Naegleria
(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). Heterolobosean amoebae form cylindrical trophozoites with eruptive limax pseudopodia. They have also been shown to have an alternate flagellated stage in their developmental cycle, although molecular phylogenetic studies have included some taxa which lack either an amoeboid or a flagellated stage. This group includes the acrasid mycetozoans (slime moulds) which form fruiting bodies and vahlkampfiid amoebae (schizopyrenids) which do not form fruiting bodies. They all form environmentally-resistant cysts. Most species are found as bactivores in soil, water or faeces, and several species are able to opportunistically infect the central nervous system of vertebrate hosts. *Naegleria fowleri* usually lives in warm freshwater environments but can invade the human body through the nasal mucosa and cause primary amoebic meningoencephalitis (PAM).

Classification:
Domain: Eukaryota (membrane-bound nucleus)
Supergroup: Excavata (with conspicuous ventral feeding groove)
Group: Discoba (diverse group supported robustly by molecular studies)
Phylum: Heterolobosea (diverse group, incl. amoebo-flagellates, most form cysts or clusters of fruiting bodies)
Order: Schizopyrenida (no fruiting bodies)
Family: Vahlkampfidae (eruptive limax amoeboid form cylindrical, most form temporary flagellated stages)
Genus: *Naegleria* (free-living amoebae in terrestrial/aquatic habitats, some opportunistically parasitic)
Species: *N. fowleri* (causes primary amoebic meningoencephalitis 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.

The Heterolobosea has a diverse membership, most forming cysts or fruiting bodies, but their grouping is supported robustly by molecular studies. The phylum includes the schizopyrenids or amoebo-flagellates; i.e. amoebae that form temporary flagellated stages for dispersal. The vahlkampfids are naked amoebae (without tests or shells) that form eruptive 'limax' pseudopodia; most species being free-living amoebae (FLA) 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*]. Around 50 *Naegleria* spp. have been described throughout the world as free-living amoebae primarily from freshwater sources. While several species have been isolated from various vertebrate hosts, only *Naegleria fowleri* has been associated with disease in humans, especially children. Clinical infections were first encountered in Australia where *N. fowleri* was associated with public water supplies, but infections have now been found in many countries in association with warm waters such as heated swimming pools, geothermal springs, tropical lakes, above-ground pipelines and discharged industrial cooling water.

***Naegleria* species**	**Hosts**	**Location**	**Clinical signs**	**Distribution**
N. aerobia	Experimental infections in Rodentia: murid (mice), caviid (guinea pig)	brain, skin, viscera	encephalitis	various laboratories
N. australiensis	Experimental infections in Rodentia: murid (mice)	brain	encephalitis	various laboratories
N. fowleri	Primates: hominid (human), Perissodactyla: tapirid (tapir); Artiodactyla: bovid (cattle); plus experimental infections in Primates: cercopithecids (macaques, rhesus), Rodentia: murid (mice), caviid (guinea pigs), cricetid (cotton rat, muskrat) sciurid (squirrels); Artiodactyla: bovid (sheep); Lagomorpha: leporid (rabbits)	brain	encephalitis	cosmopolitan
N. italica	Experimental infections in Rodentia: murid (mice)	brain	encephalitis	various laboratories
N. philippinensis	Experimental infections in Rodentia: murid (mice)	brain	encephalitis	various laboratories

Compared to the number of *Naegleria* spp. and isolates described from environmental samples, only a small number have been recovered from naturally infected hosts. *N. fowleri* has been associated with clinical disease in humans, cattle and a tapir, although experimental infections have been established in a range of laboratory, domestic and wild animals. Several other *Naegleria* spp. have been found to experimentally infect laboratory animals, *N. gruberi* has been recovered from the intestinal contents of freshwater fish while un-named species have been detected in the brain and liver of mice and rabbits. Nonetheless, natural infections may be more widespread than previously thought as evidenced by the recent molecular characterization of *Naegleria* isolates from the brain, gills, liver, spleen, kidney and skin of 12 species of freshwater fishes which resulted in the detection of ten novel genotypes and eight genotypes referable to the species *N. australiensis, N. pagei, N. fultoni* and *N. clarki*. Indeed, serological studies have detected antibodies against *Naegleria* antigens in a wide variety of wild mammals; including raccoons, opossums and various rodents. Other species recovered from freshwater and wet soils include *N. americana, N. andersoni, N. angularis, N. antarctica, N. arctica, N. byersi, N. canariensis, N. carteri, N. chilensis, N. clarki, N. dobsoni, N. dunnebackei, N. endoi, N. fultoni, N. galeacystis, N. gallica, N. gruberi, N. indonesiensis, N. jadini, N. jamiesoni, N. johanseni, N. laresi, N. lovaniensis, N. martinezi, N. mexicana, N. minor, N. morganensis, N. neoantarctica, N. neochilensis, N. neodobsoni, N. neopolaris, N. niuginiensis, N. pagei, N. paradobsoni, N. peruana, N. polaris, N. pringsheimi, N. pussardi, N. robinsoni, N. schusteri, N. spitzbergenensis, N. sturti, N. tenerifensis* and *N. tihangensis.* No endosymbiotic bacteria have been detected in environmental or clinical isolates of *Naegleria* spp.

