Amebas

Friend and foe

Facultative Pathogenicity of *Entamoeba histolytica?*

Confusing History

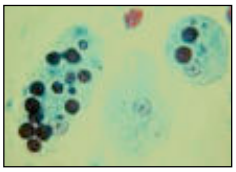
1875	**Lösch correlated dysentery with amebic trophozoites**
1925	**Brumpt proposed two species: *E. dysenteriae* and *E. dispar***
1970's	**biochemical differences noted between invasive and non-invasive isolates**
80's/90's	**several antigenic and DNA differences demonstrated** • **rRNA 2.2% sequence difference**
1993	**Diamond and Clark proposed a new species (*E. dispar*) to describe non-invasive strains**
1997	**WHO accepted two species**

Family Entamoebidae

- Family includes parasites and commensals
- Species are differentiated based on size, nuclear substructures

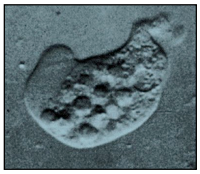
- ***Entamoeba histolytica***
- ***Entamoeba dispar***
- ***Entamoeba coli***
- ***Entamoeba hartmanni***
- ***Endolimax nana***
- ***Iodamoeba bütschlii***

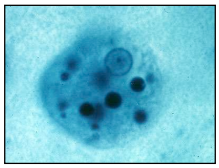
Entamoeba histolytica
one of the most potent killers in nature



Entamoeba histolytica

- **worldwide distribution (cosmopolitan)**
  - **higher prevalence in tropical or developing countries (20%)**
  - **1-6% in temperate countries**
- **Possible animal reservoirs**
- **Amebiasis - Amebic dysentery**
  - aka: Montezuma's revenge



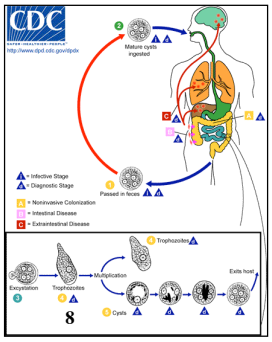


Taxonomy
- One parasitic species?
  - *E. histolytica*
  - *E. dispar*
  - *E. hartmanni*

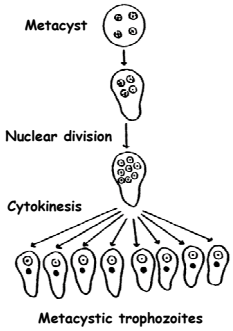
Entamoeba Life Cycle - Direct

- Fecal/Oral transmission
- Cyst - Infective stage
  - Resistant form
- Trophozoite - feeding, binary fission

- Different stages of cyst development
  - Precysts - rich in glycogen
  - Young cyst - 2, then 4 nuclei with chromotoid bodies
  - Metacysts - infective stage
  - Metacystic trophozoite - 8

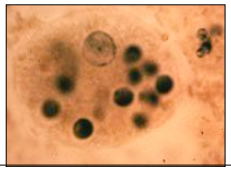


Excystation



- Cyst wall disruption
- Ameba emerges
  - Nuclear division 4→8
  - Cytokinesis
- Trophozoites go on to inhabit large intestine
  - Replicate via binary fission

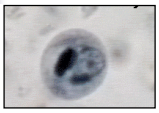
Key Features of Trophozoites

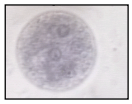


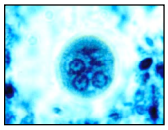
- Shape - more ovoid
- 20-30 μm
- Psuedopods rapidly extend and withdraw
- 1 nucleus
  - Central endosome

Show movies here!
Will be posted on website

Keys Features of Cysts





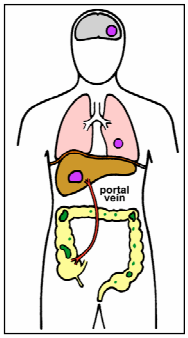


- oval or spherical shape
- 10-20 μm
- distinct cell wall set apart from cytoplasm
- Young cysts - still contain chromatoid bodies
- Mature - quadrinucleated
  - Concentric endosome
  - Periphral chromatin

