 27 of 83 (32.5%) patients that submitted three cellophane tape samples were coinfected with *Enterobius vermicularis*. Another significant outcome of the study was that 7 and 6 of 35 patients reported dermatologic complaints as itching and lesions, consecutively. The patients and/or their families were mostly educated, middle-class individuals living in apartments in city centre, using tap water for drinking and do regular hand-washing. The study results suggest that *D. fragilis* should be regarded as a causative agent in pediatric and adult patients suffering from abdominal pain, diarrhea and intense bloating, and the fresh stool samples should be examined directly or kept in a fixative solution until examination with a permanent-stained smear such as trichrome.

Keywords: *Dientamoeba fragilis, Symptom, Abdominal pain*

Lack of large-scale, case-control studies is a significant limitation for *D. fragilis* research

J Pediatr Gastroenterol Nutr. 1998 Jan;26(1):16-20.

Dientamoeba fragilis masquerading as allergic colitis.

Cuffari C[1], Oligny L, Seidman EG.

Author information

Abstract

BACKGROUND: Dientamoeba fragilis is a rare cause of chronic infectious diarrhea and colitis in children.

METHODS: Review of the clinical manifestations, diagnostic methods, and clinical course of D. fragilis infection in our hospital.

RESULTS: Eleven pediatric patients are discussed, seven of whom had a history of recent travel. Clinical manifestations of infectious diarrhea included anorexia, intermittent vomiting, abdominal pain, and diarrhea, ranging from 1 to 100 weeks in duration. Peripheral eosinophilia was present in seven patients. One patient with well-documented bovine protein allergy had intermittent episodes of diarrhea and abdominal pain, despite an appropriate elimination diet. Eosinophilic colitis documented by colonoscopy, was due to D. fragilis. Metronidazole was effective in treating five patients, and iodoquinol was effective in treating four others.

CONCLUSIONS: D. fragilis should be included in the differential diagnosis of chronic diarrhea and eosinophilic colitis. The identification of this pathogen requires clinical awareness of epidemiologic risk factors and presenting complaints, as well as the laboratory staining procedures essential to its proper identification.

Detection of Dientamoeba fragilis in Portuguese children with acute gastroenteritis between 2011 and 2013.

Júlio C[1], Furtado C[2], Rocha R[1], Escobar C[3], Brito MJ[4], Oleastro M[1].

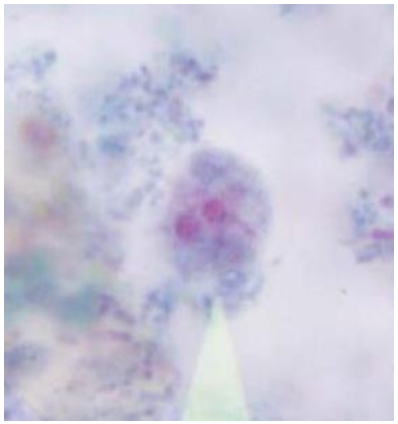
Author information

Abstract

Dientamoeba fragilis is an inhabitant of human gastrointestinal tract with a worldwide distribution. The first description considered this protozoan a rare and harmless commensal, since then it has struggled to gain recognition as a pathogen. Commercial multiplex real-time PCR was used to detect D. fragilis in fecal samples from hospitalized children (⩽18 years) with acute gastrointestinal disease, admitted to two hospitals of Lisbon area, with different demographic characteristics. A total of 176 children were studied, 103 (58·5%) male, 144 (81·8%) children between 0 and 5 years and 32 (18·2%) above 6 years old. The overall protozoa frequency considering the four tested microorganisms were 8·5% (15/176), and the most frequently found protozoan was D. fragilis, 6·3% (11/176). Dientamoeba fragilis frequency was higher among older children (21·9%), than younger children (2·8%), and greater in boys (6·8%) than in girls (5·5%). All positive children presented with diarrhoea associated with vomiting, fever and abdominal pain. Infection was associated with the age of children ($P < 0·001$), school attendance ($P = 0·002$) and consumption of certain foods ($P = 0·014$), e.g. cakes with crème and ham. The frequency of diantamoebiasis found in a cohort of hospitalized Portuguese children, with acute gastrointestinal disease, could be considered a very high value when compared with the protozoan frequency normally associated with this pathology.

KEYWORDS: Dientamoeba fragilis; Portugal; acute gastroenteritis; frequency; hospitalized children

DIAGNOSIS

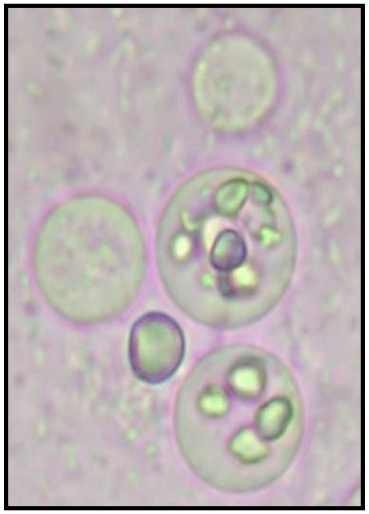


- **Microscopy**

  Permanent-stained smears required!

