Acanthamoeba
(protist: amoeba)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). Protists which move and feed using pseudopodia (false feet) are known as amoebae. Rather than forming a monophyletic group, amoebae are divided into three major disparate groups: Heterolobosea (supergroup Excavata), Rhizaria (supergroup SAR) and Amoebozoa (supergroup Amorphea). The latter contains four classes of amoebae differing in their types of pseudopodia and organelles: Tubulinea, Discosea, Gracilipodia and Archamoebae. Discosean amoebae are flattened in profile and form broad lobopodia. Acanthamoebae form finely-tapering subpseudopodia (acanthopodia) emerging from the cell-wide lobopodium and they exhibit sluggish motility. Most species form cysts and are found as free-living organisms, although some species are considered to be 'amphizoic' due to their ability to live as both free-living organisms and parasitic pathogens. Most free-living species live in aquatic or terrestrial environments where they feed on bacteria, fungi and other protozoa. Several *Acanthamoeba* species, however, are facultative/opportunistic parasites primarily in the central nervous system, skin and lungs of humans and animals, and the cornea of humans.

Classification:
Domain: Eukaryota (membrane-bound nucleus)
Supergroup: Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Phylum: Amoebozoa (locomotion by noneruptive pseudopodia, asexual development)
Subphylum: Lobosa (with lobose pseudopodia)
Class: Longamoebea (flattened elongated cells with pointed subpseudopodia)
Order: Centramoebida (finely-tapering subpseudopodia (= acanthopodia), most form cysts)
Family: Acanthamoebidae (trophozoites flattened, prominent subpseudopodia, cysts stellate)
Genus: *Acanthamoeba* (free-living in terrestrial and aquatic environments, some species opportunistic parasites)
Species: various species cause granulomatous amoebic encephalitis and cutaneous acanthamoebiasis in humans and animals, and amoebic keratitis in humans

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Amoebae form pseudopodia to move and feed. Several types of amoebae are recognized on the basis of differences in their biology and morphology, with recent molecular phylogenetic studies supporting their classification into three major disparate phyla: Heterolobosea (supergroup Excavata), Amoebozoa (supergroup Amorphea) and Rhizaria (supergroup SAR). Most species are free-living in aquatic and terrestrial habitats where they feed on other micro-organisms, but several species have become symbiotic in metazoan organisms as endocommensals or opportunistic-facultative parasites (representatives tabulated below).

Higher taxonomy	Class/Order	Family	Genus	Hosts	Tissues (disease)*
Supergroup: Excavata (with conspicuous ventral feeding groove)					
Group: **Discoba** (diverse group supported robustly by molecular studies)					
Phylum: Heterolobosea (amoebo-flagellates, most form cysts)	O: Schizopyrenida (no fruiting bodies)	Vahlkampfidae (eruptive limax pseudopodia, flagellated stages)	*Naegleria*	mammals	central nervous system (PAM)
Supergroup: Amorphea (unikonts with single flagellum, or nonflagellated amoebae)					
Phylum: **Amoebozoa** (locomotion by noneruptive pseudopodia, asexual development)					
Subphylum: Conosa (archamoebae & mycetozoa)	C: Archamoebae (amitochondriate, rounded cysts)	Entamoebidae (uninucleate, endozoic)	*Entamoeba*	mammals	colon (dysentery), central nervous system (SAM)
Subphylum: Lobosa (lobose amoebae)	C: Discosea (flattened forms, protoplasmic flow polyaxial)	Vexilliferidae (dactylopodia, parasomes)	*Paramoeba* *Neoparamoeba*	fish	gills (AGD)
	C: Longamoebea (flattened elongated cells, stellate cysts)	Acanthamoebidae (acanthopodial subpseudopodia)	*Acanthamoeba, Balamuthia*	mammals	central nervous system (GAE)

Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups robustly supported by molecular studies)		
Group: **Rhizaria** (amoebae with fine pseudopodia in simple, branching or anastomosing patterns)		
Phylum: Cercozoa	Filosa (with filopodia, naked or testate)	free-living (aquatic, terrestrial)
Phylum: Endomyxa	heterotrophic amoeboid or plasmodial cells	free-living, some parasitoids
Phylum: Retaria	Foraminifera (with reticulopodia), Radiolaria (with axopodia)	free-living (aquatic)

*PAM = primary amoebic meningoencephalitis; SAM = secondary amoebic meningoencephalitis; GAE = granulomatous amoebic encephalitis; AGD = amoebic gill disease.

