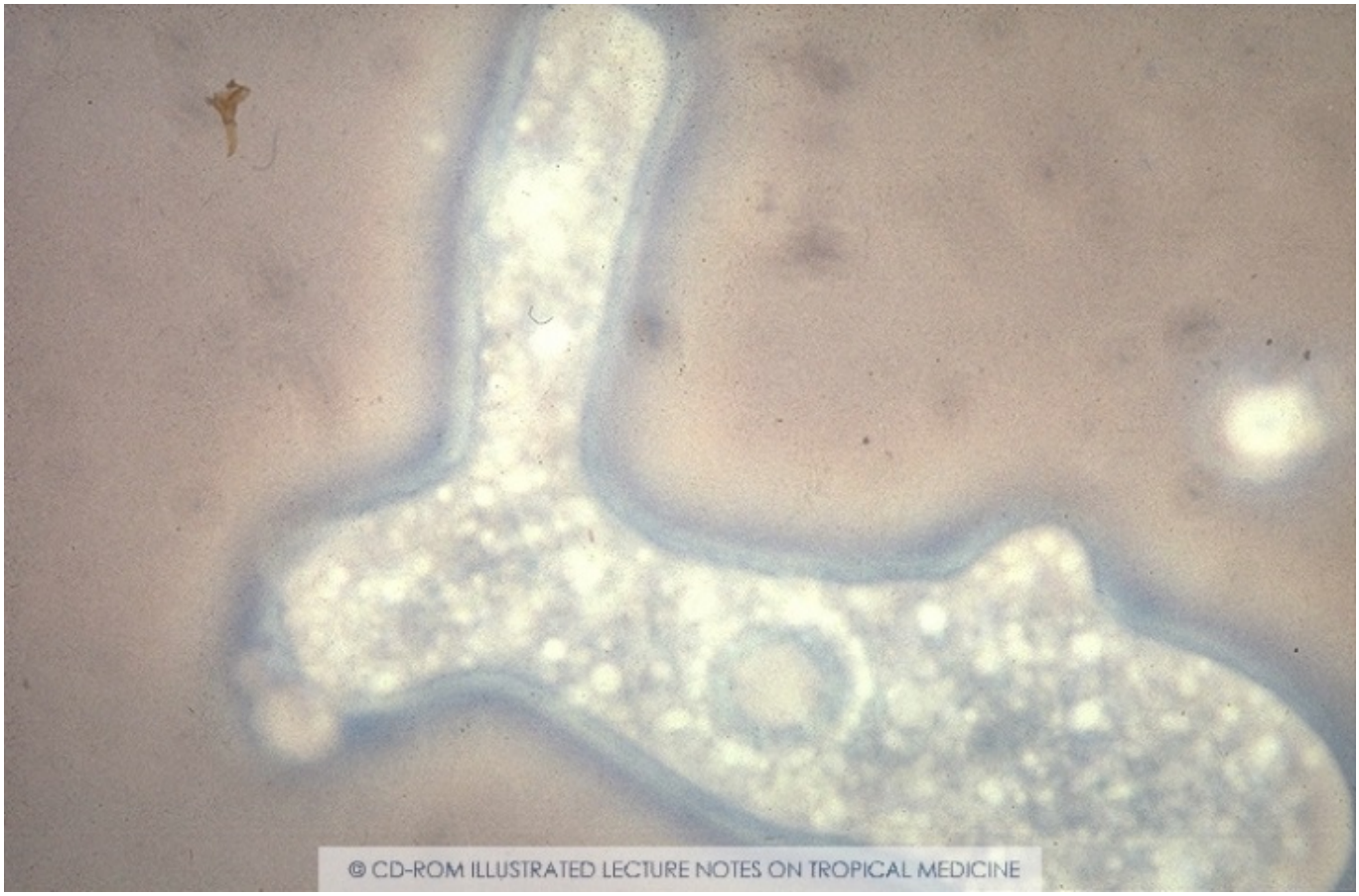