  Standard O&P examination of stool is insufficient

  (Trichrome, Hematoxylene-Eosine, Chlorazol black)



- **Culture**

  Robinson's medium, Dobell's medium

- **Molecular methods**

  PCR (*Conventional and Real Time PCR*)

TREATMENT

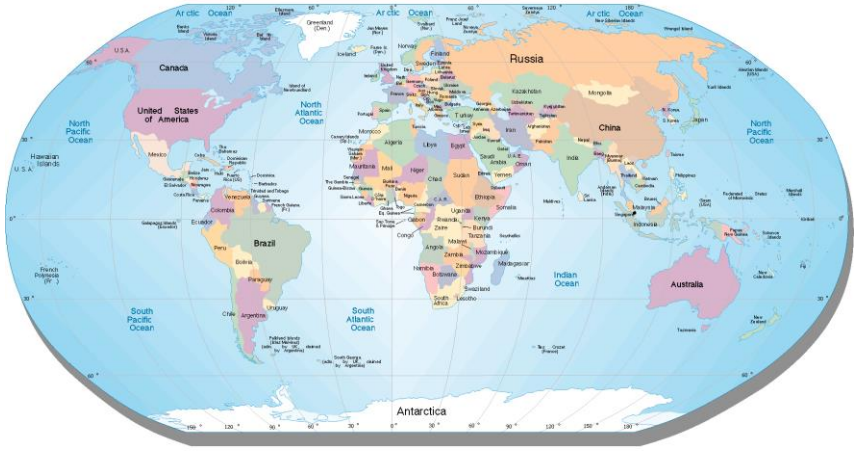
- Treatment of patients
- Drug of choice

} No consensus!

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

- ✓ **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)*

D. fragilis
EPIDEMIOLOGY



✓Cosmopolitan

✓Prevalence rates **(*BEFORE PCR*)** => 0% - 52.5% (unreliable)

*Incidence in Celal Bayar University (2004): **5.2%** (with routine trichrome staining)*

FACTORS AFFECTING THE PREVALENCE RATES (BEFORE PCR!!!)

1. Failure to examine the permanent stained smears or culture material

2. Failure to examine more than 1 stool samples

3. Experience of the laboratory staff

D. fragilis EPIDEMIOLOGY AFTER PCR

Dientamoeba fragilis in Denmark: epidemiological experience derived from four years of routine real-time PCR

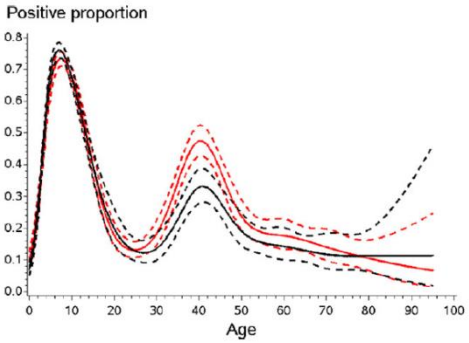
D. Röser · J. Simonsen · H. V. Nielsen · C. R. Stensvold · K. Mølbak

Received: 28 February 2013 / Accepted: 4 April 2013

Abstract The intestinal protozoon *Dientamoeba fragilis* remains a clinical entity of dubious significance. While several previous studies address questions of epidemiology, only a handful have systematically employed and reported on the results from real-time polymerase chain reaction (qPCR), the best currently available diagnostic modality, and the comparison of results from different studies is, therefore, difficult. Since 2007, Statens Serum Institut (Denmark) has utilised qPCR for *D. fragilis* as routine diagnostic work-up for intestinal parasitosis, testing more than 22,000 samples from 2008 through 2011, and the aim of this study was to report on the results and experiences gained in the process. We demonstrate a staggeringly high proportion (43 %) of investigated patients positive for *D. fragilis*, ranging from 12 to 71 % depending on age group, showing a bimodal age distribution peaking in children and adults of parental age, as well as a clear association between exposure to children an
We discuss these findin
vector hypothesis and
edge of risk factors pert

Introduction

The clinical significance
uncertain. While several
fragilis to be a potential p
D. fragilis occurs comm
asymptomatic individ
summarised 50 publicatio
D. fragilis prevalences fro
on age or gender distributi
figures is naturally diffic
ences between studies, su



The Netherlands

High Detection Rates of Enteropathogens in Asymptomatic Children Attending Day Care

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Abstract

Background: Gastroenteritis morbidity is high among children under the age of four, especially amongst those who attend day care.

Objective: To determine the prevalence of a range of enteropathogens in the intestinal flora of children attending day care and to relate their occurrence with characteristics of the sampled child and the sampling season.

Methods: We performed three years of enteropathogen surveillance in a network of 29 child day care centers in the Netherlands. The centers were instructed to take one fecal sample from ten randomly chosen children each month, regardless of gastrointestinal symptoms at time of sampling. All samples were analyzed for the molecular detection of 16 enteropathogenic bacteria, parasites and viruses by real-time multiplex PCR.

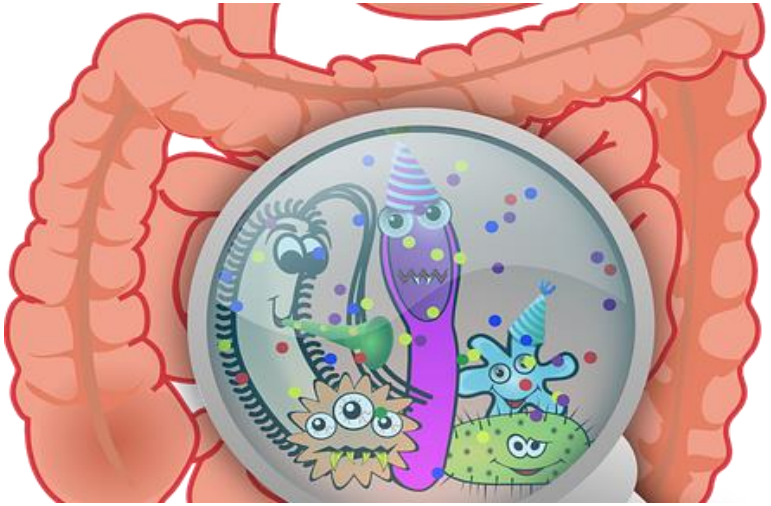
Results: Enteropathogens were detected in 78.0% of the 5197 fecal samples. Of the total, 95.4% of samples were obtained from children who had no gastroenteritis symptoms at time of sampling. Bacterial enteropathogens were detected most often (most prevalent EPEC, 19.9%), followed by parasitic enteropathogens (most prevalent: *D. fragilis*, 22.1%) and viral enteropathogens (most prevalent: norovirus, 9.5%). 4.6% of samples related to children that experienced symptoms of gastroenteritis at time of sampling. Only rotavirus and norovirus were significantly associated with gastroenteritis among day care attendees.

D. fragilis EPIDEMIOLOGY

High prevalence of *D. fragilis* after PCR

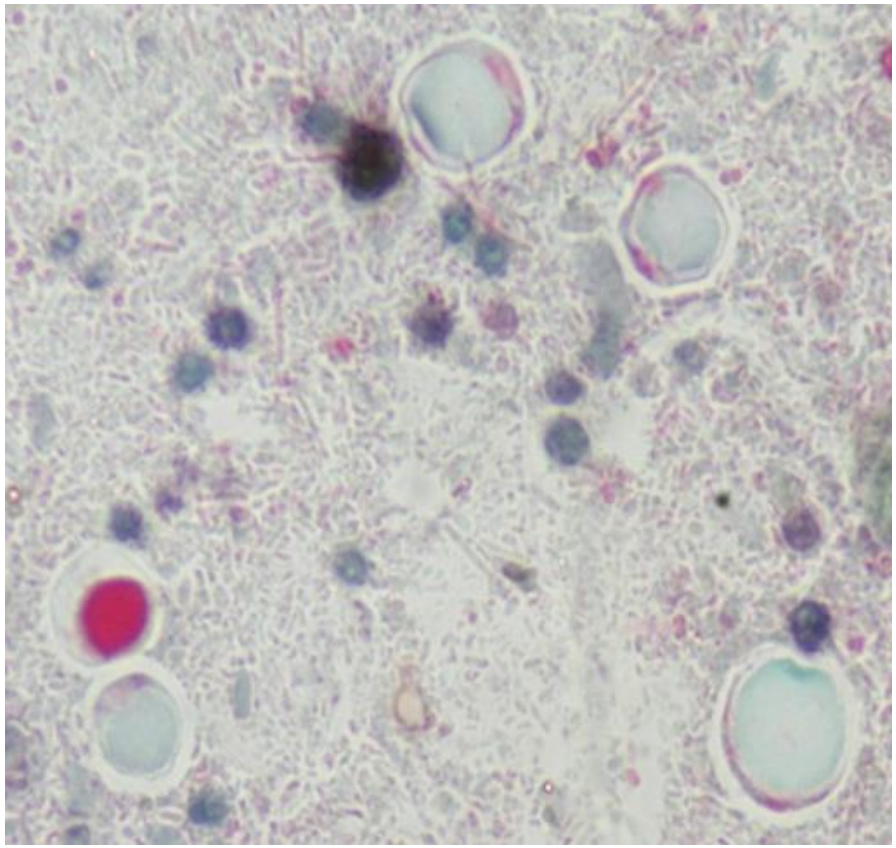
=> Infection or colonization in the gut?

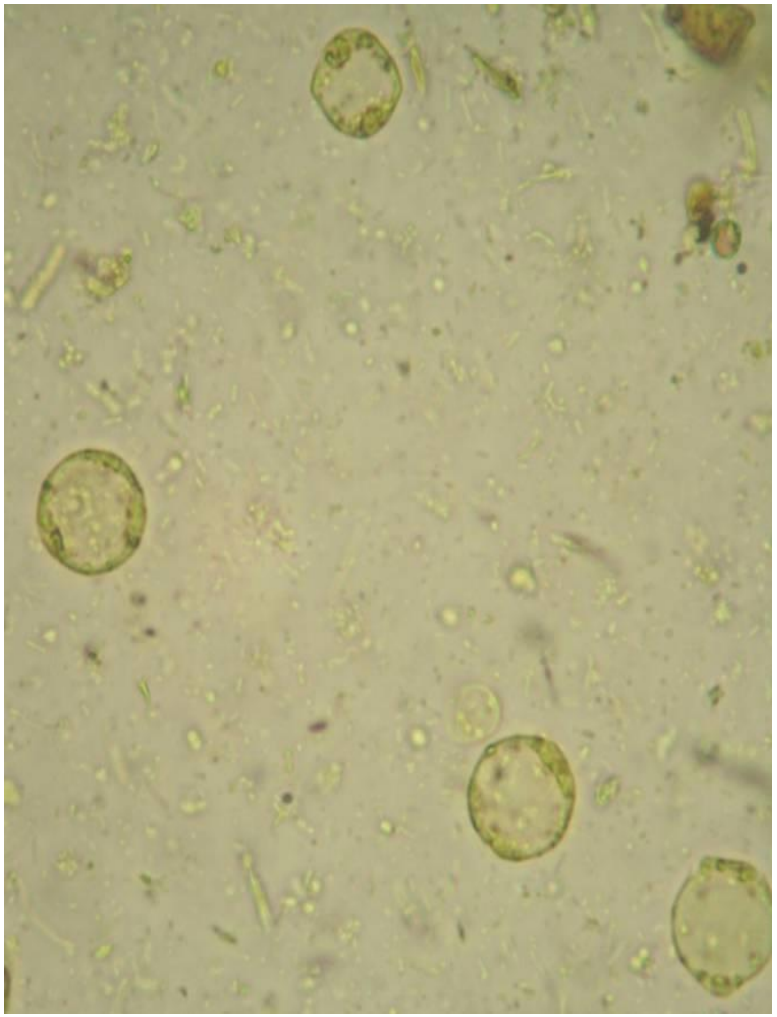
- ✓ Some subtypes are pathogenic?
- ✓ Host-microbe interactions?
- ✓ Role of immune system?



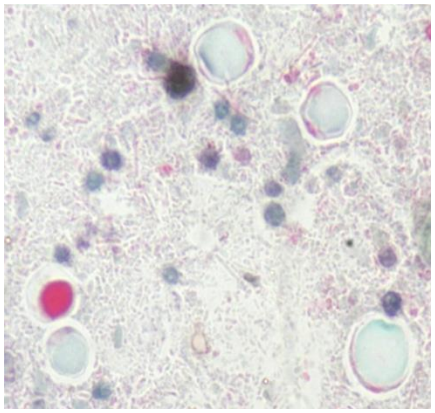
- ✓ Role of the bacteria, virus, fungi and protists in gut microbiota?

Blastocystis





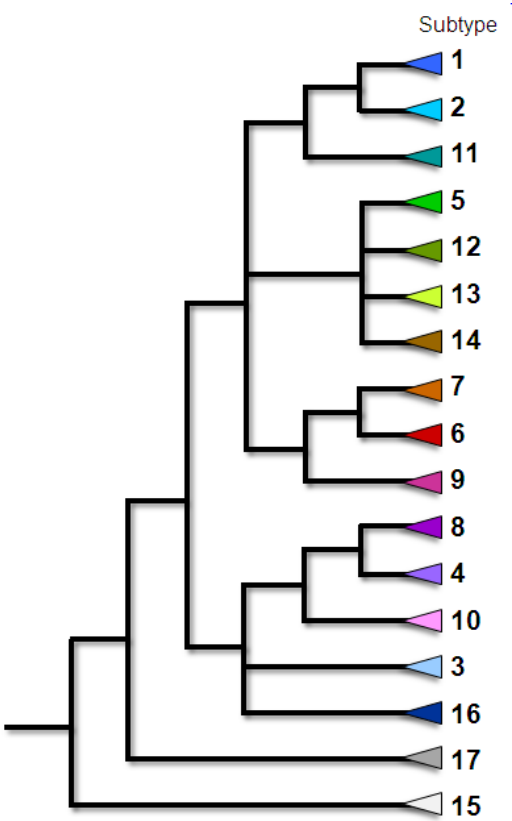
Blastocystis



- ✓ **Most common intestinal agent in surveys on "intestinal parasites" worldwide!**
  - > 1 billion people colonised…
  - > 50 % in developed countries
  - 100% in Senegalese children!