Disease Manifestations

- Ulcer formation
- Ulcer enlargement
- Perforation of intestinal wall
- Local abcesses
- Secondary bacterial infections
- Occassional ameboma

ameboma = inflammatory thickening of intestinal wall around the abscess (can be confused with tumor)



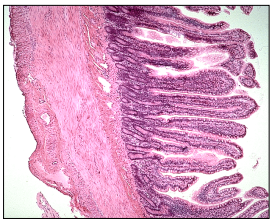
Clinical Features and Symptoms

Range of Outcomes
- Asymptomatic/cyst passer
- Symptomatic nondysenteric
- Amebic dysentery
- Extraintestinal disease

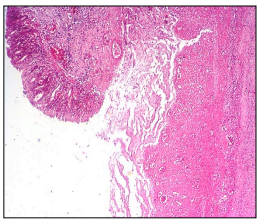
Intestinal Symptoms
- **Range**
  - **mild to intense, transient to chronic**
- **Nondysenteric**
  - **diarrhea, cramps, flatulence, nausea**
- **Dysenteric**
  - **blood/mucus in stools**
  - **cramps/pain**
- **Ameboma**
  - **palpable mass**
  - **obstruction**

E. Histolytica Pathology

Healthy Intestine



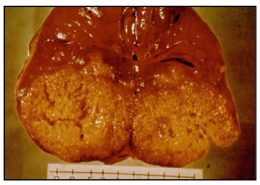
***E. Histolytica* infected Intestine**

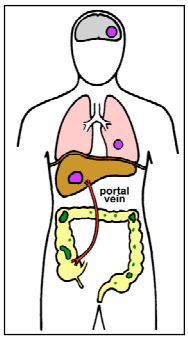


Flask-shaped ulcer
Trophozoites at the boundary

Extraintestinal Amebiasis

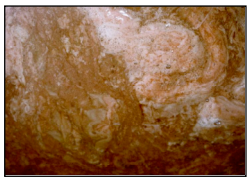
Amebic Liver Abscess
- **chocolate-colored 'pus'**
  - **necrotic material**
  - **usually bacteria free**
- **lesions expand and coalesce**
- **further metastasis, direct extension or fistula**

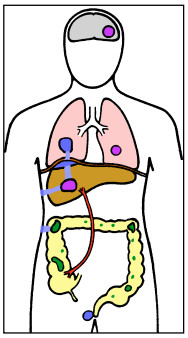




Pulmonary Amebiasis

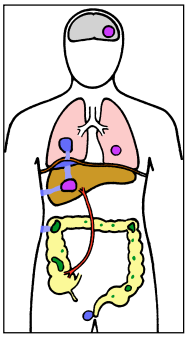
Pulmonary Amebiasis
- rarely primary
- rupture of liver abscess through diaphragm
- 2º bacterial infections common
- fever, cough, dyspnea, pain, vomica





Cutaneous Amebiasis

- intestinal or hepatic fistula
- mucosa bathed in fluids containing trophozoites
  - perianal ulcers
  - urogenital (eg, labia, vagina, penis)

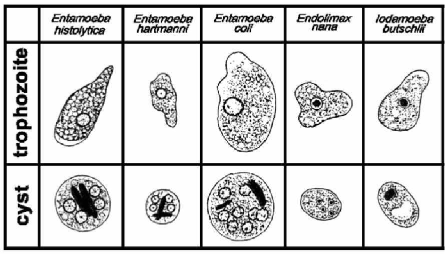


Epidemiologic Risk Factors

- Prevelance
  - Lower socioeconomics
  - Crowding
  - Human fecal waste management
  - Endemic area
  - Communal living
  - Institutionalization
- Severity
  - Children, neonates
  - Malnutrition
  - Corticosteroid use

Intestinal Amoebae

Humans harbor 9 species of intestinal amoebae

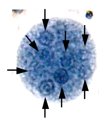


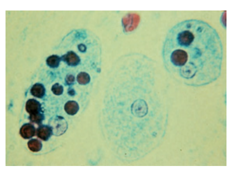
X Details of these commensals are covered in the text

Entamoeba histolytica Diagnosis

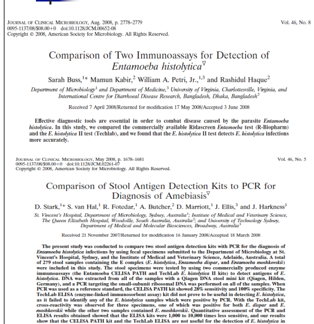
- Microscopic
  - Detection in stool samples
    - Classis stains
    - Multiple samples tested
    - Blood and mucus present
  - Culturing of samples - time
    - histolytica vs. dispar
    - impracticle
- Molecular
  - ELISA - immunlogical based via specific lectins
    - histolytica vs. dispar
  - PCR-based methods
    - 100x more sensitive

vs.





Diagnostics



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0095-1137/08/$08.00+0 doi:10.1128/JCM.00652-08

Comparison of Two Immunoassays for Detection of *Entamoeba histolytica*

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Effective diagnostic tools are essential in order to combat disease caused by the parasite *Entamoeba histolytica*. In this study, we compared the commercially available Ridascreen *Entamoeba* test (R-Biopharm) and the *E. histolytica* II test (Techlab), and we found that the *E. histolytica* II test detects *E. histolytica* infections more accurately.

Journal of Clinical Microbiology, May 2008, p. 1678–1681 Vol. 46, No. 5
0095-1137/08/$08.00+0 doi:10.1128/JCM.02261-07

Comparison of Stool Antigen Detection Kits to PCR for Diagnosis of Amebiasis

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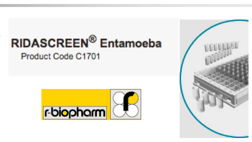
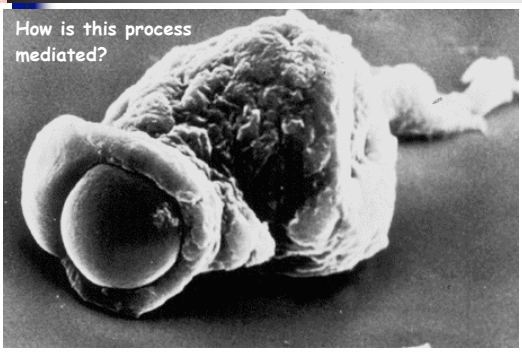
The present study was conducted to compare two stool antigen detection kits with PCR for the diagnosis of *Entamoeba histolytica* infections by using fecal specimens submitted to the Department of Microbiology at St. Vincent's Hospital, Sydney, and the Institute of Medical and Veterinary Science, Adelaide, Australia. A total of 279 stool samples containing the E complex (*E. histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii*) were included in this study. The stool specimens were tested by using two commercially produced enzyme immunoassays (the Entamoeba CELISA PATH and TechLab *E. histolytica* II kits) to detect antigens of *E. histolytica*. DNA was extracted from all of the samples with a Qiagen DNA stool mini kit (Qiagen, Hilden, Germany), and a PCR targeting the small-subunit ribosomal DNA was performed on all of the samples. When PCR was used as a reference standard, the CELISA PATH kit showed 28% sensitivity and 100% specificity. The TechLab ELISA (enzyme-linked immunosorbent assay) kit did not prove to be useful in detecting *E. histolytica*, as it failed to identify any of the *E. histolytica* samples which were positive by PCR. With the TechLab kit, cross-reactivity was observed for three specimens, one of which was positive for both *E. dispar* and *E. moshkovskii* while the other two samples contained *E. moshkovskii*. Quantitative assessment of the PCR and ELISA results obtained showed that the ELISA kits were 1,000 to 10,000 times less sensitive, and our results show that the CELISA PATH kit and the TechLab ELISA are not useful for the detection of *E. histolytica* in stool samples from patients in geographical regions where this parasite is not endemic.