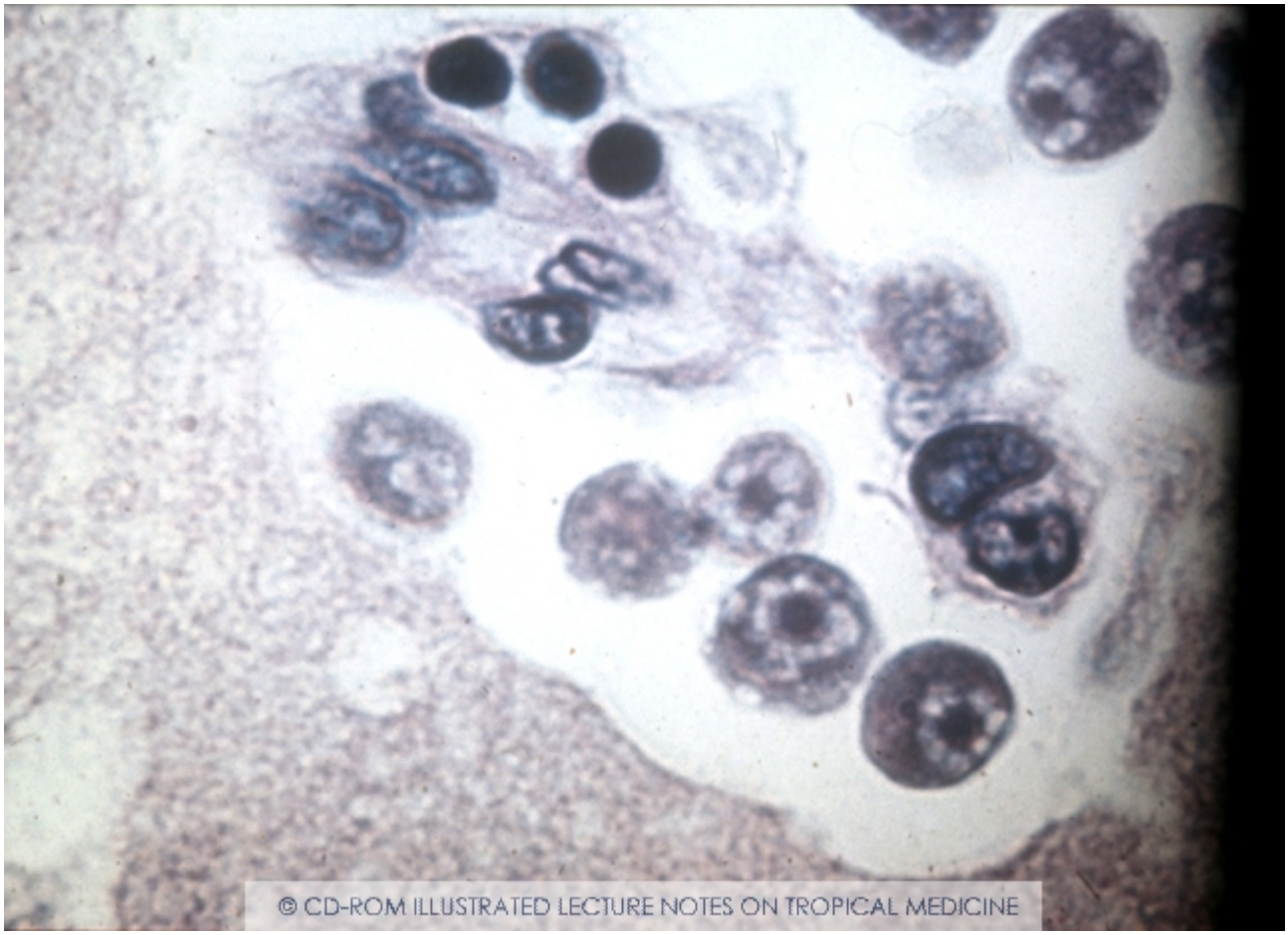
# Free-living amoebae