- ✓ Pathogenicity doubtful! "***Reported when ≥ 5 Blastocystis present in microscopy***"

- ~~✓ ***Blastocystis hominis***~~ ***NO MORE!*** ***Blastocystis***!



BLASTOCYSTIS Genetic Diversity

- *Extensive genetic diversity!*

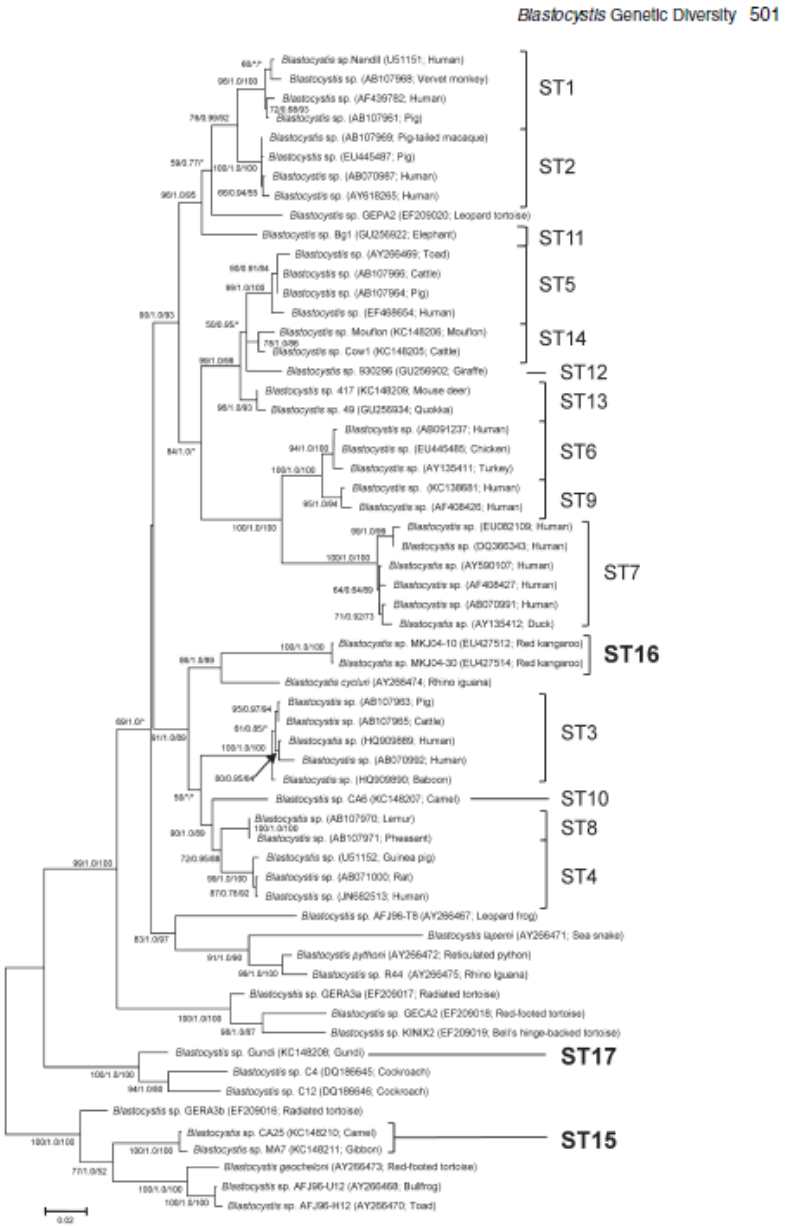
- Today => **17 different subtypes**

  Each may be an individual species

- 9 STs were recognised in humans

- ST1, 2, 3 and 4 >%90 in human cases

- Some STs are ***zoonotic*** (ST4-8)



Alfellani et al., 2013

✓Most common subtype in humans => ST3

TABLE 3. Distribution of *Blastocystis* subtypes infecting humans in different geographic regions

