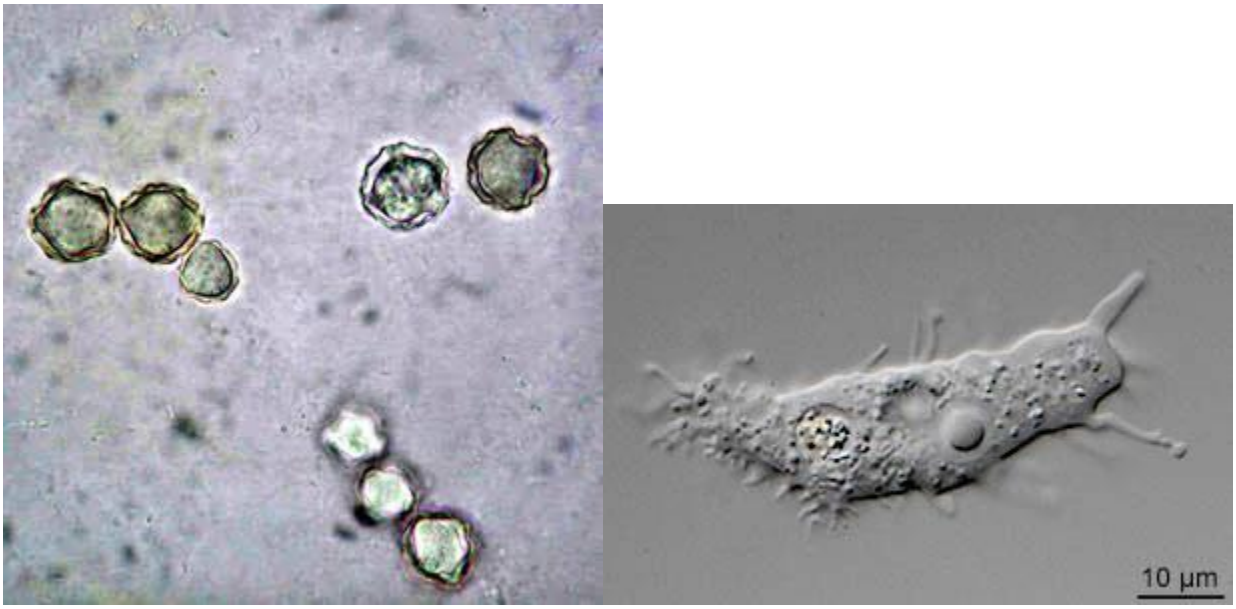
Acanthamoeba Infection, Its Outcomes, and the Laboratory Diagnosis

Dr. Mohammad Taghi Ahady

PhD in Medical Parasitology

Acanthamoebas are a group of opportunistic protozoa belonging to free-living amoebae that are widely distributed in the environment. Several studies conducted by researchers over the past few years show that *Acanthamoeba* is distributed in almost all regions of the world. These pathogenic amoebae have been isolated from various environments, including sea water, ocean sediments, sea shores, pond water, stagnant water, freshwater ponds, hot springs, frozen waters, saltwater ponds, dust, river water, surface layers of water, soil, and even air. *Acanthamoebas* have also been isolated from man-made sources, including mineral water cans, sterile water cans, factory waste, hot water baths, hydrotherapy, humidifiers, sewage, agricultural field soil, vegetables, surgical instruments, contact lenses and hospital environments. 23 species of Acanthamoeba have been named based on morphological parameters. All *Acanthamoeba* species can grow in NNA culture medium with a bacterium as a food source such as *Escherichia coli*. *Acanthamoeba* has a diameter between 13-22 micron. There are two stages and two forms in the life cycle of *Acanthamoeba*: Trophozoites: They are between 10-45 microns and vary up to 50 microns depending on the species. Spiny pseudopodas (acantopodia) are needle-shaped that move and give the trophozoite a slow motion. Cysts: The cysts are double-walled endocyst and exocyst, and the endocyst can be seen in different shapes such as star, square, triangular and even spherical. The size of the cyst varies approximately between 9-25 microns. Inside the nucleus, there is a large central karyosome.



Acanthamoeba species can be divided into three morphological groups, based on cyst measure and structure: Group 1: This group includes 4 species which have large size cyst (>18 micron), such as *A. astronyxis*. Group 2: Includes 11 species, which are the most prevalent isolates, such as *A. polyphaga*, *A. castellanii*, *A. griffin* (the size of cyst <18 micron). Group 3: Includes 5 species such as *A. culbertsoni*, *A. palestinensis* (the size of cyst <18 micron).

Molecular analysis of the 18S nuclear small-subunit rRNA gene and the sequencing of diagnostic fragment 3 (DF3) of 18S rRNA gene obtained via PCR have classified *Acanthamoeba* isolates into 23 genotypes (T1-T23). It should be emphasized that the T4 genotype is the most common and important *Acanthamoeba* genotype in Iran and the world, which is the cause of various diseases in humans, including keratitis (AK) and encephalitis (GAE).

Acanthamoebas can enter human bodies from various sources, and afterwards live as opportunistic parasites causing pathogenic consequences. Thus, environmental sources can provide important risks for human infection. These amoebae can cause granulomatous amoebic encephalitis (GAE), amoebic keratitis (AK), cutaneous lesions, pulmonary and kidney infections. Acanthamoeba is also responsible for life threatening infections in immunodeficiency patients.

Amphotericin B and sulfonamides such as sulfadiazine are recommended. Ketoconazole and microconazole are also recommended in the treatment of amebic keratitis. The prognosis of GAE treatment is unsuccessful, While AK responds well to drug therapy.

Diagnosis strategies include: 1. Isolation of *Acanthamoeba* from the cornea of the eye in order to diagnose amoebic keratitis (AK): Biopsy or Scraping from corneal lesions, Culture the sample in non-nutritional agar (NNA) medium containing *Escherichia coli* (ECNNA) as Xenic medium, or in Axenic medium (NNA without E. coli), It is recommended that before and after the culture of the samples, microscopic examination should be done directly by preparing a wet smear. Implementation of molecular PCR method: *Acanthamoeba* DNA extraction by phenol chloroform method and then amplification of a fragment of 18S rDNA gene using specific primers. Molecular methods such as PCR are more sensitive than culture and can detect even low levels of *Acanthamoeba.* 2. Isolation of *Acanthamoeba* from cerebrospinal fluid to diagnose granulomatous amoebic encephalitis (GAE): Cerebrospinal fluid (CSF) extraction or lumbar puncture (LP), this test is performed on the lower part of waist. During a lumbar puncture, a needle is inserted into the space between the vertebrae to take a sample of the cerebrospinal fluid (CSF). Direct microscopic examination of wet smear prepared from CSF, centrifuging the cerebrospinal fluid and preparing the smear from sediment and staining the smear with the Giemsa method and microscopic observation. Culture the punctured CSF fluid in ECNNA medium, repeat the same microscopic test. PCR technique can also be implemented. 3. Isolation of *Acanthamoeba* from environmental samples: Collection of water, soil, dust or other environmental samples. Filtration of the samples using nitrocellulose papers with 1.6 micrometer pores. Cutting and separating the middle part of nitrocellulose paper after filtration and cultivating it in 1.5% ECNNA medium. After a week, the colony grown in the culture medium is examined with a microscope. Molecular methods such as PCR can also be implemented.

Conclusion: *Acanthamoeab* is an important protozoan pathogen with widely distribution throughout the world. *Acanthamoeba* contaminates all parts of the environment including water, soil, dust, and air. This pathogenic amoeba is the cause of Amoebic Keratitis (AK), and Granulomatouse Amoebic Encephalitis (GAE). The treatment of *Acanthamoeba* infections is difficult and sometimes unsuccessful. Therefore, it is necessary to follow health standards and early diagnosis of *Acanthamoeba* infection should be done with the strict implementation of

laboratory diagnosis procedures including sampling, infected sample culture, microscopic examination, serological and molecular tests such as PCR.

Keywords: *Acanthamoeba*, Infection, Amoebic Keratitis, Granulomatous Amoebic Encephalitis, Laboratory Diagnosis