Balamuthia
(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. Tubulinid amoebae form tubular subcylindrical pseudopodia and the cells may be testate (surrounded by a shell) or naked. 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. *Balamuthia* was originally classified as a leptomyxid amoebae exhibiting limax, plasmodial, reticulated and/or polyaxial pseudopodia, but molecular studies have found it to be closely related to *Acanthamoeba*. The single species, *Balamuthia mandrillaris*, normally exists as a free-living amoeba in soil and aquatic environments, but can opportunistically infect humans and animals causing granulomatous amoebic encephalitis (GAE).

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: *Balamuthia* (free-living in terrestrial environments, sometimes opportunistically parasitic)
Species: *B. mandrillaris* (causes granulomatous amoebic encephalitis, disseminated disease or skin lesions 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*]. *Balamuthia* was first thought to be a leptomyxid amoebae on the basis of its thick-walled cyst and pleomorphic pseudopodia: the latter being limax (monopodial slug-like movement), plasmodial (multinucleate), reticulated (fibrous network) and/or polyaxial (movement on more than one axis). However, molecular phylogenetic studies have firmly placed *Balamuthia* as a close relative of acanthamoebae rather than other leptomyxids. *Balamuthia mandrillaris* was originally isolated from a mandrill baboon (hence the specific epithet). It was subsequently discovered to be a free-living amoebae commonly found in soil and dust, but which can opportunistically infect humans, horses, sheep and dogs but is most often seen in non-human primates. There are no intermediate hosts in the life cycle and there is no evidence that animals are reservoirs for human infections. The amoeba has a worldwide distribution and can infect both immuno-competent and immuno-suppressed hosts; notably humans with HIV/AIDS or those undergoing immuno-suppressive treatment.

***Balamuthia* species**	**Hosts**	**Location**	**Clinical signs**	**Distribution**
B. mandrillaris	Primates: hominid (human, gorilla, orangutan), hylobatid (gibbon), cercopithecid (colobus monkey, baboon); Carnivora: canid (dog); Perissodactyla: equid (horse); Artiodactyla: bovid (sheep)	brain, lungs, spleen, kidneys, adrenals, skin	granulomatous encephalitis	Europe, Americas, Australia, Asia

Parasite morphology: This amoeba forms two developmental stages; vegetative trophozoites and dormant cysts (it does not form a temporary flagellated stage). The trophozoites are 12-60 µm in diameter and most are uninucleate with an unusually large dense vesicular nucleus generally containing one large central nucleolus (although more than one nucleolus may occur). They often contain multiple nuclear-like bodies which are spherical, 2-4 µm in diameter, have a large dense central body and are found near the tips of extended pseudopodia. The shape of the trophic form is variable; sometimes appearing rounded with no pseudopodia, but often extended in a thread-like configuration with multiple and extended pseudopodia. Cysts are polygonal or spherical measuring 6-30 µm in diameter and wrinkled in appearance. They are bound by a three-layered cyst wall lacking pores; composed of a thick inner (endocyst) wall, a wrinkled outer (ectocyst) wall and an amorphous fibrillar middle (mesocyst) wall.

Site of infection: Amoebae (trophozoites and cysts) infect the skin or lungs and may spread haematogenously thereafter to the central nervous system or disseminate to other organs such as the kidneys, adrenal glands, pancreas and thyroid. There is also some evidence to suggest amoebae may invade the brain via the olfactory nerve.

Pathogenesis: Infection of the skin leads to localised dermal ulcerations which can be very slow to heal or simply fail to do so. Nasopharyngeal infections may lead to pneumonitis and subsequently spread to the brain causing granulomatous amoebic encephalitis (GAE), sometimes known specifically as *Balamuthia* amoebic encephalitis (BAE). *Balamuthia* characteristically exhibits slow growth and spread to the nervous system may take from several weeks up to two years. Symptoms are insidious and are often not recognised in the early stages of infection. Symptoms of the sub-acute stage include headache, seizures, nausea, stiff neck and increased cerebrospinal fluid in the brain. GAE is a chronic condition with high mortality due to the formation of haemorrhagic necrotising lesions in brain tissue, particularly in perivascular spaces. The description ‘granulomatous’ refers to the masses of immune cells that form at sites of infection or inflammation. Symptoms include focal paralysis, seizures, facial paralysis, difficulty in swallowing and double vision. Changes in clinical parameters include elevated levels of protein and leukocytes in

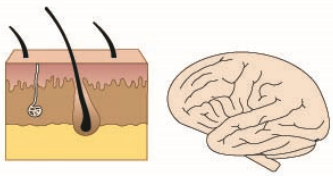
cerebrospinal fluid and blood as well as decreased levels of glucose. Infections occur in both immuno-compromised and immuno-competent individuals, particularly in children and the elderly. Unlike other free-living amoebae which opportunistically infect animals, *Balamuthia* cannot be grown in cultures containing only bacteria as a food source – the amoebae require eukaryotic cells and thrive in tissue cell cultures, especially those containing human brain microvascular endothelial cells. *Balamuthia* binds preferentially to collagen-1, laminin-1 and fibronectin in the extracellular matrix resulting in the production of 'food cups' allow them to feed on cells. Infections have also been shown to induce production of the cytokine IL-6 by brain endothelial cells leading to inflammatory responses which worsen the condition. Other putative virulence factors involve metalloprotease activity leading to degradation of the extracellular matrix (particularly collagen-1 and -3, elastin and plasminogen) and an increase in pathology associated with decreased tissue structure and function.

Developmental cycle and mode of transmission: *B. mandrillaris* is a free-living amoeba found most commonly in soil and at times in aquatic environments. Trophozoites feed mainly on other protozoa but lack of food induces encystment. Infections are thought to be acquired by hosts either by trophozoites invading cuts, wounds or abrasions in the skin and then entering the bloodstream or by cysts being inhaled into the upper respiratory tract. Zoonotic transmission has been considered as non-human primates are commonly affected by *B. mandrillaris* but there is no evidence as yet to suggest that animals act as reservoirs of infection. There is little evidence for human-to-human transmission and most cases occur as solitary infections not constrained by geography.

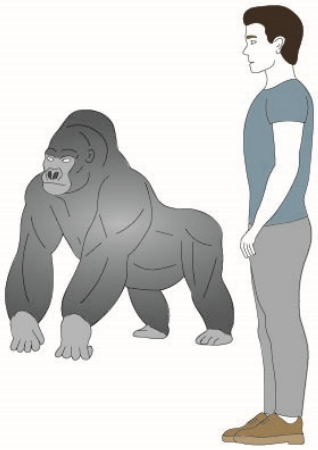
Differential diagnosis: Most cases are fatal and are diagnosed post-mortem. Cysts and trophozoites can be identified via microscopic examination of fixed and stained histological sections, or by immunofluorescent-antibody labelling using a specific antiserum (anti-*Balamuthia* antibodies raised in rabbits). Host antibodies have been detected in serum samples by enzyme immunoassays and DNA has been amplified from clinical samples by polymerase chain reaction (PCR) amplification of ribosomal RNA gene sequences. Radiological imaging techniques (CT and MRI scans) have also been used to identify symptomatic lesions in the brain. Unlike the other free-living amoebae that can cause meningo-encephalitis, *Balamuthia* is difficult to isolate and culture from cerebrospinal fluid. Instead, amoebae may be recovered from brain biopsy material cultured in the presence of mammalian cells.

Treatment and control: A major problem associated with therapy is the ability of *Balamuthia* to form cysts which are highly resistant to physical and chemical stresses. The triple layered cell wall presents a significant barrier to antimicrobials and it is highly likely that cysts may reactivate following chemotherapy leading to a recurrence of infection. Multiple drugs have been tested against trophozoites for the treatment of infections, with variable success reported for amphotericin B, zaithromycin, fluconazole, flucytosine, pentamidine and propamidine isethionates, miltefosine and sulfa drugs. Unfortunately, many have undesirable side effects when used for prolonged periods and new, less toxic drugs are required. Chemotherapy has also been complemented by immuno-therapy using specific antibodies raised against *Balamuthia* isolates. Good prognosis relies heavily upon early diagnosis and timely treatment, especially of low dose infections involving amoebae strains of low virulence. Prevention involves reducing transmission potential by avoiding contact with potential sources (using gloves when working with soil and not immersing the head when swimming or bathing in untreated waters).

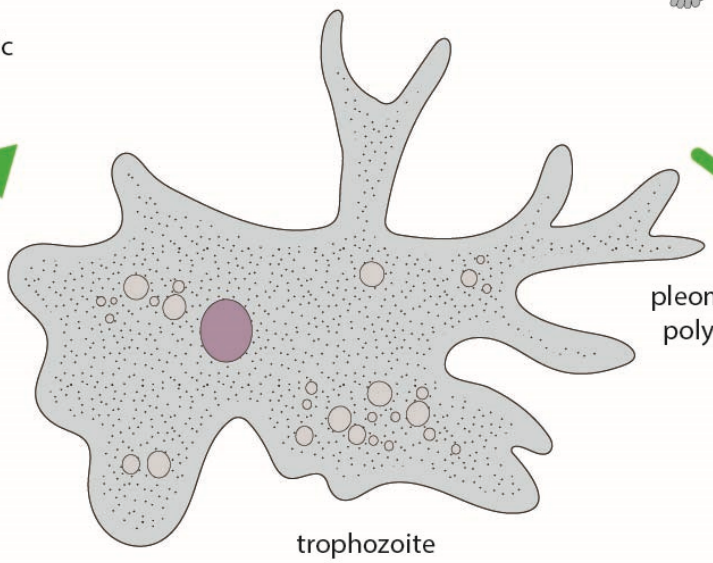
Balamuthia



skin, brain
(dermal ulcers,
granulomatous amoebic
encephalitis)



Vertebrate Hosts
(mammals, esp.
primates)

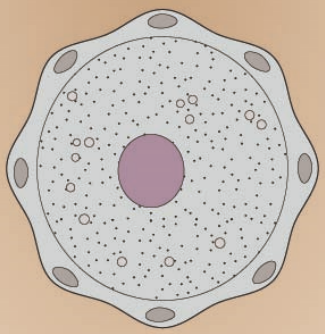


pleomorphic
polypodia

trophozoite
(12-60 µm)

exystment in contact
with skin

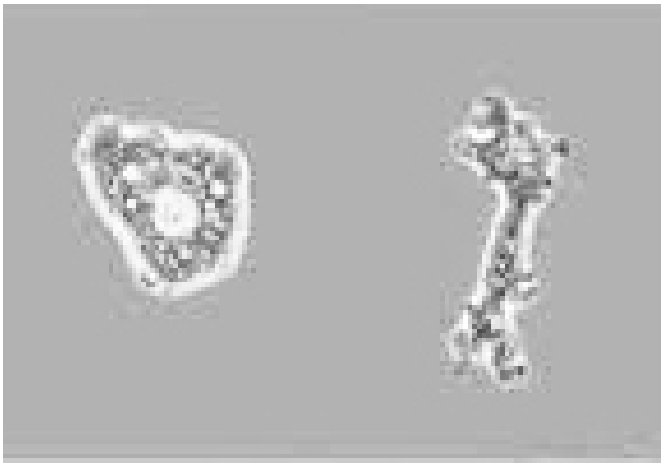
cyst formation in
external environment



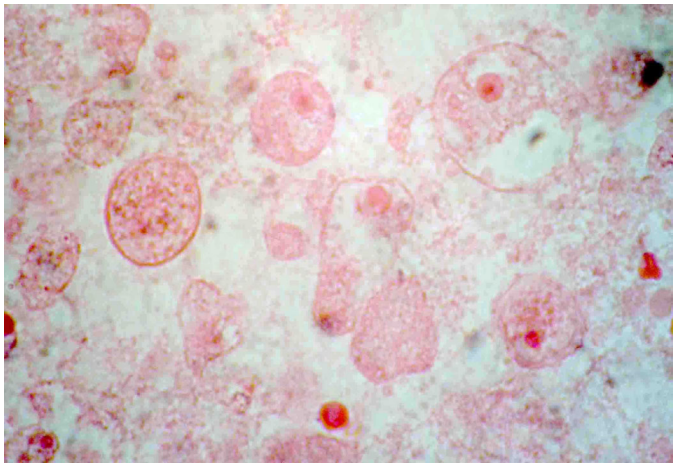
often with
wrinkled
exocyst wall

cyst
(6-30 µm)

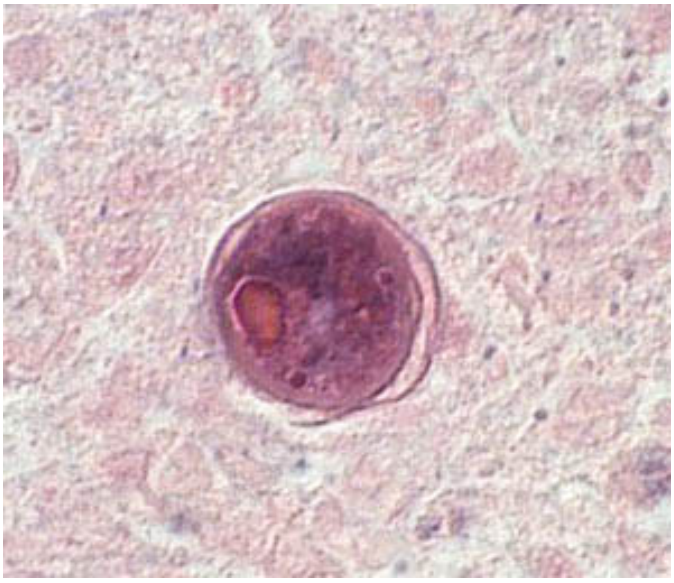
usually free-living in terrestrial and aquatic habitats,
occasionally opportunistically parasitic
(transmission by contact with soil)



Balamuthia trophozoites



Balamuthia brain lesion



Balamuthia cyst