Saprophytic amoebae from water, silt and wet soil which belong to the genera *Naegleria, Acanthamoeba, Balamuthia* and *Sappinia* are cosmopolitan and potentially pathogenic. They seldom cause infection although underreporting is probable. In industrialised countries with a moderate climate these amoebae prefer fresh water with a temperature higher than average, such as public swimming pools and warm waste water from factories or power stations. This suggests that these amoebae must be widely distributed in a tropical environment.

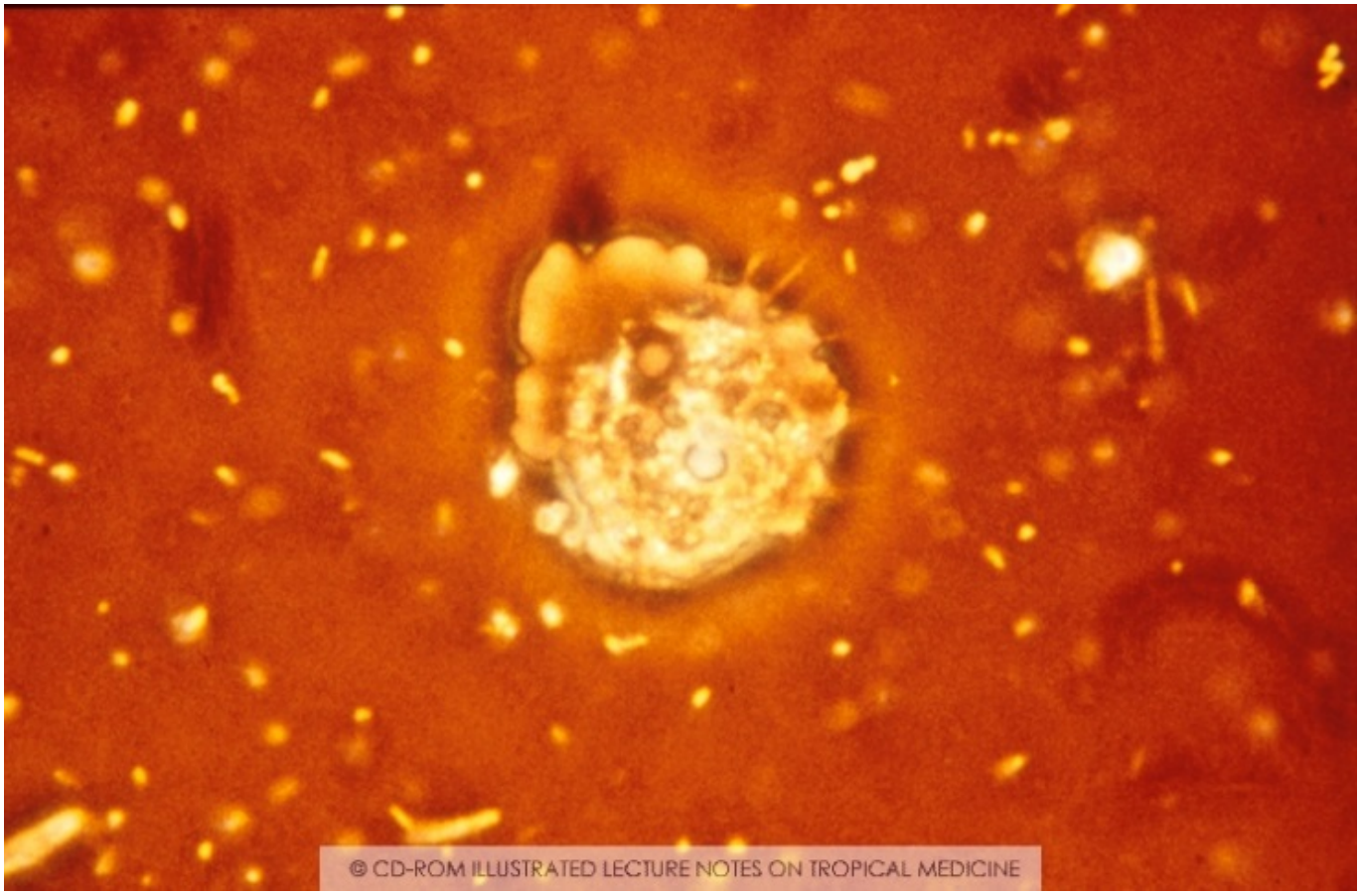
## Naegleria fowleri




Naegleria fowleri trophozoite, one of the free-living amoebae.




Naegleria fowleri amoebae. Copyright ITM




Acanthamoeba sp. Notice the typical thorn-like projections (acanthopoda). Copyright ITM

## Historical note

Culberston et al in 1958 were the first to launch the concept that free-living soil and water amoebae could cause disease in humans. In order to feed trophozoites form a kind of “cell-mouth”, called an amoebostome. This is quite spectacular in electron microscopic pictures. In addition to phagocytosis via these food cups, contact-mediated cytolysis occurs. When flagella develop, e.g. after transfer from culture or from tissue to water, they sprout at the broad blunt end. This change to the flagellate form can take 2-20 hours. The flagellate form does not divide, but is motile. When the flagella are lost, the amoeboid form is regained and the parasite resumes asexual reproduction. Cysts measure 7-15 µm, but are absent from human tissue, in contrast with *Acanthamoeba* infections.

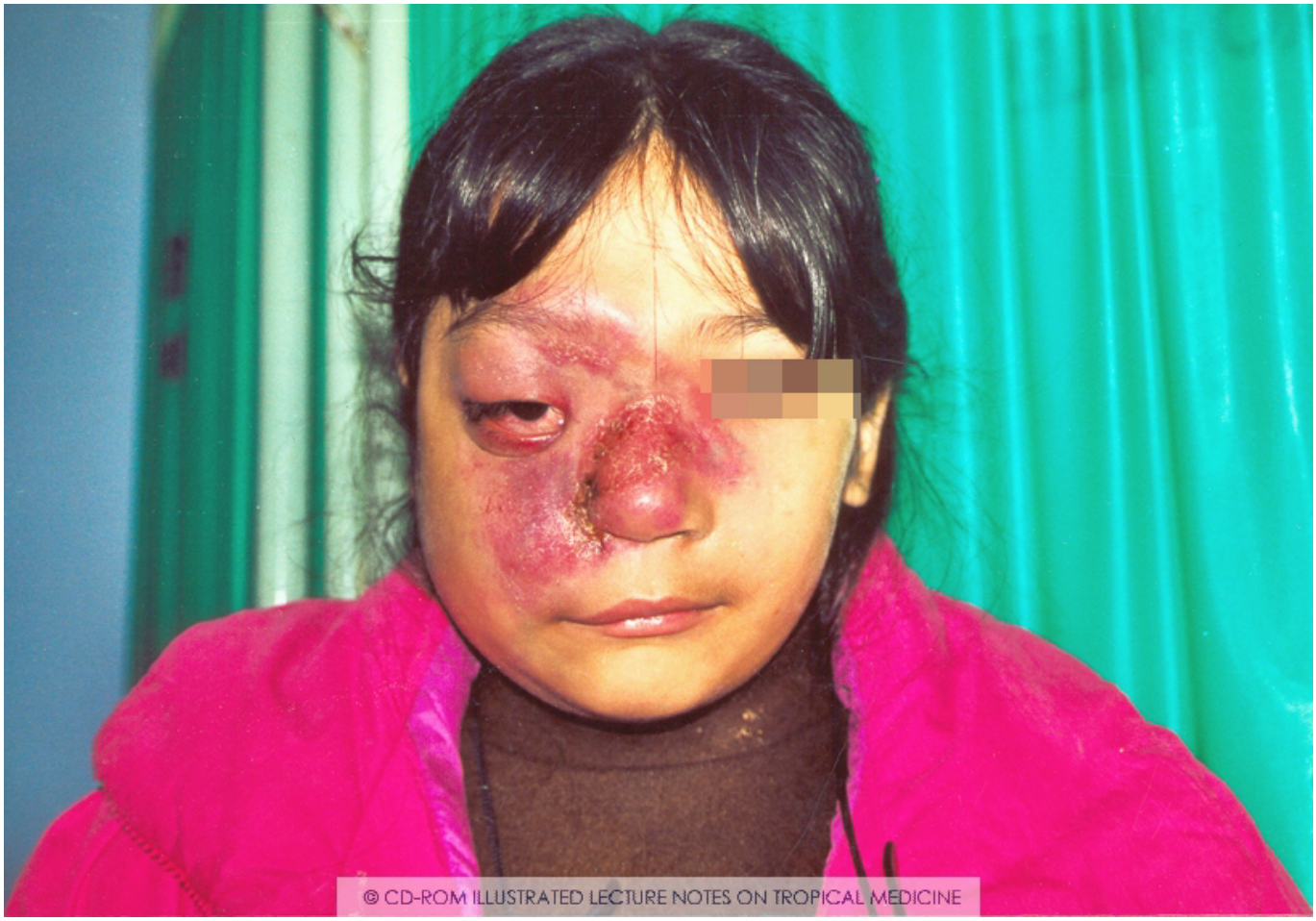
Infection with *Naegleria fowleri* is the consequence of bathing or swimming in contaminated freshwater ponds or lakes at quite high temperatures, such as fresh water lakes in the summer (e.g. southern USA) ponds, rivers and hot springs. Sampling of such warm water has indicated that *N. fowleri* is commonly present in such environments. The infection follows penetration of water into the nasal cavities. From there the lamina cribriforma of the ethmoid bone is penetrated, probably through phagocytosis of the olfactory epithelium. Via the first cranial nerve, the infection spreads to the lowermost part of the frontal cerebral lobes. Extensive tissue damage follows. The amoebae reproduce rapidly in the cerebrospinal fluid. There is virtually no inflammatory reaction. Haemorrhagic necrosis of the base of the brain, cerebral cortex and the olfactory lobes develops.

The incubation time is 2 to 15 days. Early in the infection, upper respiratory distress, severe headache, sore throat, runny or stuffy nose, altered smell and taste occur. Fever, vomiting and neck stiffness follow. Mental confusion and coma occur after 3 to 6 days. Most infections are lethal. A high index of suspicion is needed for diagnosis since CSF findings are very similar in acute bacterial meningitis. A history of bathing in surface water during the previous two weeks is significant. The disease closely resembles acute bacterial meningitis and is known as primary amoebic encephalitis (PAM).

In clinical practice, most cases will be diagnosed only at autopsy (immunofluorescense and immunoperoxidase techniques).

Immediate chemotherapy is required for survival. *N. fowleri* is sensitive to amphotericin B. Combination treatment with ampho B, miltefosine, miconazole, rifampicin, azithromycin, chloramphenicol and/or ketoconazole has been used. Specialised advice is absolutely required.

## Balamuthia mandrillaris




Balamuthia mandrillaris infection with important skin lesion. Copyright Alexander von Humboldt Institute, Peru

It is not clear how humans get infected, but transmission via swimming in contaminated surface water is one possibility. The pathogen has also been isolated from a potted plant in a home. Infection with this amoeba causes peri-orbital swelling and ulceration, followed by symptoms of granulomatous meningo-encephalitis, in both immunocompetent and immunocompromised persons. Symptoms include headache, nausea, vomiting, fever, visual disturbances, dysphagia, seizures and hemiparesis. Both trophozoites and cysts are found in CNS tissues. Differentiation with *Acanthamoeba* is difficult when using only simple light microscopy. Electron microscopy, immunofluorescence testing and histochemistry are needed for definite species identification.  In vitro studies show that *B. mandrillaris* is susceptible to pentamidine. Ketoconazole, propamidine, miltefosine, 5-flucytsosine,

clotrimazole, sulfadiazine, fluconazole and clarithromycine have all been used in treatment of patients. Treatment is not standardised yet. Experience in Peru has shown that prolonged administration of itraconazole 400 mg/day (adults) can be useful.

## Acanthamoeba sp.

Acanthamoeba sp are free-living protista which occur in numerous places (water, dust, waste). Several species have been described: A. castellani, A. culbertsoni, A. polyphaga, A. healyi, A. astronyxis, A. hatchetti, A. rhysodes, A. griffini, A. quina, A. lugdunensis.

Acanthamoeba species are responsable for several clinical problems: (1) granulomatous amoebic encephalitis, (2) keratitis, (3) disseminated lesions, including skin ulcers, but also lesions in adrenals, kidneys, liver, spleen, thyroid….

**1. Granulomatous amoebic encephalitis (GAE)**: unlike with Naegleria, infection of the central nervous system progresses slowly and occurs where there is immunosuppression or in the course of a severe general illness. Infections are more common in AIDS patients with a low CD4-count. Generally it presents as a subacute meningo-encephalitis with signs of a brain abscess and develops in two to three weeks (range 7 days – 5 months). The cerebral hemispheres tend to be involved with an inflammatory exudate covering the cortex, granulomatous necrosis of the brain parenchyma and thrombosed blood vessels. Such infections can mimic malignancies, fungal infections or abscesses. In AIDS patients, the differential diagnosis with cerebral toxoplasmosis can be very difficult.

**2. Keratitis.** This is more common than cerebral inflammation. The amoebae may infect small wounds of the cornea and then trigger a dangerous ulcerative keratitis which may develop into painful uveitis with hypopyon, scleritis and panophthalmitis. