Free-living amoebas:

Several species of free-living amoeboflagellates or amoebas have been isolated and cultured from the spinal fluid of patients who died of meningoencephilitis. The free living amebas included:

1. ***Naegleria fowleri***
2. ***Acanthamoeba castelloni***
3. ***Balamuthia mandrillaris***

Most cases have been developed in children who were swimming and diving in worm soil, contaminated pools.

- Recently two important theories claimed for the free living organisms:

1. Free living amoeba as allergens:

Naegleria and Acanthamoeba have been claimed to be responsible for allergic pneumonia this is believed to be due to inhalation of amoebic antigens derived from amoeba growing in the humidifiers of air conditioning places.

2. Free living amoeba as carriers:

Some free-living water amoebae may sometimes harbour pathogenic bacteria such as legionella, pseudomonas and cholera vibrios. The bacteria can grow and multiply in the amoebae and survive in the cysts, resisting adverse environments, for example in chlorinated water. This may be significant in hospital infection if the water is contaminated with free-living amoebae. These water amoebae have been shown to be acceptable hosts for *Chlamydia pneumoniae, Legionella pneumophila* and some enteroviruses.

1.Naegleria fowleri :

Is a free-living excavate form of protist typically found in warm fresh water, from 25–35 °C (77–95 °F) in an amoeboid or temporary flagellate stage. It belongs to a group called the Percolozoa or Heterolobosea. *N. fowleri* can invade and

attack the human nervous system; although this occurs rarely, such an infection will nearly always result in the death of the victim.

In humans, *N. fowleri* can invade the central nervous system via the nose, more specifically the olfactory mucosa and cribriform plate of the nasal tissues. The penetration initially results in significant necrosis of and hemorrhaging in the olfactory bulbs. From there, amoebae climb along nerve fibers through the floor of the cranium via the cribriform plate and into the brain.

The amoebae begin to consume the cells of the brain piecemeal by means of a unique sucker apparatus extended from their cell surface. It then becomes pathogenic, causing primary amoebic meningoencephalitis (PAM or PAME). PAM is a syndrome affecting the central nervous system, characterized by changes in olfactory perception (taste and smell), followed by vomiting, nausea, fever, headache, and the rapid onset of coma and death in two weeks.

PAM usually occurs in healthy children or young adults with no prior history of immune compromise who have recently been exposed to bodies of fresh water. Amphotericin B is effective against *N. fowleri* in vitro.

N. fowleri can be grown in several kinds of liquid axenic media or on non-nutrient agar plates coated with bacteria. Escherichia coli can be used to overlay the non-nutrient agar plate and a drop of CSF sediment added to it. Plates are then incubated at 37 degrees and checked daily for clearing of the agar in thin tracks, which indicate that the trophozoites have fed on the bacteria.

Detection in water is performed by centrifuging a water sample with *Escherichia coli* added, and then applying the pellet to a non-nutrient agar plate. After several days the plate is microscopically inspected and *Naegleria* cysts are identified by their morphology. Final confirmation of the species' identity can be performed by various molecular or biochemical methods.

Confirmation of *Naegleria* presence can be done by so called flagellation test, when amoeba is exposed to hypotonic environment (distilled water). *Naegleria* in contrast to other amoebae differentiates within two hours into flagellar state.

Pathogenicity can be further confirmed by exposition to high temperature (42°C): *Naegleria fowleri* is able to grow at this temperature, but the non-pathogenic *Naegleria gruberi* is not.

Naegleria fowleri is only the amoebas with three known morphological form (Amoebic trophozoite, flagellated trophozoite and cyst form).

1.Amoebic trophozoite:

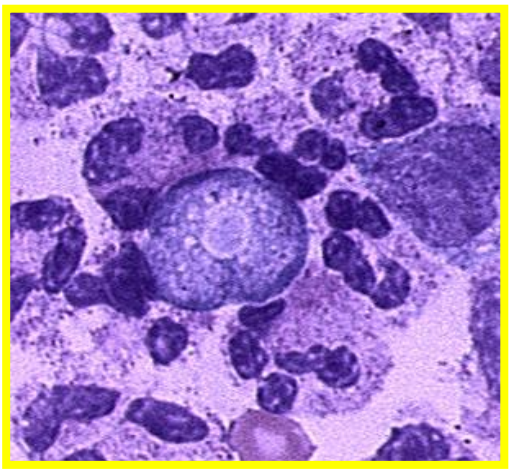
Size about 8-22 µ, motility is sluggish with pseudopodia; number of nuclei is one with karyosome, no peripheral chromatin. The cytoplasm is granular and vacuolated.

2.Flagellated trophozoite:

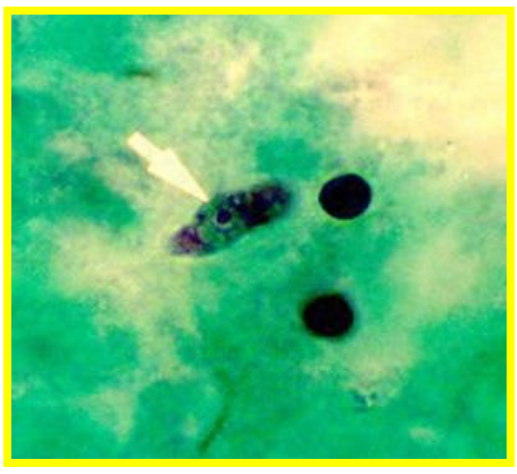
The size about 7-15 µ, the motility by 2-4 flagellates, and one nucleus identical to amoeboid troph. with central karyosome.

3.Cyst form:

The size about 9-12 µ *Naegleria fowleri* does not form cysts in human tissue. Cysts in the environment and culture are spherical, have a smooth, single-layered wall. Cysts have a single nucleus with thick cell wall, the nucleus on large central karyosome no peripheral chromatin. The cytoplasm is granular and contains vacuoles.

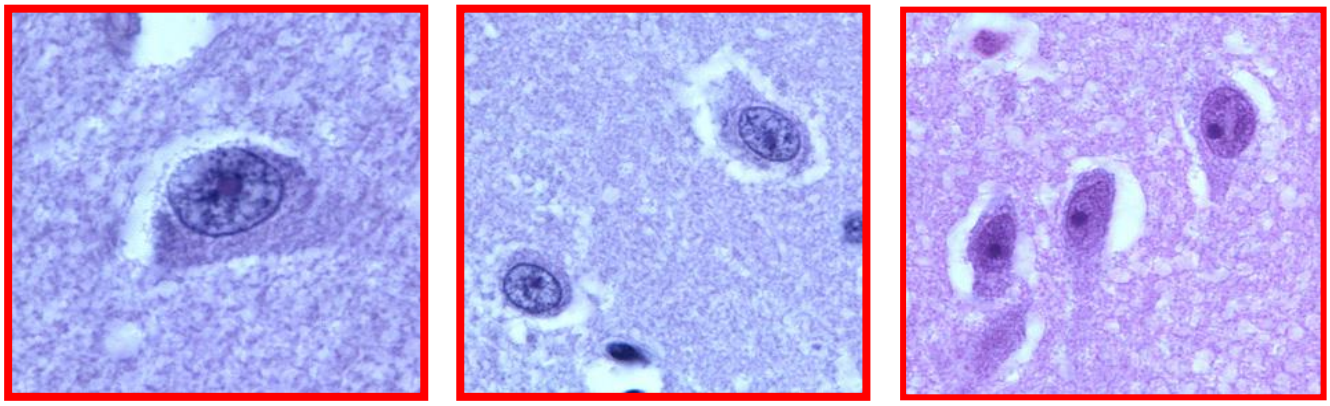


A



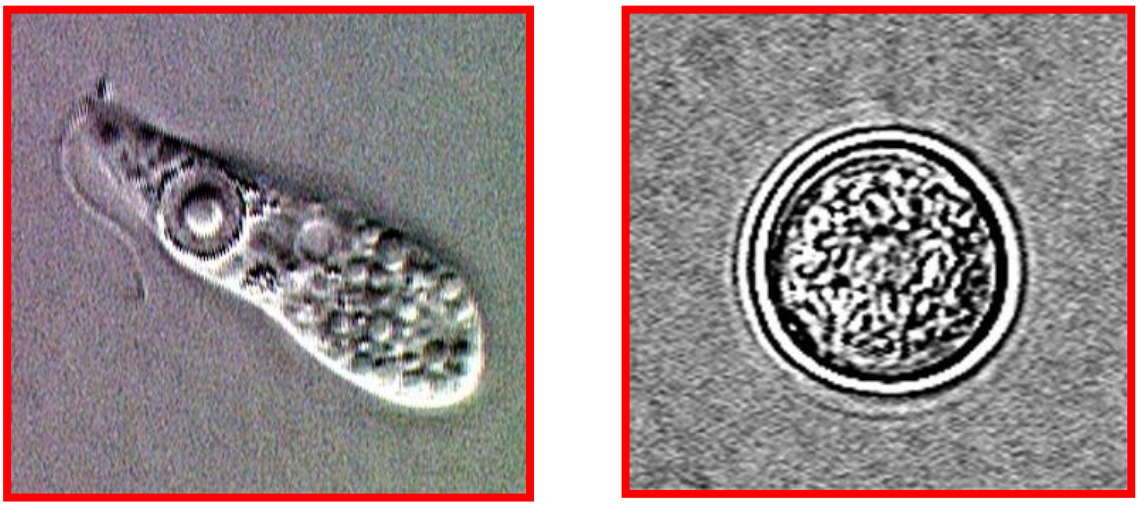
B

A: Trophozoite of *N. fowleri* in CSF, stained with hematoxylin and eosin (H&E). B: Trophozoite of *N. fowleri* in CSF, stained with trichrome.



C **D** **E**

C, D,E : Trophozoites of *N. fowleri* in brain tissue, stained with H&E.



F **G**

F: Ameboflagellate trophozoite of *N. fowleri* **G: Cyst of *N. fowleri* in culture.**