Country/region and type of isolates (no. of infected individuals studied)	Subtype distribution (%)[a]										No. of positive isolates/total no. of isolates (%)[b]	Reference
	1	2	3	4	5	6	7	8	9	Unknown/ mixed		
Bangladesh (26)	7.7	—	92.3	—	—	—	—	—	—	—	NA	304
Guangxi, China (35)	37.1	—	40	—	—	—	5.7	—	—	17.2	NA	294
Yunnan, China (78)	20.5	1.3	70.5	1.3	—	—	—	—	—	6.5	NA	138
Denmark (29)	3.4	20.7	51.7	24.1	—	—	—	—	—	—	NA	231
Denmark (28)	17.9	32.1	46.6	3.8	—	—	—	—	—	—	NA	227
Egypt (44)	18.2	—	54.5	—	—	18.2	9.1	—	—	—	NA	105
Germany (166)	21	1	66	7	—	—	—	—	—	5	NA	31
Germany (12)	25	16.7	41.7	16.7	—	—	—	—	—	—	12/67 (17.9)	304
Greece (45)	20	13.3	60	2.2	—	2.2	2.2	—	—	—	NA	147
Japan (55)	20	21.8	43.6	10.9	—	—	—	—	—	3.6	NA	113
Japan (50)	8	—	52	4	—	22	10	—	4	—	50/2,037 (2.45)	304
Pakistan (10)	20	—	70	—	—	10	—	—	—	—	NA	304
Singapore (9)	22.2	—	77.8	—	—	—	—	—	—	—	9/276 (3.3)	291
Thailand (153)	90.2	—	4.6	—	—	—	1.3	—	—	3.9	334/924 (36.1)[c]	271
Turkey (87)	9.2	13.8	75.9	—	—	—	1.1	—	—	—	NA	180
Turkey												
Isolates from pediatric patients (51)	21.6	19.6	52.9	—	—	—	—	—	—	5.9	51/161 (31.7)	62
Isolates from adult patients (41)	14.6	24.4	58.5	—	—	—	—	—	—	2.4	41/125 (32.8)	62

[a] —, subtype not detected.
[b] NA, not available.
[c] Three hundred thirty-four *Blastocystis*-positive samples were obtained by in vitro culture of stool specimens. Out of these 334 isolates, only 153 were amenable to PCR amplification.

BLASTOCYSTIS => ZOONOTIC DISEASE

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Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Blastocystis subtypes detected in humans and animals from Colombia

Juan David Ramírez [a,b,*], Laura Viviana Sánchez [a], Diana Carolina Bautista [a], Andrés Felipe Corredor [a], Astrid Carolina Flórez [c], Christen Rune Stensvold [c,d]

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Protist

ELSEVIER

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

ORIGINAL PAPER

Genetic Diversity of *Blastocystis* in Livestock and Zoo Animals

Mohammed A. Alfellani[a,d], Derya Taner-Mulla[a], Alison S. Jacob[a], Christine Atim Imeede[a], Hisao Yoshikawa[b], C. Rune Stensvold[c], and C. Graham Clark[a,1]

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Monitoring Editor: Michael Melkonian

Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia

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Diagnosis

Treatment
Metronidazole
Co-Trimaxazole

- ✓ **Microscopy**

  Standard O&P, Permanent-stained smears

- ✓ **Culture**

  Jones medium! Easy-to cultivate

- ✓ **PCR**

  Best diagnostic option*

  Useful for public health screenings

* *Roberts T et al, 2011.* Comparison of Microscopy, Culture and Conventional Polymerase Chain Reaction for Detection of *Blastocystis sp.* in Clinical Stool Samples

Pathogenicity of Blastocystis

- More data available compared to *D. fragilis*!
- Yet, mostly on experimental models, not in vivo models!

Parasitol Int. 2012 Sep;61(3):437-42. doi: 10.1016/j.parint.2012.02.007. Epub 2012 Feb 25.

Characterization of two cysteine proteases secreted by Blastocystis ST7, a human intestinal parasite.

Wawrzyniak I, Texier C, Poirier P, Viscogliosi E, Tan KS, Delbac F, El Alaoui H.

Clermont Université, Université Blaise Pascal, Laboratoire Microorganismes: Génome et Environnement, BP 10448, F-63000 Clermont-Ferrand, France.

Abstract

Blastocystis spp. are unicellular anaerobic intestinal parasites of both humans and animals and the most prevalent ones found in human stool samples. Their association with various gastrointestinal disorders raises the questions of its pathogenicity and of the molecular mechanisms involved. Since secreted proteases are well-known to be implicated in intestinal parasite virulence, we intended to determine whether Blastocystis spp. possess such pathogenic factors. In silico analysis of the Blastocystis subtype 7 (ST7) genome sequence highlighted 22 genes coding proteases which were predicted to be secreted. We characterized the proteolytic activities in the secretory products of Blastocystis ST7 using specific protease inhibitors. Two cysteine proteases, a cathepsin B and a legumain, were identified in the parasite culture supernatant by gelatin zymographic SDS-PAGE gel and MS/MS analysis. These proteases might act on intestinal cells and disturb gut function. This work provides serious molecular candidates to link Blastocystis spp. and intestinal disorder

Parasitology. 2016 Nov;143(13):1713-1722. Epub 2016 Sep 9.

On Blastocystis secreted cysteine proteases: a legumain-activated cathepsin B increases paracellular permeability of intestinal Caco-2 cell monolayers.

Nourrisson C[1], Wawrzyniak I[2], Cian A[3], Livrelli V[1], Viscogliosi E[3], Delbac F[2], Poirier P[1].

Author information

Abstract

Blastocystis spp. pathogenic potential remains unclear as these anaerobic parasitic protozoa are frequently isolated from stools of both symptomatic and asymptomatic subjects. In silico analysis of the whole genome sequence of Blastocystis subtype 7 revealed the presence of numerous proteolytic enzymes including cysteine proteases predicted to be secreted. To assess the potential impact of proteases on intestinal cells and gut function, we focused our study on two cysteine proteases, a legumain and a cathepsin B, which were previously identified in Blastocystis subtype 7 culture supernatants. Both cysteine proteases were produced as active recombinant proteins. Activation of the recombinant legumain was shown to be autocatalytic and triggered by acidic pH, whereas proteolytic activity of the recombinant cathepsin B was only recorded after co-incubation with the legumain. We then measured the diffusion of 4-kDa FITC-labelled dextran across Caco-2 cell monolayers following exposition to either Blastocystis culture supernatants or each recombinant protease. Both Blastocystis culture supernatants and recombinant activated cathepsin B induced an increase of Caco-2 cell monolayer permeability, and this effect was significantly inhibited by E-64, a specific cysteine protease inhibitor. Our results suggest that cathepsin B might play a role in pathogenesis of Blastocystis by increasing intestinal cell permeability.

Microbiology (2010), **156**, 1284–1293 DOI 10.1099/mic.0.034025-0

Staurosporine-induced programmed cell death in *Blastocystis* occurs independently of caspases and cathepsins and is augmented by calpain inhibition

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Previous studies have shown that the protozoan parasite *Blastocystis* exhibits apoptotic features with caspase-like activity upon exposure to a cytotoxic monoclonal antibody or the anti-parasitic drug metronidazole. The present study reports that staurosporine (STS), a common apoptosis inducer in mammalian cells, also induces cytoplasmic and nuclear features of apoptosis in *Blastocystis*, including cell shrinkage, phosphatidylserine (PS) externalization, maintenance of plasma membrane integrity, extensive cytoplasmic vacuolation, nuclear condensation and DNA fragmentation. STS-induced PS exposure and DNA fragmentation were abolished by the

Selena W. S. Sio · Manoj K. Puthia · Alex S. Y. Lee · Jia Lu · Kevin S. W. Tan

Protease activity of *Blastocystis hominis*

Received: 12 January 2006 / Accepted: 12 January 2006 / Published online: 4 March 2006

Abstract Parasite-derived proteases are important for the parasite life cycle and the pathogenesis of the disease they produce. Proteases of intestinal protozoan parasite *Blastocystis hominis* were studied for the first time with azocasein assays and gelatin SDS-PAGE analysis. Parasitic lysates were found to have high protease activity and nine protease bands of low (20–33 kDa) and high (44–75 kDa) molecular weights were reported. Proteases were found to be pH-dependent and highest proteolytic activity was observed at neutral pH. Inhibition studies showed that *B. hominis* isolate B, like many other protozoan parasites, contains mainly cysteine proteases.

a number of diseases. Proteases were assigned a number of roles in host–parasite relationship (Mckerrow et al. 1993). Several species of parasitic protozoa contain multiple proteases that have functions in life cycle, morphogenesis, and infectivity of parasites (Williams and Coombs 1995). Proteases, cysteine proteases in particular, are known to have cytopathic effects on host cells and are considered a virulence factor in many protozoan parasites.

Blastocystis is a unicellular protozoan parasite of the intestinal tract of humans and animals. Pathogenicity of *Blastocystis* is still ambiguous and there are various inconsistent reports that either implicate or exonerate the

Discussion:

"Projections to future: Are They Friends or foes?"

In the Era of Microbiota Research

- *37 different eukaryotes have been identified!*
- *Blastocystis prevalence >50% with PCR*
- *Blastocystis carriage of 6-10 years reported!*

Resident of a healthy gut!

Comparison of D. fragilis in asthmatic children (n=50) and healthy age-matched controls (n=46) :

D. fragilis (+) **52% of asthmatic children**

78.3% of healthy individuals

(Kurt Ö, et al, unpublished data; Acıbadem Uni& Cerrahpasa Uni)

Current Conclusion: "Blastocystis and *D. fragilis* **may cause clinical manifestations in humans** under certain circumstances."

- ✓Morphology
  Ameboid forms of Blastocystis
- ✓Subtype
- ✓Virulance factors
- ✓Immune status of the host
- ✓Parasitic load
- ✓Composition of gut microbiota

Interactions between Blastocystis and *D. fragilis* Gut Microbiota

Scand J Gastroenterol. 2013 May;48(5):638-9. doi: 10.3109/00365521.2013.780094. Epub 2013 Mar 25.

Active ulcerative colitis associated with low prevalence of Blastocystis and Dientamoeba fragilis infection.

