**Sixth lecture. by Dr. Hiro M. Obaid**

Differentiation of pathogenic *Entamoeba histolytica* and the morphologically identical nonpathogenic *Entamoeba dispar*.

It has been proposed that pathogenic and nonpathogenic strains though morphologically identical may represent 2 distinct species—the pathogenic strains being *E. histolytica,* and the nonpathogenic strains reclassified as *E. dispar*. Trophozoites of *E. dispar* contain bacteria, but no RBCs.

*Entamoeba histolytica* must be differentiated from other intestinal protozoa including: *E. coli*, *E. hartmanni*, *E. gingivalis*, *Endolimax nana*, and *Iodamoeba buetschlii* (the nonpathogenic amebae); *Dientamoeba fragilis* (which is a flagellate not an ameba); and the possibly pathogenic *Entamoeba polecki*. Differentiation is possible, but not always easy, based on morphologic characteristics of the cysts and trophozoites. The nonpathogenic *Entamoeba dispar*, however, is morphologically identical to *E. histolytica*, and differentiation must be based on isoenzymatic or immunologic analysis. Molecular methods are also useful in distinguishing between *E. histolytica* and *E. dispar* and can also be used to identify *E. polecki*. Microscopic identification of cysts and trophozoites in the stool is the common method for diagnosing *E. histolytica*. This can be accomplished using:

- Fresh stool: wet mounts and permanently stained preparations (e.g., trichrome).
- Concentrates from fresh stool: wet mounts, with or without iodine stain, Concentration procedures; however, are not useful for demonstrating trophozoites.

In addition, *E. histolytica* trophozoites can also be identified in aspirates or biopsy samples obtained during colonoscopy or surgery.

**Immunodiagnosis**

**Antibody Detection**

Enzyme immunoassay (EIA) kits for *Entomoeba histolytica* antibody detection as well as EIA kits for antigen detection are commercially available. Antibody detection is most useful in patients with extraintestinal disease (i.e., amebic liver abscess) when organisms are not generally found on stool examination. Antigen detection may be useful as

an assistant to microscopic diagnosis in detecting parasites and can distinguish between pathogenic and nonpathogenic infections.

The indirect hemagglutination (IHA) test has been replaced by commercially available EIA test kits for routine serodiagnosis of amebiasis. The EIA test detects antibody specific for *E. histolytica* in approximately 95% of patients with extraintestinal amebiasis, 70% of patients with active intestinal infection, and 10% of asymptomatic persons who are passing cysts of *E. histolytica*. If antibodies are not detectable in patients with an acute presentation of suspected amebic liver abscess, a second specimen should be drawn 7-10 days later. If the second specimen does not show seroconversion, other agents should be considered. Detectable *E. histolytica*-specific antibodies may persist for years after successful treatment, so the presence of antibodies does not necessarily indicate acute or current infection. Specificity is 95% or higher: false-positive reactions rarely occur. Although the immunodiffusion test is as specific, it is slightly less sensitive than the IHA and EIA and requires a minimum of 24 hours to obtain a result, in contrast to 2 hours required for the IHA or EIA tests. However, the simplicity of the procedure makes it ideal for the laboratory that has only an occasional specimen to test. The IHA and EIA tests are more suitable for laboratories that have frequent requests for amebiasis serology. Although detection of IgM antibodies specific for *E. histolytica* has been reported, sensitivity is only about 64% in patients with current invasive disease. Several commercial EIA kits for antibody detection are available .

**Antigen Detection**

Antigen consists of a crude soluble extract of axenically cultured organisms. Antigen detection may be useful as an aide to microscopic diagnosis in detecting parasites and to distinguish between pathogenic and nonpathogenic infections. Recent studies indicate improved sensitivity and specificity of fecal antigen assays with the use of monoclonal antibodies which can distinguish between *E. histolytica* and *E. dispar* infections. At least one commercial kit is available which detects only pathogenic *E. histolytica* infection in stool; several kits are available which detect *E. histolytica* antigens in stool but do not exclude *E. dispar* infections. (E histolytica II Test, Tech Lab, Blacksburg, VA, USA, Sensitivity 96.9–100, Specificity 94.7–100)

**Molecular Diagnosis**

**Conventional PCR**

In reference diagnosis laboratories, molecular analysis by PCR-based assays is the method of choice for discriminating between the pathogenic species (*E. histolytica*) and the nonpathogenic species (*E. dispar*).

### Real-Time PCR

A TaqMan real-time PCR approach has been validated at CDC and is used for differential laboratory diagnosis of amebiasis. The assay targets the 18S rRNA gene with species-specific TaqMan probes in a duplex format, making it possible to detect both species in the same reaction vessel.