Recognition of Host Cells



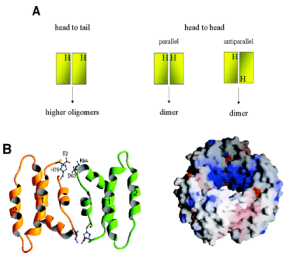
Virulence factors

- Molecules that help:
  - Establish infection in host
  - Cause pathogenesis
  - Allow transmission from host to host
  - Evade host immune defenses
- General types of virulence factors
  - Adherence factors
  - Invasion factors
  - Endotoxins
  - Exotoxins
  - Siderophores

Amoebic Factors Implicated in Pathogenesis

Factor	Suggested role in pathogenesis
GalNAc lectin	Adherence to mucin/cells, serum resistance
Fibronectin/collagen Receptors	Adherence to extracellular matrix
Cysteine proteinases	Invasion through the extracellular matrix
Amoebapore	Lysis of target cells
Phospholipases	Lysis of target cells
Cytoskeleton	Adhesion plates, endocytosis, motility

Amoebapores - virulence factor

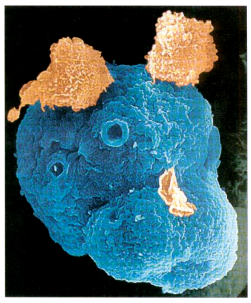


- Family of small (77 AA) proteins contained in secretory granules
- Similar in structure and function to NK lysins
- Used to kill bacteria and host cells
- Amoebapores insert into target membranes and form ion channels
- Amoeba mutants which make less amoebapores cause less disease in animal model studies

Originally 3 isoforms identified: A, B, and C.

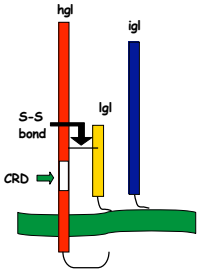
Entamoeba virulence factor

- Gal/GalNAc lectin is a multifunctional virulence factor
  - Lectin - proteins which specifically bind carbohydrates
  - Classification is based on carbohydrate specificity
- Plays roles in adherence, cytolysis, invasion, resistance to lysis by complement, and encystation.



Gal/GalNAc Adherence Lectin

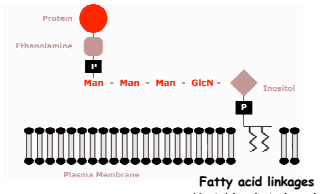
- Heterotrimer
  - Heavy subunit (hgl)
    - 170kDa
    - Integral membrane protein
    - CRD: carbohydrate recognition domain
    - Cytoplasmic tail - signaling
  - Lipid-anchored light subunit (lgl)
    - 31-35kDa form -glycosylation
    - Covalent S-S bond to hgl
  - Intermediate subunit (igl)
    - 150 kDa lectin
    - Non-covalent association



30 igl homologues identified

GPI anchor

- Glycosylphosphatidylinositol
  - Glycolipid that anchors a protein to the cell surface
  - Roles in cell surface localization, signaling, surface molecule turnover



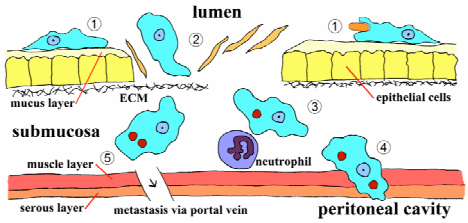
Man - mannose

GlcN - glucosamine

Fatty acid linkages
Variable chain length
C14-C22
(C18 is most common)