Parasite morphology: *Naegleria* spp. have three morphological stages in their life cycle: amoeboid trophozoites, flagellated trophozoites (amoebo-flagellates) and cysts. Under adverse environmental conditions, including cold or dry environments, the parasite will encyst in order to survive. Cysts are ovoid measuring 8-12 μm in diameter and are bound by a porous wall of 1-2 layers, depending on the species. The pores are large and plugged with mucus. Regenerated trophozoites emerge through the pores when environmental conditions allow. Amoeboid trophozoites predominate as motile feeding and dividing stages. They are pleomorphic and constantly changing shape, from thin elongate forms to robust round forms. When rounded, *Naegleria fowleri* trophozoites measure 10-15 μm in diameter. They are uninucleate and have a characteristic dense nucleolus surrounded by a large transparent nuclear halo. They have a granular cytoplasm containing numerous vacuoles and they often form peripheral 'food cups' used to engulf bacteria, yeasts or cellular debris as a nutrient source, as well as providing an attachment mechanism. Trophozoites exhibit a limax (slug-like) monopodial pattern of movement, wherein they extend finger-like lobopodia to move forward. Trophozoites reproduce by binary fission to produce two identical offspring. Amoeboid trophozoites may also form temporary bi-flagellated stages 10-20 μm in length which act as non-feeding non-dividing dispersal forms in aquatic media.

Site of infection: *Naegleria fowleri* infects the central nervous system, initially the olfactory and frontal lobes but progressing through perivascular regions to the base of the brain, the brain stem and the cerebellum. A single case was also reported where amoebae were detected in an immuno-compromised patient outside the brain in lesions in the stomach, intestines, lymph nodes and lungs. Cyst formation has not been reported in host tissues.

Pathogenesis: *Naegleria* species and strains vary markedly in their infectivity and pathogenicity so many infections may be asymptomatic and go unnoticed. Indeed, *Naegleria* amoebae (including *N. fowleri*) have been isolated from the nasal mucosa of healthy asymptomatic individuals. However, invasive infections by *N. fowleri* most often result in fulminant (sudden and severe) central nervous system disease, and eventually death. Once the organism has gained access to the brain, strains with a low level of pathogenicity consume nerve tissue in a piecemeal fashion, using food cups. In contrast, highly pathogenic strains apparently absorb the hosts' nervous tissue by releasing cytolytic enzymes to lyse cells thus allowing the amoebae to consume the cellular debris. It is this process that leads to the disease known as primary amoebic meningoencephalitis (PAM), a combination of cell lysis and inflammation involving brain tissues and the surrounding meninges. Onset of symptoms occurs within days of exposure, and includes severe headache, nausea and vomiting, stiff neck, variable fever of (38.5-41°C) and behavioural abnormalities. Thus far, most infections have involved children and the disease PAM has almost invariably proven fatal after 1-2 weeks.

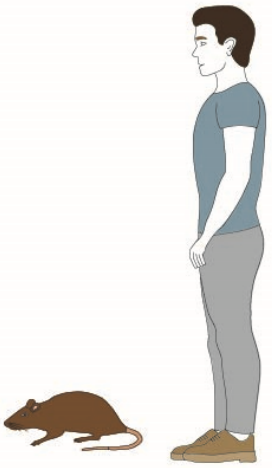
Developmental cycle and mode of transmission: Infections are acquired when amoeboid trophozoites and cysts in water come into contact with the olfactory epithelium of the nasal mucosa. Trophozoites penetrate the epithelium and track along the olfactory nerve crossing the cribriform plate to the olfactory bulb in the subarachnoid space. From there, trophozoites are free to move throughout the rest of the central nervous system. Obviously, infections are more likely in people who spend a long time swimming, bathing or submerging their heads regularly in water, particularly untreated warm water sources (including shallow pools, spas and geothermal springs). Thermotolerant amoebae thrive in warm waters (*N. fowleri* grows fastest at 42°C) and they can tolerate suboptimal chlorine disinfection. Children are more at risk as they are likely to spend more time splashing and diving in water. There is also some evidence to suggest that *Naegleria* cysts may also be inhaled as dust formed from dried freshwater lakes, although *N. fowleri* cysts have been shown to have poor resistance to desiccation and thus only rarely occurs in soil.

Differential diagnosis: Presentation with meningitis-like symptoms is the first indicator of PAM. A recent history of immersion in freshwater could alert a physician to consider the possibility of *Naegleria* infection. Direct diagnosis may be made by microscopic examination of brain biopsy material of cerebrospinal fluid (CSF) for motile amoebae. Samples mounted in a drop of distilled water may further aid identification, as *Naegleria* trophozoites quickly become flagellated when exposed to water (as opposed to those of *Acanthamoeba*). Amoebae can also be cultured on agar plates spread with bacteria incubated at 37°C for 24 hours. Serological (ELISA) and molecular (PCR) tests have recently been developed to aid diagnosis but are used mainly for research. Molecular characterization techniques have been used to explore genotypic variation between and within species. Isoenzyme electrophoresis, restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) analyses led to the recognition of three major geographic variants of *N. fowleri* (European, Oceanic and American), but subsequent studies using ribosomal RNA gene sequences have so far identified six major genotype groups; three corresponding to geographic regions but another three being more widespread.

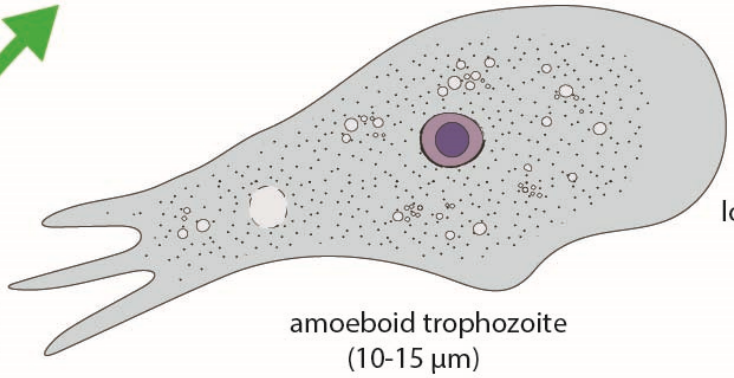
Treatment and control: The currently accepted treatment for infection with *Naegleria fowleri* is aggressive treatment with high doses of intravenous and intrathecal amphotericin B, and in some cases miconazole. PAM is generally fatal within 1–2 weeks of infection, thus early diagnosis is essential for successful treatment. Meningitis is a relatively common condition; however, *Naegleria fowleri* as a cause of these symptoms is rare. It is therefore unlikely to be suspected and diagnosed early enough for effective treatment. Doctors need to be aware of the risk of infection and public education campaigns should be mounted in high-risk areas (such as around hot springs and warm pools). Signs should warn bathers not to immerse their heads and to avoid diving, splashing and inhaling mists. Swimming pools and spas should be regularly chlorinated (at appropriate dosages) and monitored for the presence of amoebae. Chlorine has been shown to kill all life-cycle stages of *N. fowleri* if thoroughly mixed into water supplies, and chloramination has been shown to be most effective.

Naegleria

nasal mucosa, brain
(cell lysis, inflammation,
primary amoebic
meningoencephalitis)



Vertebrate Hosts
(mammals, some
other animals)



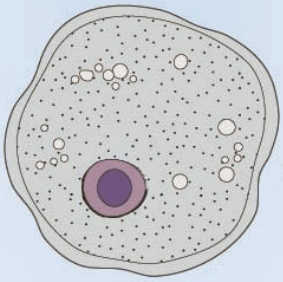
leading
'limax'
lobopodia

trailing
filopodia

amoeboid trophozoite
(10-15 µm)

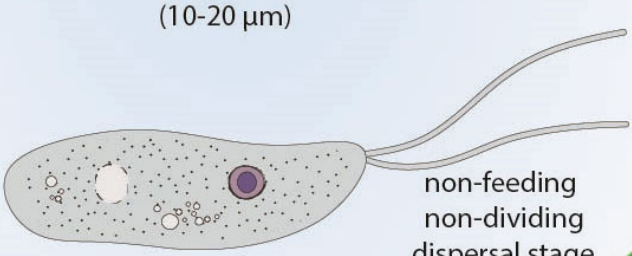
exystment in contact
with nasal mucosa

cyst formation in
external environment



porous
cyst wall

cyst
(10-20 µm)

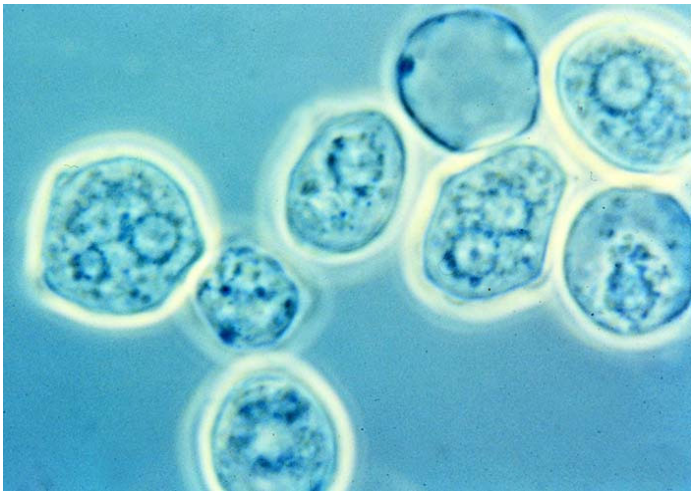


non-feeding
non-dividing
dispersal stage

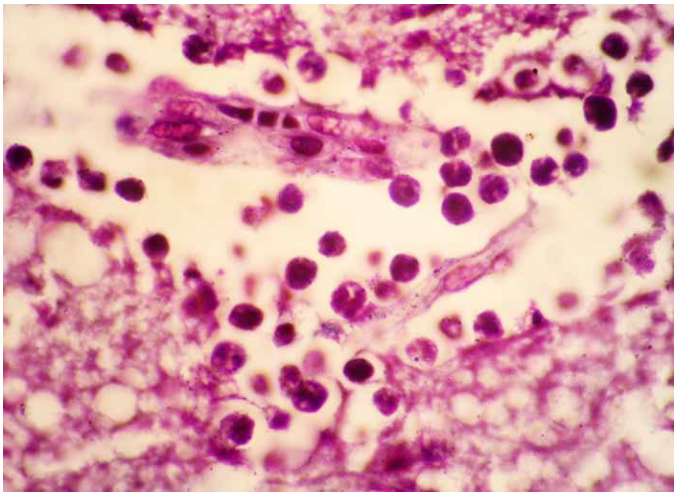
unique biflagellated
trophozoite (10-20 µm)

many species free-living in aquatic and terrestrial habitats,
some opportunistically parasitic
(transmission by contact when bathing)

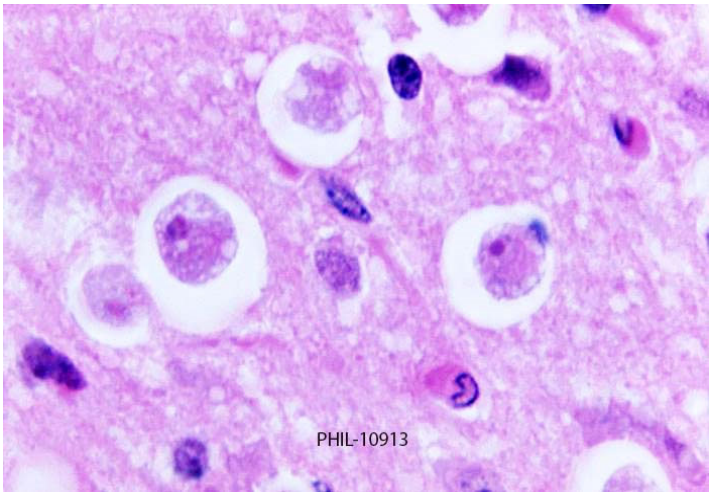
Naegleria trophozoites



Naegleria cysts



Naegleria brain lesion



Naegleria brain lesion