## Free-Living Amebas: *Naegleria, Acanthamoeba* and *Balamuthia*

Three genera of free-living amebas, Naegleria, Acanthamoeba, and Balamuthia are known to infect humans. Naegleria fowleri causes an acute and almost fatal encephalitis, which, fortunately, is rare. Several species of Acanthamoeba and B mandrillaris can cause lung and skin infections, as well as encephalitis, in immunocompromised patients. In addition, Acanthamoeba may cause an ulcerative keratitis, which is usually associated with improper sterilization of soft contact lenses. These amebas live freely in soil and in fresh and coastal waters. The resistant cysts can be transported in dust.

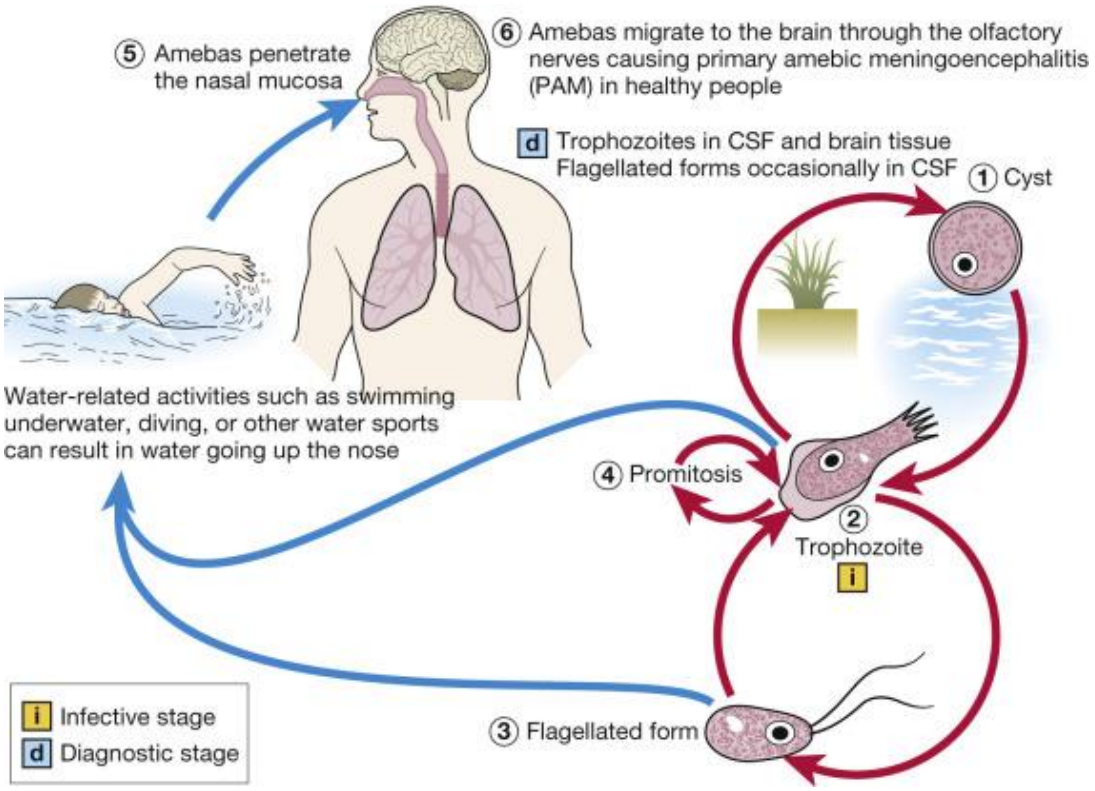
### Naegleria Fowleri

#### Structure

The trophozoites are 10 to 15 μm in diameter and produce broadly rounded lobopodia. Cysts are single-walled, spherical and 8 to 12 μm in diameter. The trophozoites can also transform to a flagellated form.

#### Multiplication and Life Cycle

The trophozoites are free-living inhabitants of soil and warm fresh water. They reproduce by binary fission.




**Pathogenesis and Clinical manifestation**

Amebas splashed or inhaled onto the olfactory epithelium migrate up the olfactory nerve to the brain and spread via the subarachnoid space. Clinical manifestation Naegleria fowleri causes primary amebic meningoencephalitis, a rare, rapidly fatal disease with sudden onset of headache, fever, stiff neck, lethargy, and coma in otherwise healthy people.

**Epidemiology**

The organism is found worldwide in soil and warm fresh water. Infectious cysts may be carried in dust.