Petersen AM, Stensvold CR, Mirsepasi H, Engberg J, Friis-Møller A, Porsbo LJ, Hammerum AM, Nordgaard-Lassen I, Nielsen HV, Krogfelt KA.

PMID: 23528075 [PubMed - indexed for MEDLINE]

OPEN ACCESS Freely available online PLOS ONE

Blastocystis Is Associated with Decrease of Fecal Microbiota Protective Bacteria: Comparative Analysis between Patients with Irritable Bowel Syndrome and Control Subjects

Céline Nourrisson[1,2,3], Julien Scanzi[4,5], Bruno Pereira[6], Christina NkoudMongo[1], Ivan Wawrzyniak[2,3], Amandine Cian[7], Eric Viscogliosi[7], Valérie Livrelli[1,8], Frédéric Delbac[2,3], Michel Dapoigny[4,5], Philippe Poirier[1,2,3]*

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- Gut microbiota in healthy individuals is ***different*** compared to individuals with IBD, IBS and other intestinal diseases.
- Blastocystis is dependent on other components of microbiota for colonization in the gut!
- **Blastocystis & D. fragilis => low or negative in active UC patients, diarrhea-predominant IBS patients!**

Interactions between Blastocystis and Gut Microbiota

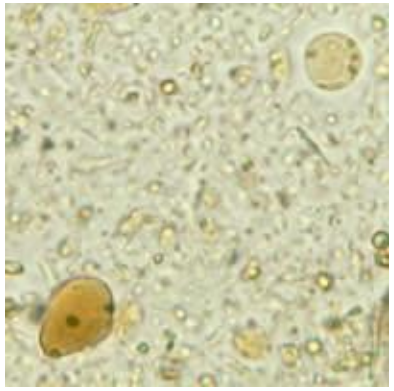
- Retrospective analyses of fecal DNA metagenomic data
  (Andersen et al, 2015)
- *316 individuals => 110 obese/ 62 overweight / 143 thin (lean)*
- ***Blastocystis => positive correlation with Ruminococcus & Prevotella***

  => negative correlation with Bacteriodes!

  Bacteriodes ⇔ low microbial diversity (unhealthy status)
- ***Significant correlations => Blastocystis & low BMI***

  Blastocystis & High bacterial diversity

Interactions between Blastocystis And Gut Microbiota



✓ *"Blastocystis positive in thin individuals (p=0.008)"*

High bacterial diversity in thin individuals also requires the presence of Blastocystis

Future Research

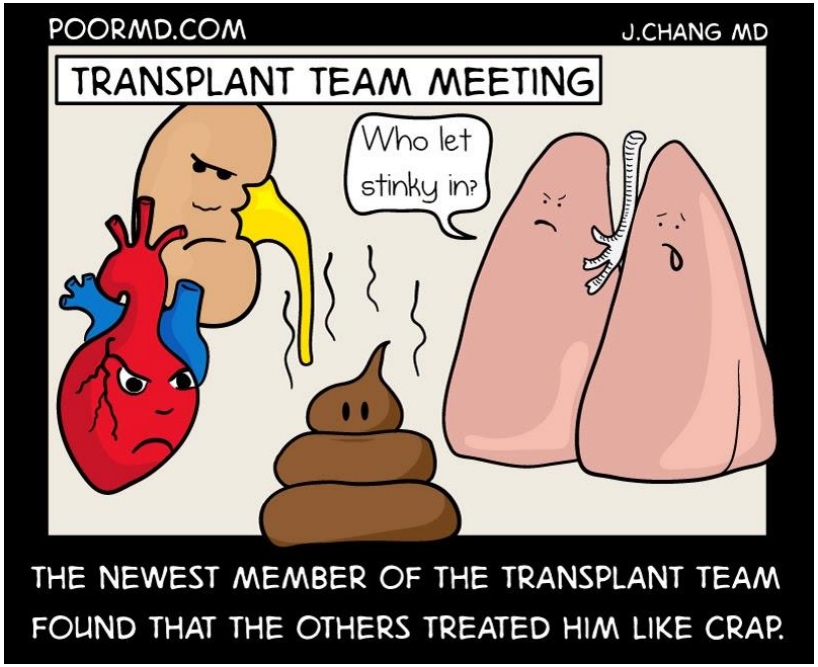
- **Diversity of gut microbiota including the effects of their metabolic functions (*METABOLOMICS*)**
- *Role of the Microbiota Composition in Diseases*
  - Functional and inflammatory bowel diseases
  - Colon cancer
  - Autoimmune diseases
  - Metabolic syndrome

Multidisciplinery Projects required!

Clinical Microbiology / Gastroenterology / Public Health &
Bioinformatic Data Interpretation is essential

Future Research

✓**FMT** (fecal microbiota transplantation) – treatment of recurrent *Clostridium difficile* infections



✓Use of protists as **probiotics!**


Thank you…