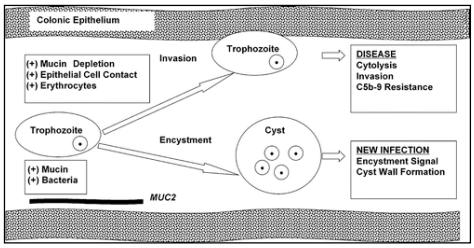
Possible pathogenic mechanism

Contact dependent killing of epithelial cell - lectin mediated
Breakdown of tissue (extracellular matrix) - cytolysis
Amoebapores - pore-forming proteins (~5 kDa)
Surface cysteine proteases? Still unclear



Gal/GalnAc Lectin Signaling

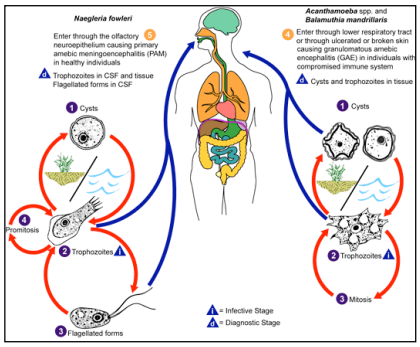
Determinants of invasion: correlation with variation in disease



MUC2 - predominant secreted mucin
Allelic variation!

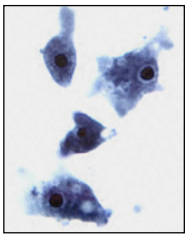
Bacterial flora - influence balance between trophozoite vs. cysts formation. Some bacterial combinations promote better.

Free-living opportunistic amoeba



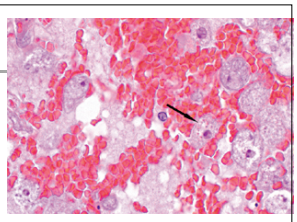
Naegleria fowleri

- **Geographic Range:** Cosmopolitan
  - Found throughout world in freshwater.
    - Three life forms: amoeba, flagellate, cyst
  - Infections generally occur around thermal pools where population of amoeba is high.
  - Also very common in water above 80ºF
  - Most cases of human infections are from the United States
    - Particularly from Florida, Texas, Colorado
    - Other countries reporting cases include Czech Republic, Mexico, Africa, New Zealand, and Australia.



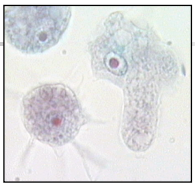
Pathology

- Causes Primary Amebic Meningoencephalitis (PAM)
- Very rapidly causes the death of host
  - Rapid destruction of brain tissue

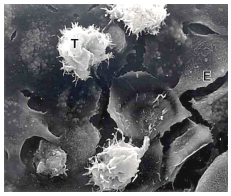


- Symptoms very similar to other types of meningitis and encephalitis.
  - Headaches, fever, stiff neck, etc. progressing to dementia and death.
  - But much less common and usually mistaken for more common bacterial and viral forms

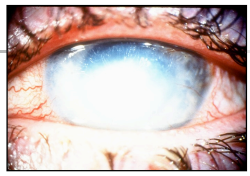
Acanthamoeba sp.



- **Geographic Distribution:** Cosmopolitan
  - Found in freshwater almost everywhere
    - Amoeba and cyst forms
  - Also found is soil, dust, sewage
  - Cannot survive in thermal pools
- **Location in Host**:
- Most common in eye and skin. Rarely invades brain.
- **Pathology:** Rarely causes damage in people with intact immune systems except contact lens wearers.



Acanthamoeba



- Most common cause of corneal ulcers and keratitis in contact lens wearers
  - Keratitis is an inflammation of the cornea
  - Can lead to blindness.
  - Most common in people who make their own saline solution.
  - May require abrasion by the contact lens

Immunocomprised - cutaneous lesions