**Diagnosis**

Diagnosis

Primary amebic meningoencephalitis cannot be distinguished clinically from acute pyogenic or bacterial meningoencephalitides. The disease usually occurs in children and young adults in good health who have recently swum in warm water. Computed tomography of the brain shows obliteration of the cisternae surrounding the midbrain and of the subarachnoid space over the cerebral hemispheres. The disease may be diagnosed rapidly by examining one or two drops of fresh cerebrospinal fluid under a light microscope for N fowleri. The organism may also be cultured from cerebrospinal fluid or brain tissue for a definitive diagnosis. Diagnoses have been

made by examining paraffin-embedded brain tissue sections stained with hematoxylin and eosin..

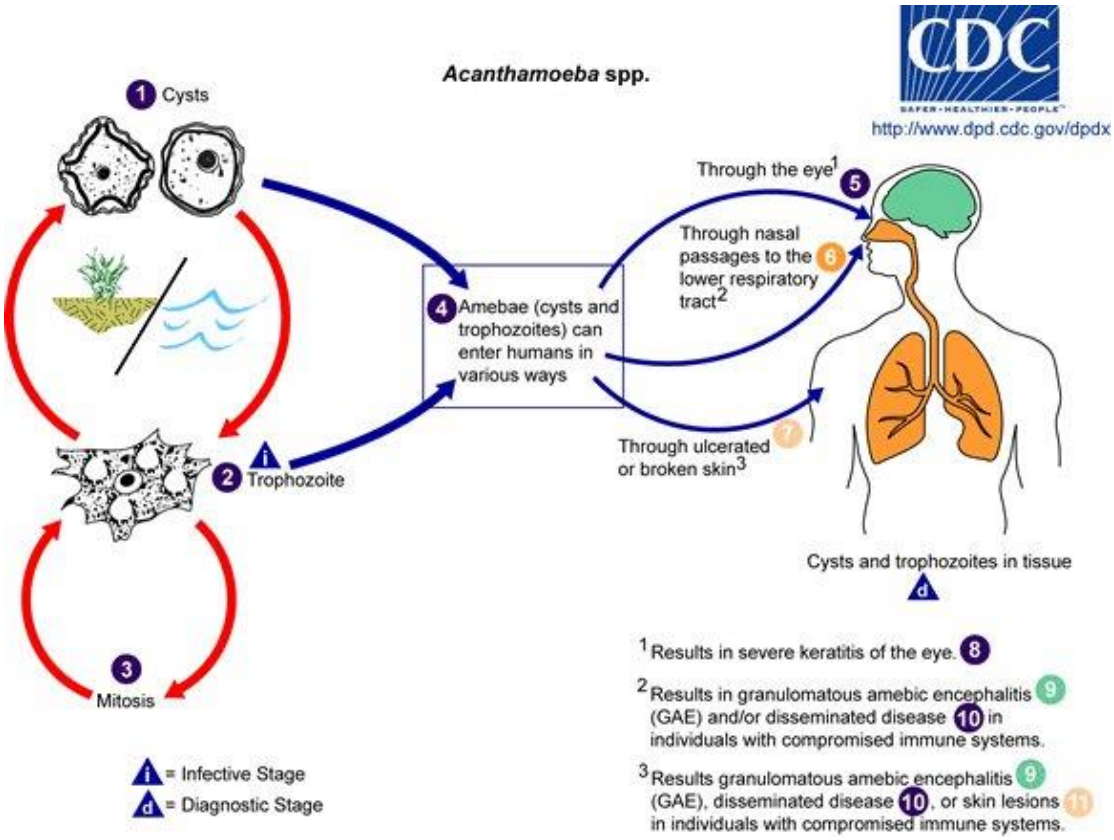
## Acanthamoeba Species

### Structure

Acanthamoeba castellanii: trophozoites are 25 to 40 µm in diameter with characteristic spine-like pseudopodia. Cysts are double-walled, usually polygonal and spherical, and 15 to 20 µm in diameter.

### Multiplication and Life Cycle

The trophozoites are free-living inhabitants of soil and of fresh and salt water. They reproduce by binary fission.




### Pathogenesis and Clinical Manifestation

Encephalitis is caused by the hematogenous spread from superficial or pulmonary lesions to the brain. Keratitis results from contamination of superficial corneal scrapes. Clinical Manifestation, Acanthamoeba species and Balamuthia mandrillaris usually act as opportunistic pathogens in immunocompromised or debilitated individuals in whom they cause pneumonitis or dermal ulcerations. From these lesions the amebas may spread to the brain to cause slowly progressive, and usually fatal encephalitis called granulomatous amebic encephalitis. In healthy individuals,

Acanthamoeba spp can cause an ulcerating keratitis, which is often associated with the use of improperly sterilized contact lenses.

