FREE LIVING AMOEBAE

are aerobic, mitochondriate, eukaryotic microorganisms widely distributed in nature and the human environment. From many genera of free-living amoebae that exist in nature, only four are involved in human and animal infections. Among them there are several of ***Acanthamoeba*** species, ***Balamuthia mandrillaris***, ***Naegleria fowleri*** and ***Sapinia pedata*** causes infections .

These amoebae are ubiquitous, and have been found in water, soil and air, but also in sewage, swimming pools, flowerpots, water tubs, humidifiers, aquaria, eye wash solutions and hospital environment, e.g. dialysis and dental treatment units . These protozoa are called **amphizoic** because of their ability to complete their life cycle inside host organism as well as in the environment.

- Human infections with these amoebae are rare but have been reported all over the world.

- FLA can cause localized systemic diseases as well as disseminated infections.

-All four genera cause acute and almost always fatal infections of the central nervous system (CNS). Some of them are also responsible for extra-CNS infections of the skin, eyes, sinuses, lungs and kidneys. Diseases occur both in immunocompetent and immunocompromised individuals, including patients with AIDS.

-Of greatest concern is the comparatively high mortality rate between infected individuals and lack of well-established recommended treatment.

- Early diagnosis of these infections is very important although it may be difficult due to non-specific symptoms.

1-*Naegleria Fowleri (brain – eating amoeba)*:

It causes **primary amoebic meningoencephalitis (PAM)** in humans. It is cosmopolitan, mainly in North America, Western Europe, Africa, Japan and Australia.

The amoebae found in warm fresh water of ponds, lakes, pools and moist soil. rivers, and hot springs. It is also found in soil, near warm-water discharges of industrial plants, and in poorly chlorinated, or

un-chlorinated swimming pools, in an amoeboid or temporary flagellate stage. There is no evidence of this organism living in salt water

Morphology: It is the only amoebae which is found in 3 forms; **flagellate trophozoite, amoeboid trophozoite** and **cyst stages**. Both amoeboid and flagellates are infective stage.

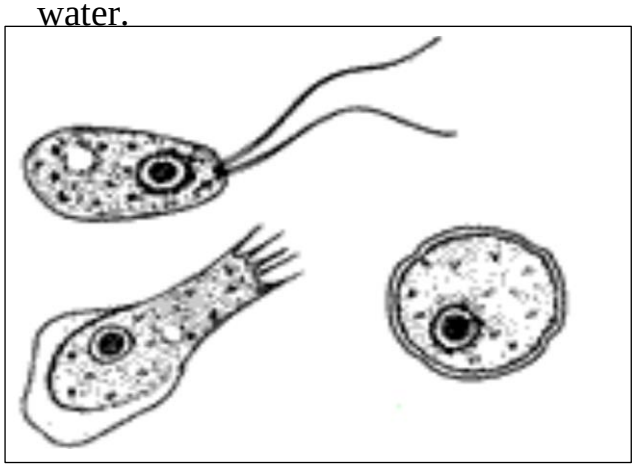
1.Amoeboid Trophozoite: It is the only form known to exist in humans. It is characterized by having single nucleus surrounded by halo and it move by pseudopodia. The pseudopodia form at different points along the cell, thus allowing the change direction. In their free 2– living state, the trophozoite feed on bacteria, in tissues, trophozoite phagocytize RBCs, white blood cells and destroy.

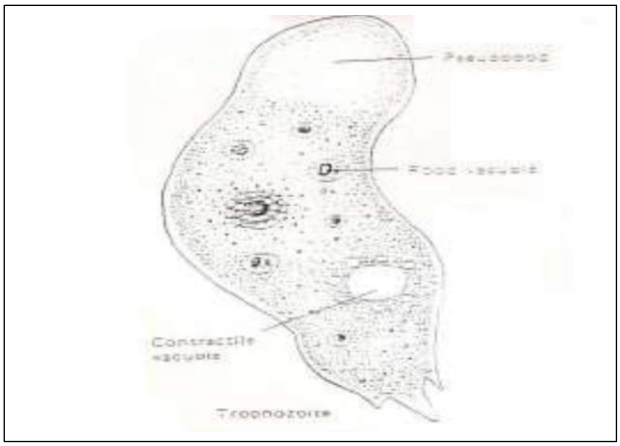
Trophozites are about 10-20 micrometers in diameter, mobile. Movement occurs by the organism extending broad, blunt pseudopodia (called lobodium), and then having the intracellular fluid and contents flow into the projection.