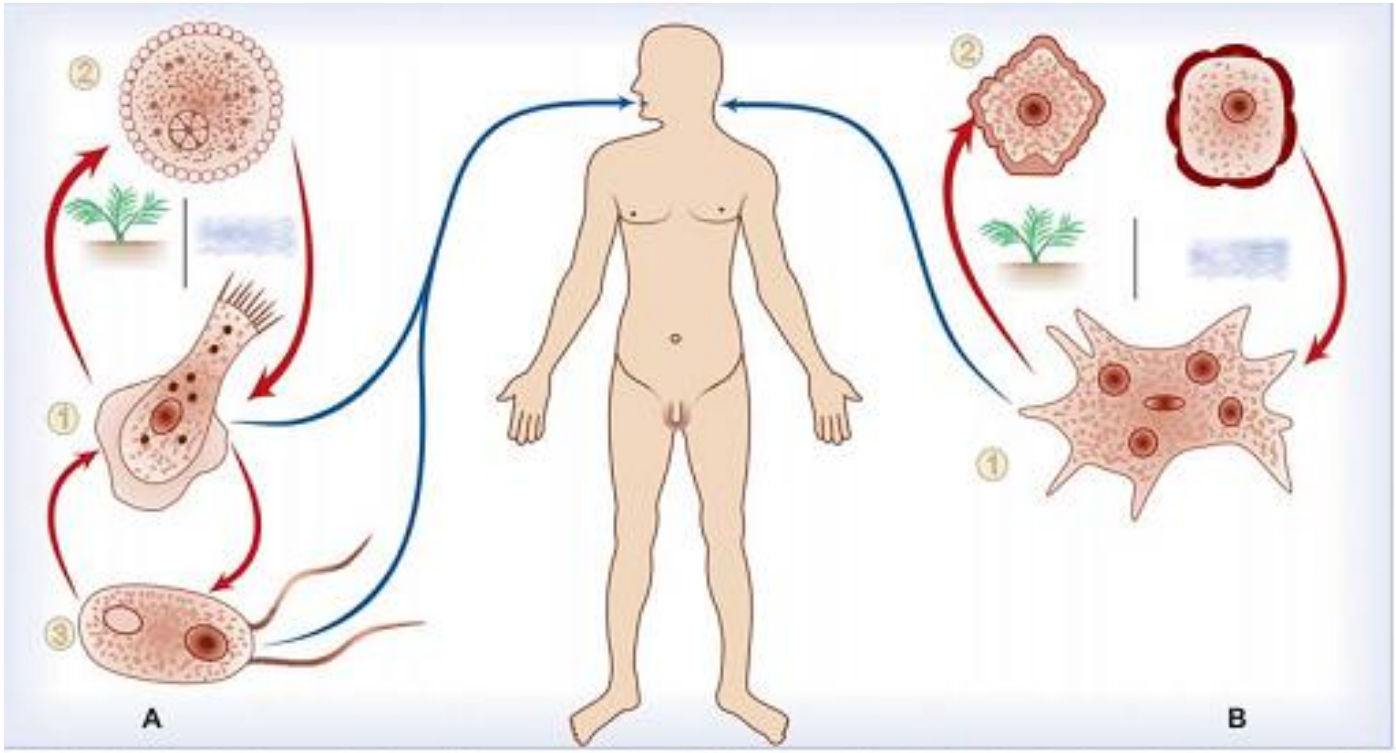


FIGURE 3.8: Pathogenic free-living amoebae. (A) *Naegleria fowleri:* 1—Amoeboid trophozoite showing lobopodia, nucleus with large endosome, and vacuoles. 2—Cyst. 3—Pear-shaped flagellate form showing flagella. (B) *Acanthamoeba culbertsoni:* 1—Trophozoite, showing spinous acanthopodia. 2—Cyst

Life Cycle of *Naegleria fowleri*

Laboratory diagnosis:

A. Direct observation of the motile amoebae in unstained C.S.F. or nasal discharge.
B. Stained smear.
C. Stained section of the brain at autopsy.
D. Culture on specific medium.
E. Serological tests.

Treatment:

Amphotericin B may be used for *Naegleria fowleri*.

2. Acanthamoeba sp.:

Is a genus of amoebae, one of the most common protozoa in soil, and also frequently found in fresh water and other habitats. Most species are free-living,

but some are opportunists that can cause infections in humans. Entry of this parasite to the C.N.S. usually from skin ulcers or traumatic penetration such as keratitis from puncture of the corneal surface or ulceration from contaminated saline used with contact lenses has also been reported.

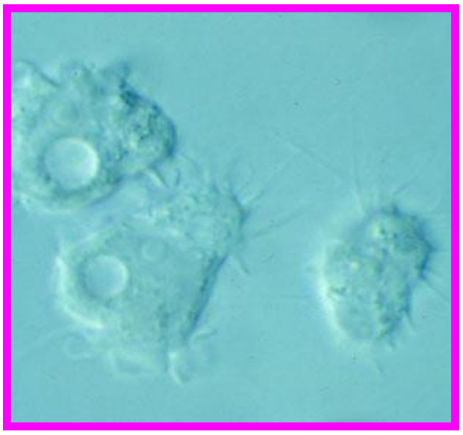
Acanthamoeba is a microscopic, free-living ameba commonly found in the environment that can cause rare but severe illness. Acanthamoeba causes three main types of illness involving the eye (chronic amoebic keratitis -CAK), the brain and spinal cord GAE(Granulomatous Amebic Encephalitis), and infections that can spread from an entry point to the entire body (disseminated infection).

Trophozoite:

The Trophozoites are small, usually 15 to 35 µm in length and oval to triangular in shape when moving. The pseudopods form a clear hemispherical lobe at the anterior, and there are various short filose extensions from the margins of the body. These give it a spiny appearance, which is what the name *Acanthamoeba* refers to. Trophozoites contain a large nucleus with a large, centrally-located karyosome but no peripheral chromatin. There is no flagellated trophozoite stage in *Acanthamoeba* spp.

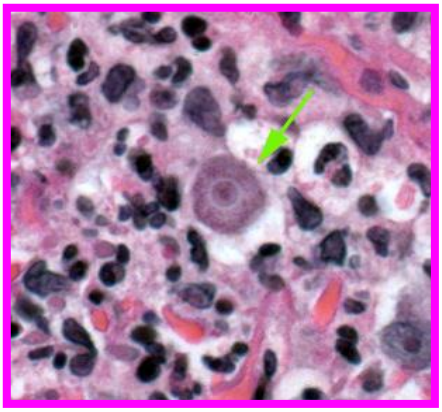
Cyst:

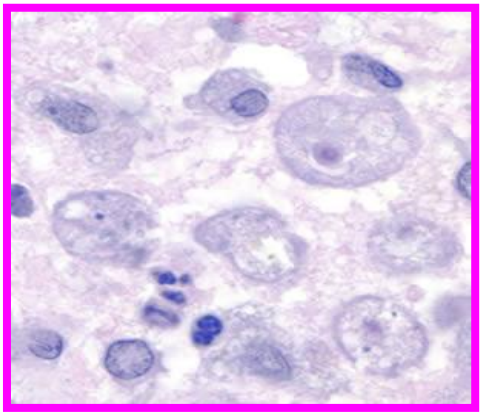
The cysts of *Acanthamoeba* spp. are typically 10-25 µm in diameter. The cysts have two walls: a wrinkled fibrous outer wall (exocyst) and an inner wall (endocyst) that may be hexagonal, spherical, star-shaped or polygonal. Cysts contain only one nucleus with a large karyosome. Cysts may be found in the brain, eyes, skin, lungs and other organs.



A **B**

A, B: Trophozoites of *Acanthamoeba* spp. from culture. Notice the slender, spine-like acanthapodia.

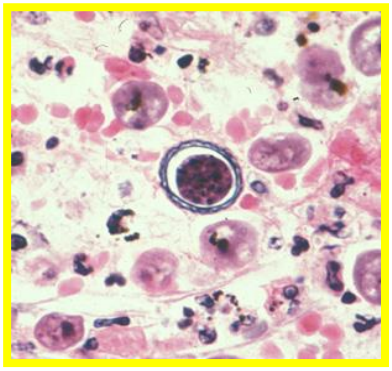


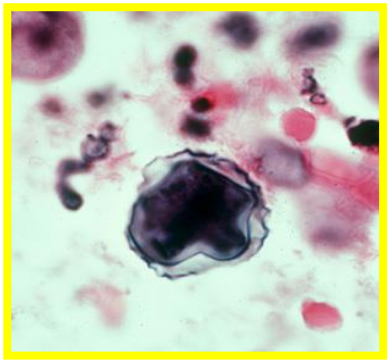


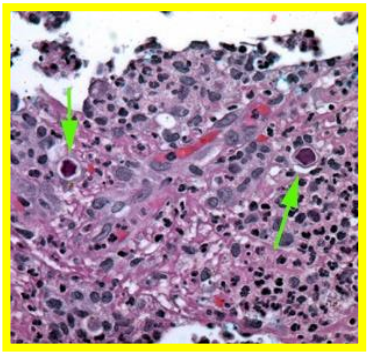
C D

C: Trophozoite of *Acanthamoeba* sp. in tissue, stained with hematoxylin and eosin (H&E).

D: Trophozoites of *Acanthamoeba* sp. in a corneal scraping, stained with H&E.



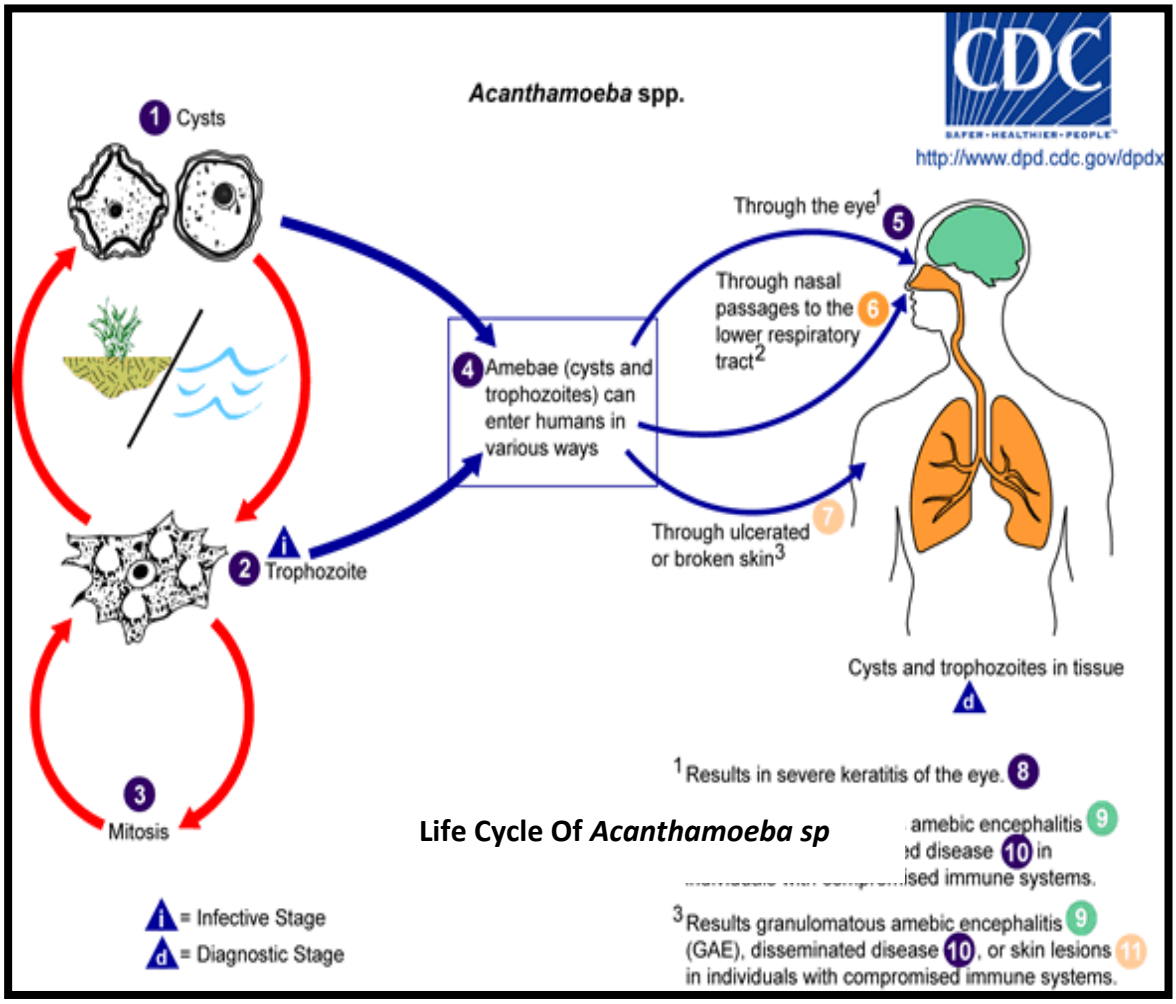




A **B** **C**

A, B: Cysts of *Acanthamoeba* sp. from brain tissue, stained with hematoxylin and eosin (H&E).

C: Cysts of *Acanthamoeba* sp. (green arrows) in tissue, stained with H&E.



Pathology:

A post-mortem biopsy reveals severe oedema and hemorrhagic necrosis. A patient that has contracted this illness usually displays subacute symptoms including altered mental status, headaches, fever, neck stiffness, seizures, focal neurological signs such as cranial nerve palsies and coma all leading to death within one week to several months. Due to the rarity of this parasite and our lack of knowledge there are currently no good diagnoses or treatments for *Acanthamoeba*.

Infection usually mimics that of bacterial leptomeningitis, tuberculous meningitis, or viral encephalitis. The misdiagnosis often leads to erroneous treatment that is ineffective.

Laboratory diagnosis:

1. Identification of trophozoite and cyst by using Giemsa or periodic acid Schiff. Stained.
2. Smear from scrapings or corneal biopsy specimens, staining with IFA have also been used.
3. Culture; require growth on non- nutrient agar plates seeded with bacteria E. coli or Klebsiella.
4. Serological, IF test and immno peroxidase.

3. Balamuthia mandrillaris

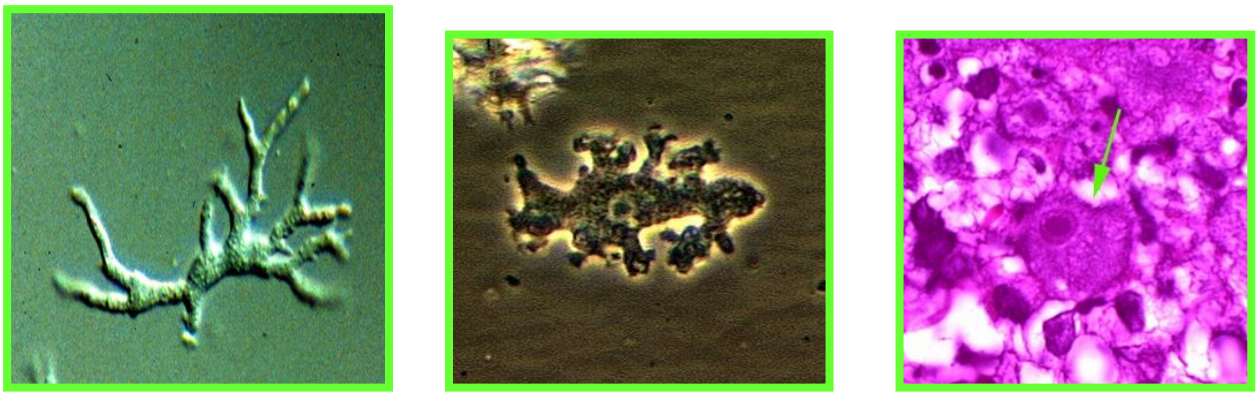
Is a free-living leptomyxid amoeba which is known to cause amoebiasis in humans, especially the deadly neurological condition known as primary amoebic meningoencephalitis. *Balamuthia* has not been definitively isolated in nature, but it is believed to be distributed throughout the temperate regions of the world. This is supported somewhat by the presence of antibodies to *Balamuthia* present in healthy individuals. The Balamuthia genus is named in honor of the late Professor William Balamuth for his contributions to the studies of parasitic and free-living amoebas.

Morphology:

Balamuthia mandrillaris is a free-living, heterotrophic amoeba, consisting of a standard complement of organelles surrounded by a three-layered cell wall, and with an abnormally large, vesicular nucleus. On average, a *Balamuthia* trophozoite is approximately 30-120 μm in diameter. The cysts fall approximately in this range as well.

Life cycle:

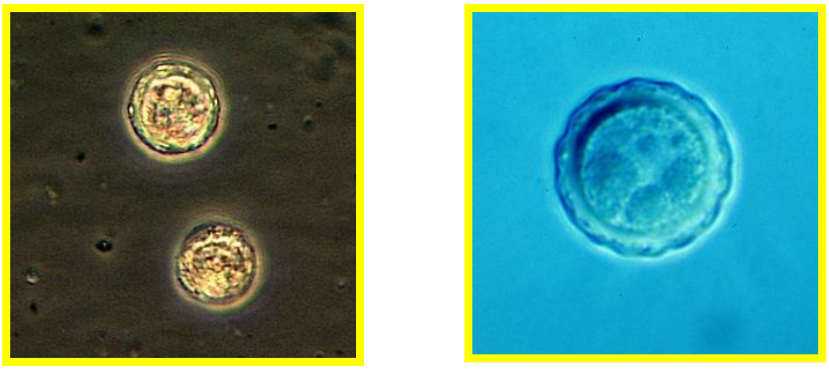
Balamuthia's life cycle consists of a cystic stage and a trophozoite stage, both of which are infectious, and both of which can be identified as inclusions in the brain tissue on microscopic examination of brain biopsies performed on infected individuals.



A **B** **C**

A, B: Trophozoites of *B. mandrillaris* in culture.

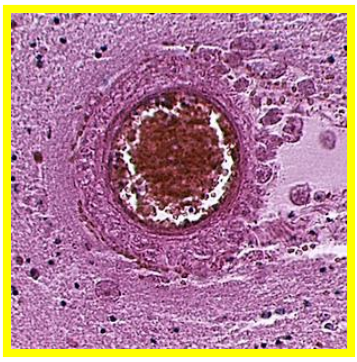
C: single trophozoite (green arrow) of *B. mandrillaris* in brain tissue, stained with H&E.