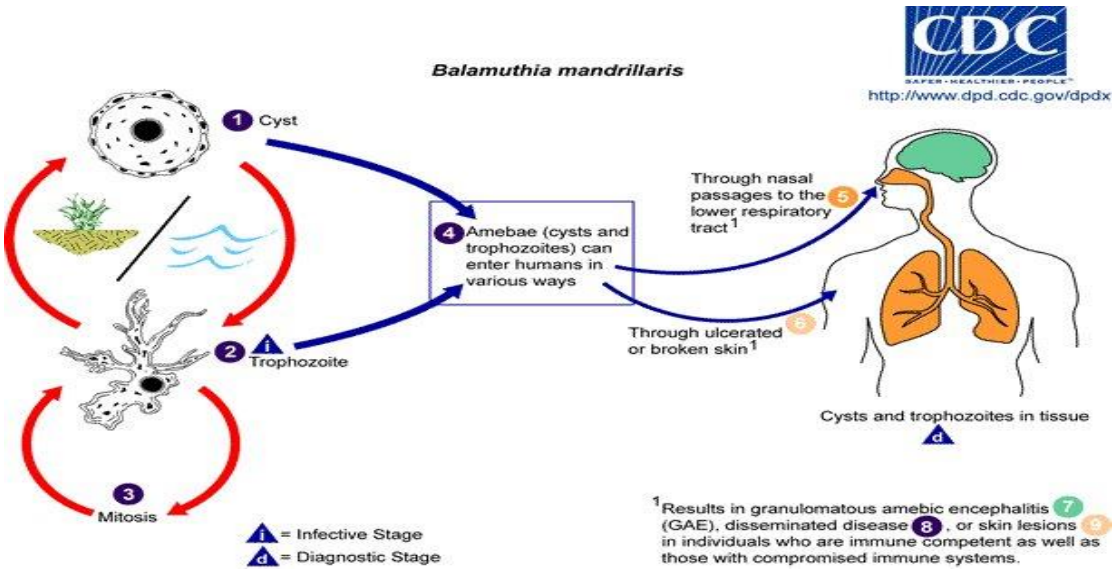
## Epidemiology

*Acanthamoeba* and *B mandrillaris* organisms live worldwide in soil and fresh and salt water. They may contaminate contact lens solution, physiotherapy pools, air-conditioning units, etc.

## Diagnosis

In many cases, granulomatous amebic encephalitis is not diagnosed until after or, at best, shortly before death. Immunosuppression or other predisposing factors may provide important clues. Computed tomography and magnetic resonance imaging of the brain are important diagnostic tests, as is examination of cerebrospinal fluid and brain biopsy specimens. The diagnosis usually is made after examination of brain tissue with light a microscope. Amebic “dermatitis” is often diagnosed by microscopic examination of a skin biopsy. Both trophozoites and cysts are usually visible.

In the case of amebic keratitis, scrapings of the corneal ulceration and biopsy specimens may contain amebic trophozoites and cysts. Both light and electron microscopy may be useful. Amebic cysts in the corneal stroma may be demonstrated by staining with hematoxylin and eosin, trichrome, calcofluor-white, or immunofluorescence techniques. Alternatively, amebas may be cultured at 37°C on non-nutrient agar with Page's saline containing Escherichia coli, Enterobacter aerogenes, or other Gram-negative bacteria. Cysts and trophozoites may be identified on the basis of morphology and locomotion; isoenzyme electrophoresis may be used to further classify species.



# Blastocystis hominis

was previously considered as a yeast, but recently it has been reclassified as a protozoan.

Habitat

It is a strict anaerobic protozoa found in large intestine of humans.

Morphology

B. hominis has 3 morphological forms:

Vacuolated form

is usually seen in stool specimen. It measures 8 µm in diameter and is characterized by its large central vacuole, which pushes the cytoplasm and the nucleus to the periphery. It multiplies by binary fission.

Amoeboid form

is a polymorphrous cell slightly larger than the vacuolated form occasionally seen in the feces. It multiplies by sporulation.

Granular form

measures 10–60 µm in diameter and is seen exclusively in old cultures.

Pathogenicity and Clinical Features

The pathogenecity of B. hominis is doubtful its believed to cause the irritable bowel syndrome. However, recent studies have shown the parasite to be associated with diarrhea. Clinical manifestations include diarrhea, abdominal pain, nausea, vomiting, fever, and chills. More than half of the patients suffering from infection with B. hominis have been found to be immunologically compromised.

Diagnosis

The condition is diagnosed by demonstration of the organism in stool smear stained by Giemsa or iron hematoxylin or Trichrome stains.