2.Flagellate Trophozoite: Pear shape bi-flagellated form, occurring when the amoeboid trophozoite exposed to a change in ionic concentration such as in distilled water. The transformation of amoeboid form to flagellated form occurs within few minutes .

The flagellate is pear-shaped and has two flagellum. This form can be used to distinguish *N. fowleri* from other pathogenic free-living amoeba, which do not have a flagellate form. The sample in question can be placed in distilled water, inducing the trophozite to convert to the flagellate form if the organism is *N. fowleri*.

3. Cyst Stage: Amoeboid trophozoite encysted due to unfavorable conditions e.g., crowding, desiccation and cold temperature below 10oc. the cyst has thick cystic wall and there are pores in cystic wall, the nucleus is similar to amoeboid form. Cysts are formed in culture and in water.

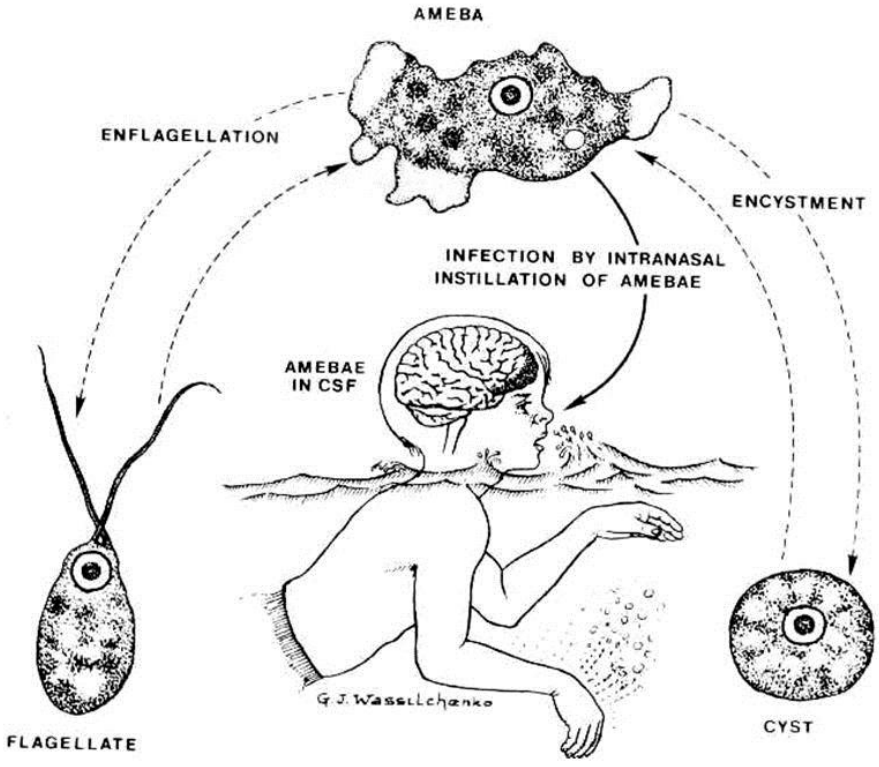




The spherical cyst is single-walled and 8-12 micrometers in diameter. The ameba encysts under severe conditions. When conditions improve, the ameba can escape the cyst through the pore, or ostiole, seen in the middle of the cyst.

Life Cycle:

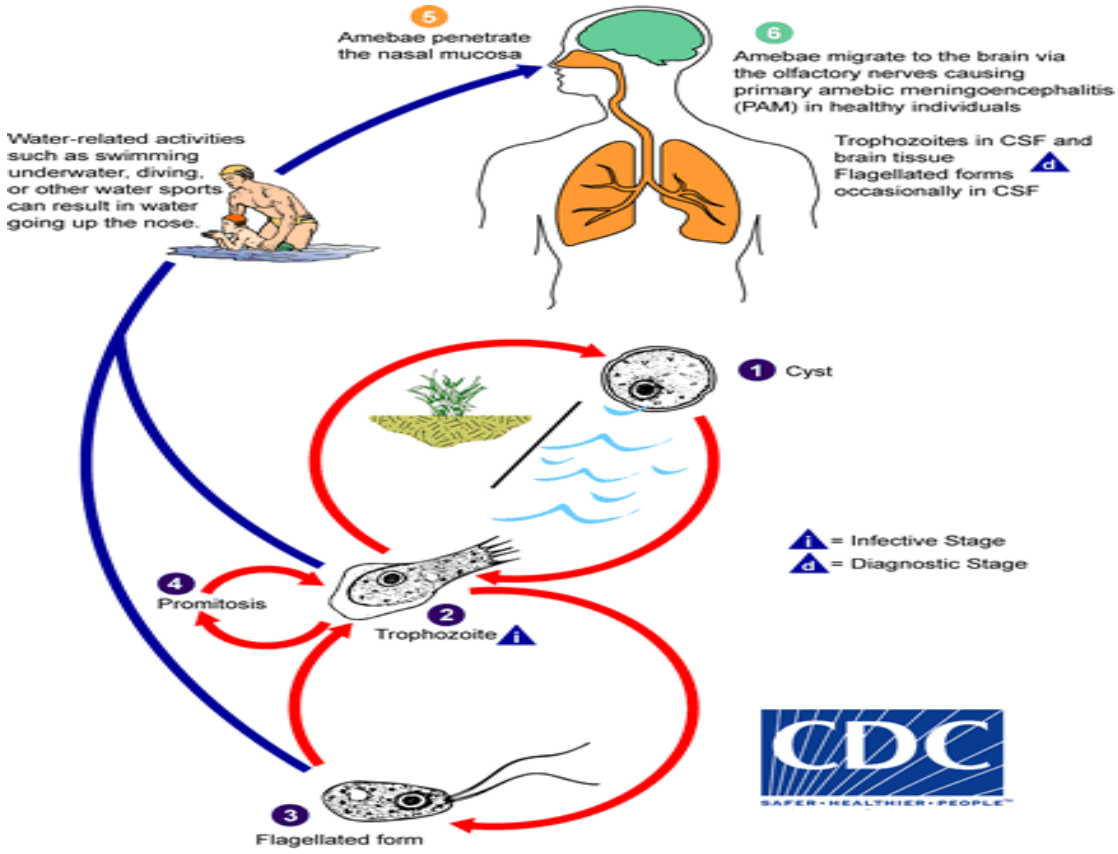
The life cycle of *N. fowleri* has three stages: trophozite (ameba), flagellate, and cyst stage. In the ameboid trophozite stage, the organism feeds on bacteria and replicates through promitosis, a type of binary fission where the nuclear membrane remains intact. When there is a chance in ionic concentration, for instance if the organism is placed in distilled water, or when nutrients are low, the trophozite can temporarily change into the flagellate form. The trophozite will generally revert back to the trophozite form when conditions are restored. When conditions are very unfavorable, for instance if the surrounding temperature is too cold, the trophozite will encyst.



Dr. RAAFAT MOHAMED

In primary amebic meningoencephalitis, usually only the trophozite is found in the tissue and cerebral spinal fluid, although sometimes the flagellate form can be found in the CSF. While trophozites feed on

bacteria in the external environment, in the body they feed on red and white blood cells as well as tissue.



Transmission to humans occurs primarily through inhalation of infested water. Increased contact of the nasal mucosa with infested water predispose humans to infection. Most injured person are young adults and children with a history of contacts with water containing the amoebae, about a week prior to the onset of neurological symptoms.

Pathogenesis and clinical features

The onset of illness occurs within 5–7 days and may develop rapidly after 24hrs. Because of the lack of distinctive clinical features، PAM can be easily confused with pyogenic, bacterial or viral meningitis. For that reason, any information about previous contact with warm water is essential .

The portal entry into the CNS is the olfactory neuroepithelium reached by

the nasal passages. The amoebic trophozoites penetrate the olfactory mucosa and migrate along the olfactory nerve، crossing the cribriform plate and reach the olfactory bulbs. Trophozoites induce an inflammatory

response associated with lytic necrotic haemorrhage. Then, numerous superficial haemorrhagic areas are seen in the cortex. Lesions are observed mainly around the base of the orbitofrontal and temporal lobe, base of the brain, hypothalamus, midbrain، pons, medulla oblongata, and the upper part of the spinal cord.

The earliest symptoms are

1- bifrontal or bitemporal headaches,
2- high temperature (38.5–41 °C), followed by nausea، vomiting and behavioral abnormalities, such as irritability and restlessness.

3-Subsequently, photophobia and neurological abnormalities develop, including lethargy, seizures، confusion, coma, diplopia or bizarre behavior may occur.

4-Cranial nerve palsies (third, fourth and six cranial nerves(may indicate cerebral oedema and herniation.