A **B**

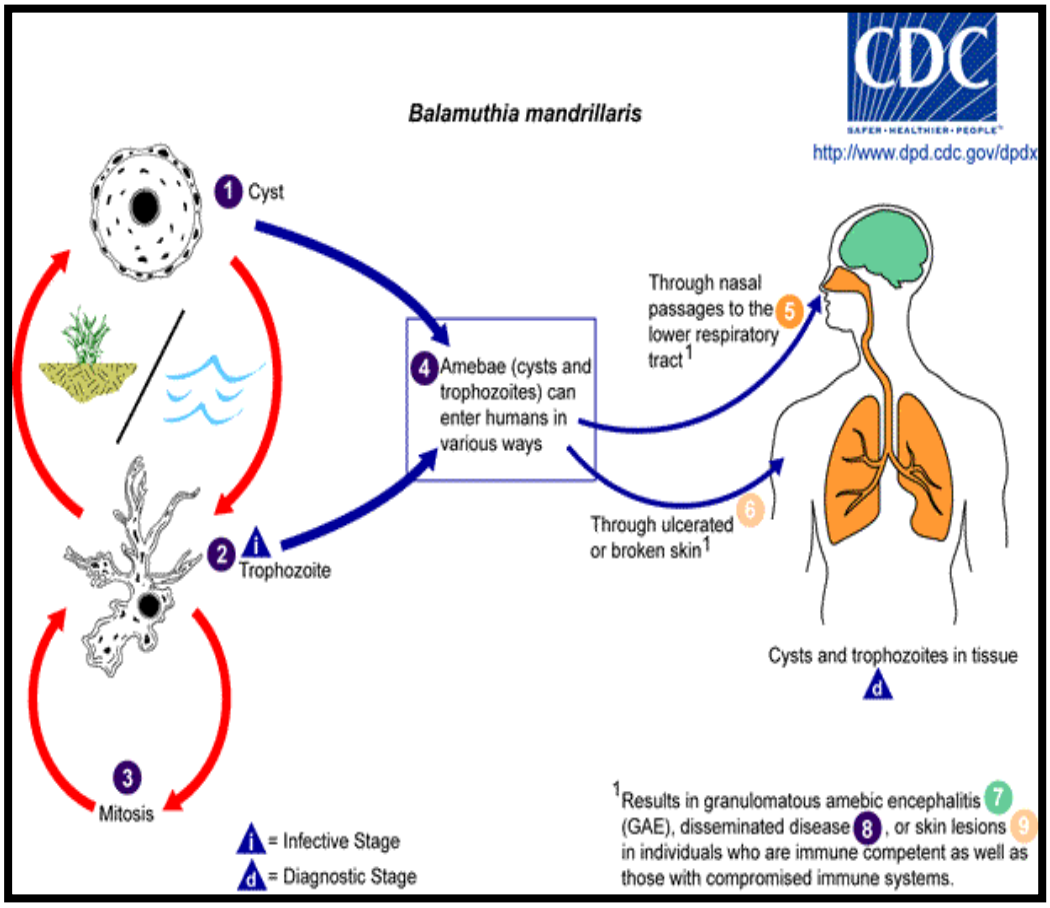
A & B: Cysts of *B. mandrillaris*.

C



D

C, D: Cysts of *B. mandrillaris* in brain tissue, stained with hematoxylin and eosin (H&E).



Pathology:

Balamuthia mandrillaris may enter the body to the lower respiratory tract or through open wounds. Upon introduction, the amoebas may form a skin lesion, or migrate to the brain. Once in the brain, *Balamuthia* causes a condition known as granulomatous amoebic encephalitis (GAE), which is usually fatal.

The symptoms of infection by *Balamuthia* are unclear, as only a few definitive cases of *Balamuthia* infection have been described thus far. *Balamuthia*-induced GAE can cause focal paralysis, seizures, and brainstem symptoms such as facial paralysis, difficulty swallowing, and double vision.

Balamuthia is also known to cause a variety of non-neurological symptoms, and often causes skin lesions, through which the amoeba may enter the bloodstream and migrate to the brain. Many patients experiencing this particular syndrome report a skin lesion (sometimes similar to those caused by *Staphylococcus aureus* or other bacteria), which does not respond well to dermatologic treatment. The lesion is usually localised and very slow to heal, or fails to heal altogether. *Balamuthia* encephalitis is an extremely deadly disease, and as of 2008, only seven recoveries had been reported, all with lasting brain damage.

A B

A, B: Gross specimens of brain tissue from a patient who died of granulomatous amebic encephalitis (GAE) caused by *Balamuthia mandrillaris*.