Amoebae that move using noneruptive pseudopodia are placed in the phylum Amoebozoa, either in the subphylum Lobosa (with lobose pseudopodia) or Conosa (with microtubular cones). Lobose amoebozoans include the Dactylopodida with finger-like subpseudopodia (= dactylopodia) and the Centramoebida with finely-tapering subpseudopodia (= acanthopodia). Acanthamoebae are flattened centramoebid amoebae which form stellate cysts. They are naked (without tests or shells) and have simple life-cycles (without temporary flagellated stages), most being free-living amoebae (FLA) abundant in aquatic and terrestrial environments. Some FLA species are amphizoic and may opportunistically infect vertebrate hosts, usually causing neurological conditions. Primary meningoencephalitis (PAM) is caused by *Naegleria* spp. while granulomatous amoebic encephalitis (GAE) is caused by *Acanthamoeba*, *Balamuthia* or *Sappinia* spp. [note that secondary amoebic meningoencephalitis (SAM) is caused by extraintestinal infections by the parasite *Entamoeba histolytica*]. FLA do not form a natural assemblage but have been split by recent molecular studies into different phyla: *Naegleria* being classified within the Heterolobosea (amoebo-flagellates), and *Acanthamoeba*, *Balamuthia* and *Sappinia* within the Amoebozoa (noneruptive pseudopodia) [together with the parasite *Entamoeba*]. Early studies often did not differentiate between the genera *Acanthamoeba* and *Hartmannella* (and sometimes even *Naegleria*), so the validity of many initial case reports remains questionable. *Acanthamoeba* spp. have since been found to be one of the most prevalent protozoa found around the world. They have been isolated from soil, dust, air, freshwater, seawater, sediments, vegetation and as contaminants in bacterial, yeast and mammalian cell cultures. Around 26 species have been described, 15 being recorded as opportunistic pathogens in mammals, birds, reptiles, amphibians and fish. Serological studies have detected antibodies against *Acanthamoeba* spp. in up to 80% of human populations, indicating a much greater prevalence of exposure than the low (rare) incidence of clinical disease would suggest. Recently, nuclear (Rns) or mitochondrial (rns) ribosomal RNA gene sequencing have been used to identify various genotypes from clinical and environmental samples (encoded T1-T20). Genotypes T7, T8, T9, T17 and T18 belong to group I; genotypes T3, T4, T11, T13 and T19 to group II; and genotypes T1, T2, T5, T6, T10, T12 and T15 to group III. Some genotypes remain to be grouped morphologically (T16, T20) and some species remain to be genotyped (e.g. *A. echinulata*). In addition, several genotypes have been detected that are not referable to named species, while other genotypes may represent several species (especially T4), Most genotypes have been recovered from environmental samples, while a growing number are being associated with clinical disease in humans and animals. Genotypes recovered from hosts but not referable to known *Acanthamoeba* spp. include T1, T6, T13 and T17 from clinical samples; T14 from human faeces; and T20 from the internal organs of a toucan.

***Acanthamoeba* species**	**Genotype (rDNA)**	**Cyst morphotype**	**Hosts**	**Location**	**Clinical signs**
un-named species	T1	II	humans	brain	encephalitis
A. palestinensis	T2	III	humans	eye, brain	keratitis, encephalitis
A. griffini	T3	II	humans	eye	keratitis
A. castellanii	T4	II	humans	eye, brain, skin, lung, prostate, bone, muscle, sinus	keratitis, encephalitis, cutaneous lesions
A. lugdunensis	T4	II	humans	eye	keratitis
A. polyphaga	T4	II	humans, fish^{+}	eye, brain	keratitis, encephalitis
A. rhysodes	T4	II	humans	eye, brain	keratitis, encephalitis
A. triangularis	T4	II	humans	eye	keratitis
A. lenticulata	T5	III	humans	eye, brain	keratitis, encephalitis
un-named species	T6	III	humans	eye	keratitis
*A. astronyxis**	T7	I	humans	brain, adrenal, lymph node, sinus, thyroid, skin	encephalitis
A. culbertsoni	T10	III	humans	eye, brain, liver, spleen, uterus, skin	keratitis, encephalitis
A. hatchetti	T11	II	humans	eye	keratitis
A. quina	T11	II	humans	eye	keratitis
A. healyi	T12	III	humans	eye, brain	keratitis, encephalitis

un-named species	T13	II	humans	eye	keratitis
A. jacobsi	T15	III	humans	eye	keratitis
un-named species	T17	I	humans	eye, brain	keratitis, encephalitis
A. byersi	T18	I	humans	brain, skin	encephalitis, cutaneous lesions

*This record is considered by some to represent a case of culture contamination.

+Trophozoites recorded from gills, urinary bladder, spleen, gall bladder and blood of freshwater fish (white sucker, common shiner, blue tilapia, largemouth bass, striped bass, channel catfish, carp, goldfish and rainbow trout)

Experimental infections by *Acanthamoeba* spp. have been established in a range of animals (mice, rats, hamsters, rabbits, pigs) to model disease (both AK and GAE). Naturally acquired infections have also been detected in gorillas (GAE), cattle (GAE, lungs, faeces, some involving genotype T4), water buffalo (lungs), sheep (GAE), horses (placentitis due to *A. hatchetti*), dogs (GAE due to *A. castellanii* and *A. culbertsoni*, as well as un-named species in lungs and viscera), cats (AK due to T4), muskrats (faeces), ground squirrels (gut), rabbits (liver, *A. polyphaga*), kangaroos (GAE), birds (*A. polyphaga* in intestines of turkeys, genotypes T4 and T5 in cornea of sparrowhawks, T20 in internal organs of toucan, un-named species in faeces of ducks and gulls and intestines of pigeons), reptiles (faeces of snakes and lizards), amphibians (faeces of toads and frogs) and fish (gills, spleen, urinary bladder and blood of wild caught and ornamental freshwater species). Free-living species which have not been associated with disease include: *A. astronyxis* (group I, genotype T7), *A. comandoni* (I, T9), *A. divionensis* (II, T4), *A. echinulata* (I), *A. mauritaniensis* (II, T4), *A. micheli* (II, T19), *A. paradivionensis* (II, T4), *A. pearcei* (I, T3), *A. pustulosa* (III, T2), *A. royreba* (III, T4), *A. stevensoni* (II, T11), *A. terricola* (II, T4), *A. tubiashi* (I, T8) as well as genotypes T16 and T17. Free-living species have been isolated from soil and water samples; including coastal seawater, freshwater lakes, streams, rivers, swimming pools, thermal springs, power plants and sewage works.

Many *Acanthamoeba* spp. have also been found to harbour microbial endosymbionts (*Candidatus Caedibacter acanthamoebae, Candidatus Odyssella thessalonicensis, Candidatus Paracaedibacter acanthamoebae, Candidatus Paracaedibacter symbiosus, Comamonas acidovorans, Legionella pneumophila, Pseudomonas aeruginosa*, megavirus, mimivirus and pandoravirus) [note that the term *Candidatus* is used to denote interim taxonomic status for bacteria that cannot be maintained in culture], as well as supporting the development and multiplication of other bacterial species (*Aeromonas, Afipia felis, Bacillus cereus, Bartonella, Burkholderia picketti, Campylobacter jejuni, Chlamydia pneumoniae, Coxiella burnetii, Cytophaga, Escherichia coli 0157, Flavobacterium, Francisella tularensis, Helicobacter pylori, Listeria monocytogenes, Mycobacterium avium, Mycobacterium leprae, Pasteurella multocida, Prevotella intermedia, Porphyromonas gingivalis, Rickettsia, Salmonella enterica, Salmonella typhyimurium, Shigella, Simkania negevensis, Staphylococcus aureus, Vibrio cholera* and *Waddlia chondrophila*).

Parasite morphology: Acanthamoebae form two developmental stages: motile feeding trophozoites and dormant resting cysts (they do not form temporary flagellated stages). Trophozoites range in size from 15-40 µm, contain a single nucleus with a large central dense nucleolus, a contractile vacuole and form numerous spiny pseudopodia (acanthopodia). Cysts are uninucleate and bound by a proteinaceous ectocyst wall and a cellulosic endocyst wall. The cysts are roughly ovoid, range from 10-20 µm in diameter and are frequently wrinkled and stellate in appearance. Individual *Acanthamoeba* species were initially classified according to cyst size and shape into three morphotypes: group I forming large cysts (>18 µm) with smooth ectocysts and stellate endocysts meeting radially at operculate pores; group II forming medium cysts (<18 µm) with wrinkled ectocysts and stellate to polygonal endocysts; and group III forming smaller cysts (<15 µm) with smooth ectocysts and spherical endocysts. Subsequent studies, however, found that these characters varied according to encystation and/or culture conditions.

Site of infection: Amoebae (both trophozoites and cysts) have been found in various organs; including the skin, upper respiratory tract, brain, liver, kidneys, adrenals, prostate, lymph nodes, bone marrow and pancreas of humans, primates and some domestic animals, as well as in the eyes but only in humans.

Pathogenesis: Most free-living amoebae are active predators feeding on bacteria, algae, yeasts and other protistans in their aquatic or terrestrial environments by phagocytosis or engulfment through pseudopod, food cup and/or food vacuole formation. In addition, some are able to take up soluble nutrients through pinocytosis and/or membrane transporters. Several *Acanthamoeba* spp. are regarded to be opportunistic parasites when they infect vertebrate hosts, often by taking advantage of temporary weaknesses in host immunity (such as traumatic lesions in immune barriers or suppressed innate or adaptive immune responses). Infections have been associated with three disease syndromes: amoebic keratitis (AK) exclusive to humans; granulomatous amoebic encephalitis (GAE) in humans and wild and domestic animals (such as horses, canines, bovines, ovines, monkeys, gorillas, and kangaroos) and cutaneous acanthamoebiasis (CA) reported in humans, canines, gorillas and several monkey species, often alongside GAE. The T4 genotype has frequently been associated with clinical disease in humans, including AK, GAE, CA and even disseminated infections involving the nasal passages, sinuses, lungs and gut. In addition, genotypes T3 and T11 (rarely T2, T5, T6 and T15) may cause AK;

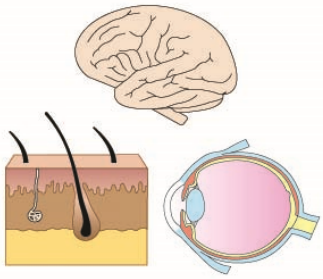
and genotypes T1, T10 and T12 (rarely T2, T5, T7 and T17) may cause GAE. If amoebae gain access to the body via the skin, CA can develop and persist over months being characterized by firm, non-tender erythematous nodules on the skin that drain a purulent exudate and can become ulcerated. Amoebae may invade the brain via haematogenous spread from the skin or directly via the upper respiratory tract, especially through the nasal mucosa to the olfactory bulb. GAE is a late-onset, protracted and usually fatal disease of the central nervous system (CNS) with a variable incubation period of several weeks to months. GAE is characterized by inflammatory necrosis of the brain due to parasite infiltrates and activity (esp. secretion of proteases) and host immune responses (proinflammatory) resulting in brain swelling, haemorrhagic necrosis, fibrin thrombi and neuronal damage. The description 'granulomatous' refers to the masses of immune cells that form at sites of infection or inflammation. Multifocal lesions present in the brain stem, midbrain, corpus callosum, and cerebellum. Symptoms include fever, headaches, stiff neck, aphasia, hemiparesis, changes in mental state (confusion, hallucinations, personality changes, irritability, sleep disturbances and decreased consciousness), vomiting, nausea, focal neurologic deficits, cranial nerve palsies, increased cranial pressure, seizures and coma, eventually leading to death. Moderate to severe oedema occurs in the cerebral hemispheres, and a chronic exudate consisting of inflammatory cells is present over the cortex. Trophozoites and cysts are scattered throughout neurological tissues, and occasionally other organs (liver, kidneys, adrenals, lungs). Multinucleated giant cells form granulomas around colonies of amoebae in immuno-competent patients; but this response is mild or completely absent in immuno-compromised hosts. Tissue damage may be caused directly by feeding trophozoites, induced by pro-inflammatory cytokine responses, or both. Dissemination to the lungs can occur and leads to pneumonitis. AK is a progressive sight-threatening corneal infection and is common amongst contact-lens wearers or people who have sustained corneal trauma. Amoebae invade the corneal epithelium and breech Bowman's membrane penetrating to the underlying collagenous stroma which they invade using collagenases. Trophozoites cluster around the corneal nerves producing radial keratoneuritis and severe pain. Other symptoms include radial neuritis, eyelid ptosis, photophobia, corneal opacity, blurred vision, lacrimation, conjunctival hyperaemia, epithelial ulcers, ring-like stromal infiltrates and scleritis. AK has not been found to lead to systemic infection or death, but is complicated by cataracts, increased intraocular pressure and hypopyon (inflamed anterior chamber) such that eye-sight can be threatened. GAE and CA are generally contracted by chronically ill patients (associated with diabetes, alcoholism and dialysis) or immuno-compromised individuals (due to congenital or acquired immunodeficiencies or immunosuppressive therapy for cancer or transplantation), whereas AK patients and most infected animals are immunocompetent. Pathogenicity has been associated directly with parasite adhesins, invasion, phagocytosis, acanthopodia activity, cytolytic enzyme production (phospholipases metalloproteinases, glycosidases, elastases, collagenases, myelinases, fibrinolytic enzymes, ecto-ATPases, neuroaminidases, superoxide dismutases), plasminogen activation, acanthaporin (pore-forming toxin); and indirectly with physiological tolerance (osmolarity, temperature, pH), biofilms, chemotaxis, drug resistance, cellular differentiation, cyst formation and host immunity (various cytokines, host cell apoptosis).

Developmental cycle and mode of transmission: Acanthamoebae are prevalent throughout the world in aquatic and terrestrial habitats, being found in raw and potable freshwater sources, seawater, sediments, soil, dust, air, sewage and numerous engineered facilities (swimming pools, spas, air-conditioning units, dialysis units, dental treatment units, eyewash stations). Infections occur when hosts come into direct contact with organisms in water and soil sources, and possibly associated aerosols. Organisms may enter the skin through cuts, wounds or abrasions, infect the nasal mucosa by inhalation or contact with contaminated water (immersion while bathing) or infect the eye by direct contact (often with contact lenses or cases washed in contaminated water). *Acanthamoeba* cysts are formed when environmental conditions become adverse for trophozoites (e.g. desiccation, food deprivation, pH and temperature extremes) and they excyst when conditions improve. The released trophozoites feed, grow and multiply asexually by mitotic division (binary fission). Trophozoites are the infective stage, but both cystic and trophic forms can enter the host, and both stages can be isolated from diseased and healthy individuals. There is no evidence to implicate horizontal transmission from one person to another or from one species to another.

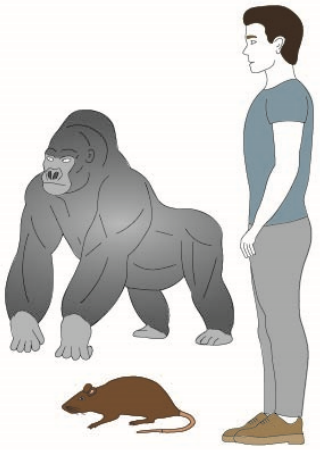
Differential diagnosis: Microscopic examination of biopsy samples (skin, brain, cornea, cerebrospinal fluid, bronchoalveolar lavage) is conventionally used to detect characteristic trophozoites and cysts; either in wet mounts, cytospin preparations or stained smears. Organisms may be stained using haematoxylin and eosin, trichrome, periodic acid-Schiff's or Gomori methenamine silver stains. Other identification techniques include chemofluorescent staining (Calcofluor, acridine orange), immunoflourescent or immunoperoxidase antibody labelling, confocal microscopy as well as organism cultivation (monoxenic plates/flasks, axenic media culture or tissue culture on mammalian cell monolayers). Ophthalmologic examination can reveal dendriform patterns on the epithelium of the cornea. DNA extracted from tissue samples has also been successfully screened by polymerase chain reaction (PCR) amplification of nuclear and/or mitochondrial ribosomal RNA gene sequences. Genotyping at these loci is recommended for molecular epidemiology. For GAE, a lumbar puncture for a cerebrospinal fluid examination can be performed, which does not test for the presence of amoebae but for elevated white blood cell counts, reduced glucose levels and elevated protein levels. CT scans are usually performed before lumbar puncture to ensure the technique is not contraindicated because of the risk of herniation. Regrettably, most cases of GAE are diagnosed post-mortem.

Treatment and control: Prognosis is poor for GAE and CA patients, primarily due to the low efficacy of current diagnostic methods, the inefficiency of current drugs, cyst formation within host tissues and the compromised immunity of most patients. Nonetheless, GAE has been successfully treated with various antimicrobial multi-drug regimens guided by *in vitro* antibiotic sensitivity testing. Drugs have included various combinations of cotrimoxazole, clotrimazole, fluconazole, flucytosine, isethionate, itraconazole, ketoconazole, pentamidine, sulfadiazine, amphotericin B, rifampin, miconazole, paromomycin, polymyxin and trimethoprim-sulfamethoxazole. Topical chlorhexidine and ketoconazole have been used to treat skin lesions in CA. Early diagnosis is vital for a successful treatment of AK as cysts are highly resistant to therapy. Propamidine, micronazole nitrate and neomycin have been used successfully in combination, as have cationic antiseptics (chlorhexidine or polyhexamethylene biguanide) with diamides (propamidine or hexamidine). The duration of the therapy is a minimum of 3-4 weeks but may require up to 6-12 months. If drugs prove unsuccessful, the infected cornea can be debrided and a penetrating keratoplasty performed. Prevention of AK revolves around the proper sanitation of contact lenses and their cases which are major sources of the disease. Decontamination in hydrogen peroxide solutions has proven effective. Sterile solutions must be used to clean and store lenses. Avoiding complete contact with acanthamoebae is difficult because they are ubiquitous and prevalent in the environment. Cysts are resistant to most biocides, antibiotics and routine water chlorination procedures, and they have been shown to remain viable for years in water at low temperatures. Precautions should be taken when swimming or bathing in untreated waters (avoid facial immersion and inhalation of water or aerosols). Patient cohorts most at risk (immunocompromised individuals) should avoid potentially contaminated sites and undergo regular health monitoring.

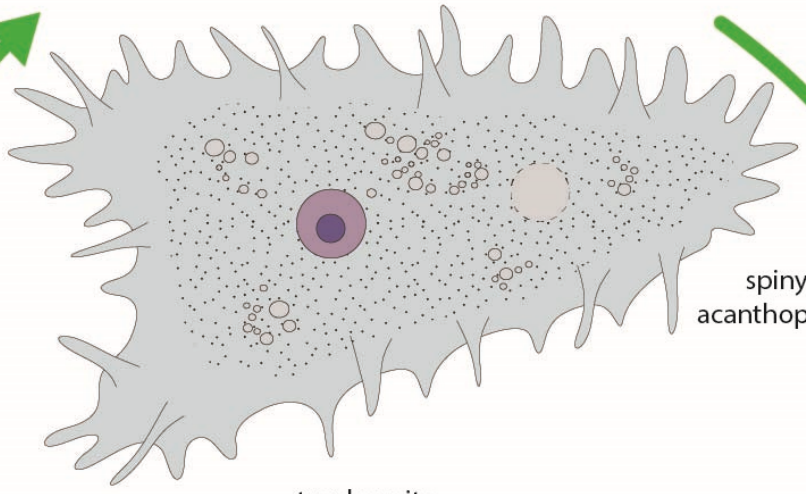
Acanthamoeba



skin, brain, eye
(cutaneous lesions,
amoebic keratitis,
granulomatous amoebic
encephalitis)



Vertebrate Hosts
(mammals, some
other animals)

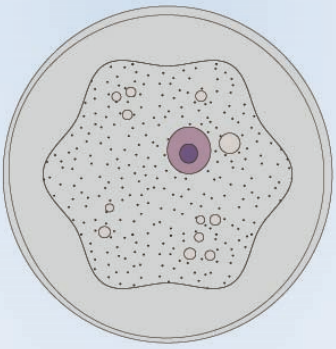


spiny
acanthopodia

trophozoite
(15-40 µm)

exystment in contact
with skin/cornea

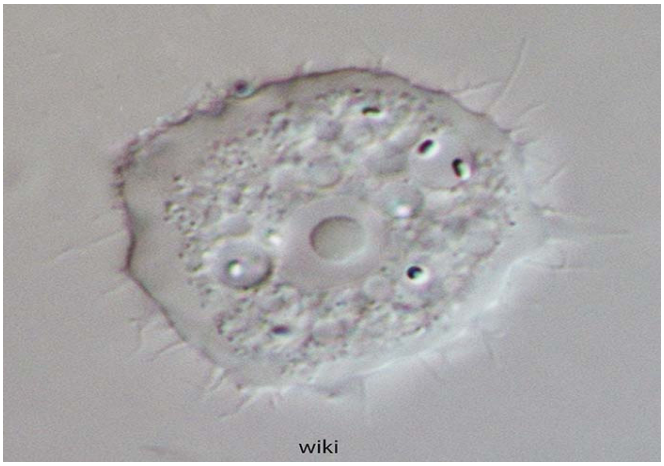
cyst formation in
external environment



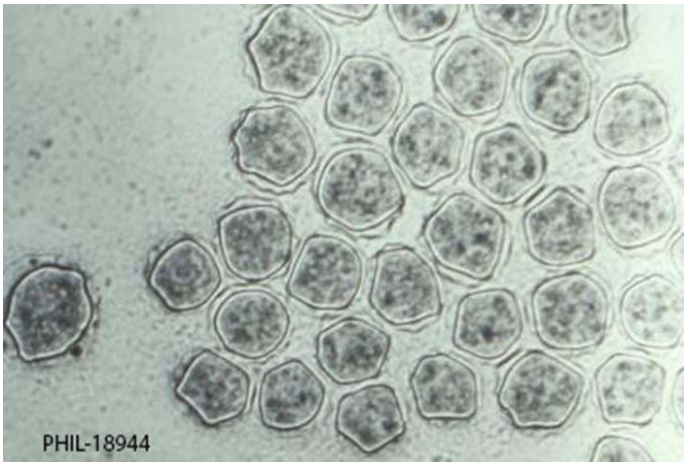
often stellate
in appearance

cyst
(10-20 µm)

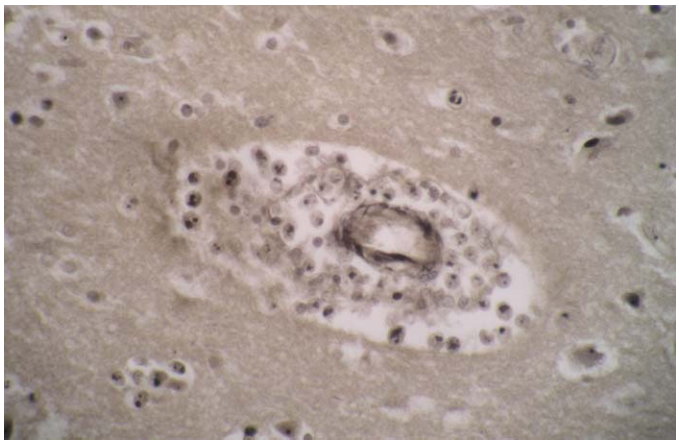
many species free-living in terrestrial and aquatic habitats,
some opportunistic/facultative parasites
(transmission by contact when bathing)



Acanthamoeba trophozoite



Acanthamoeba cysts



Acanthamoeba brain lesion