5- Intracranial pressure is usually raised. Cardiac rhythm abnormalities and myocardial necrosis may occur in some cases

Lab Diagnosis :

1-Direct demonstration of motile amoebae in unstained CSF or nasal

discharge

2- Stained smear of CSF.

3 -Stained section of brain tissue at autopsy.

4 -Culture on non-nutrient agar medium coated with E. coli bacteria.

5- Serological tests.

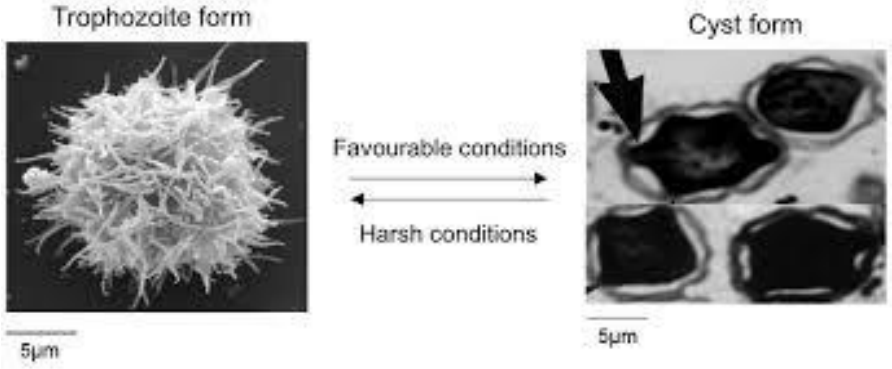
Treatment:

amphotericin has been the drug of choice. Most evidence is based on case reports and amphotericin is usually combined with rifampicin and other broad-spectrum antibiotics. Drugs are usually administered intravenously but intrathecal use has also been described

2-Acanthamoeba spp.

It causes granulomatous amoebic encephalitis (GAE) in immunocompromised patients and amoebic keratitis. It is cosmopolitan but is not necessarily associated with warm water, it is found in moist soil and in the air and water. It is found in only two form؛ the trophozoite and cyst, and either of these can be a source of infection.

Amoeboid trophozoite has spikey pseudopodia and a nucleus with large, central karyosome similar to the nucleus of Naegleria. Cyst: polygonal or thickly biconvex. Acanthmoeba organisms are slightly larger than *Neglaria fowleri*



Mode of infection can be acquired by:

•1 .inhalation., 2.ingestion , 3.through traumatized skin or eyes

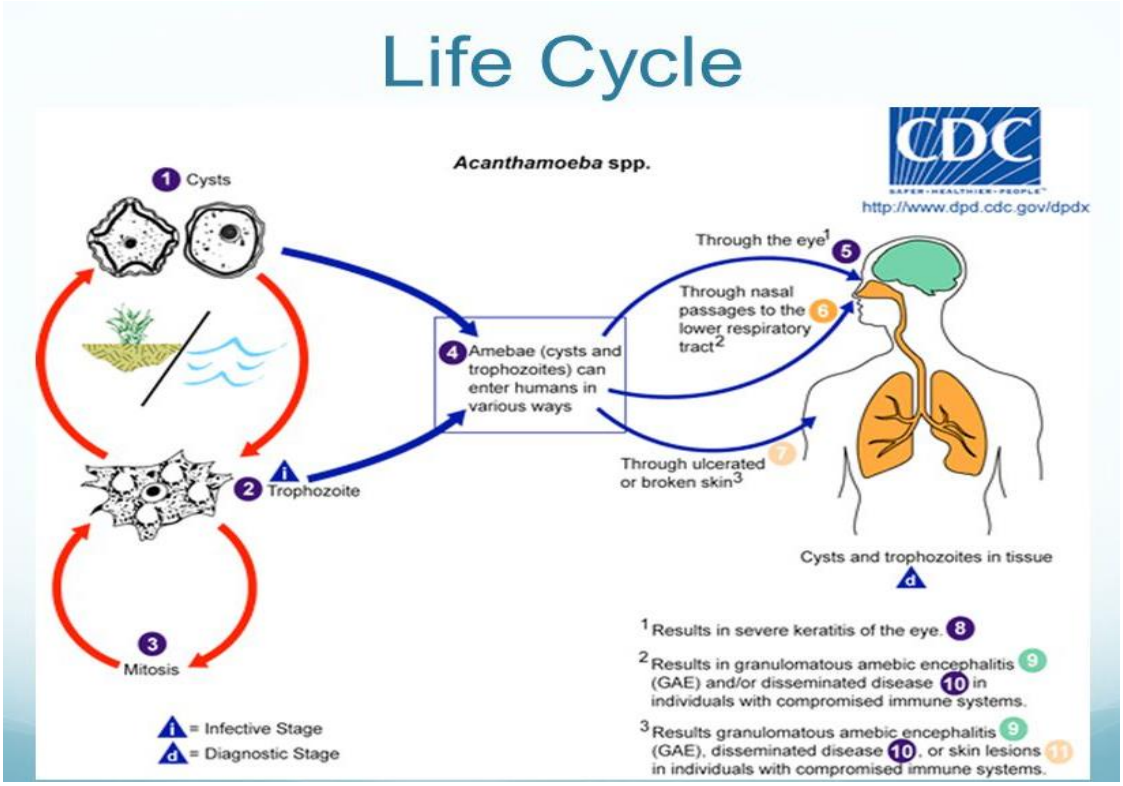
Acanthmoeba :CNS infection is not as in Naeglaria infection, and invasion of the CNS is secondary to infection elsewhere in the body. Amoebae reach the brain by way of bloodstream, most likely from lower

respiratory tract or through ulcer of the skin or mucosa, affected the immunocompromiesd patients while keratitis affects healthy persons.

Acanthamoeba spp. have been found in soil; fresh, salty, and sea water; sewage; swimming pools; contact lens equipment; medicinal pools; dental treatment units; dialysis machines; heating, ventilating, and air conditioning systems; mammalian cell cultures; vegetables; human nostrils and throats; and human and animal brain, skin, and lung tissues.

Unlike N. fowleri, Acanthamoeba has only two stages, in its life cycle. No flagellated stage exists as part of the life cycle. The trophozoites replicate by mitosis (nuclear membrane does not remain intact) .The trophozoites are the infective forms, although both cysts and trophozoites

gain entry into the body through various means. Entry can occur through the eye , the nasal passages to the lower respiratory tract , or ulcerated or broken skin . When Acanthamoeba spp. enters the eye it can cause severe keratitis in otherwise healthy individuals, particularly contact lens users . When it enters the respiratory system or through the skin, it can invade the central nervous system by hematogenous dissemination causing granulomatous amebic encephalitis (GAE) or disseminated disease , or skin lesions in individuals with compromised immune systems. Acanthamoeba spp. cysts and trophozoites are found in tissue.



A. Acanthamoeba (granulomatous amebic encephalitis)(GAE)

The amoebae probably enter respiratory system, or perhaps the skin, then migrate to CNS via the blood, once in the brain, it cause granulomatous encephalitis, which means that a more or less discrete mass of inflammatory cells and amoebae are found in the superficial layers of the brain. The lesion develop slowly and if treatment is not administered the progress of disease is unstoppable leading to death of the patient. Most patients with GAE do not have normal immune system(immunocompromised). GAE has been seen in AIDS patients. Diabetes, malignancies, malnutrition، systemic lupus erythematosus, or alcoholism.

B. Amoebic Keratitis:

Acanthamoeba spp. Caused ulcers of the cornea of eye in humans.

Cornea is invaded when there is trauma in the eye or the presence of amoebae in water. In most instances, there is an association with wearing contact lenses and a failure to clean them properly. Corneal lesions are painful and differentiation must be made from herpes simplex virus.

Diagnosis:

1.By finding amoebae in wet mount (10% KOH) of corneal ulcer scraping or in stained smear.

2.By isolation of amoebae form contact lenses or washing solutions.

3.Flood a culture plate with distilled water to distinguishing between Naegleria and Acnthomoeba species if flagellate observed it is Naegleria spp.

- **Treatment**:
  - GAE has been treated with pentamidine, usually in combination with one or more of the following: ketoconazole, hydroxystilbamidine, paromomycin, 5-fluorocytosine polymyxin, sulfadiazine, trimethoprim-sulfamethoxazole and azithromycin.
  - Centers for Disease Control and Prevention (CDC) are now investigating the use of miltefosine (also used to treat leishmaniasis). This drug has shown amoebicidal activity against several free-living species of amoeba in the laboratory and has been used successfully to treat patients infected with *B. mandrillaris* and disseminated *Acanthamoeba* spp.[9]
  - Similar medications are used in the treatment of *B. mandrillaris*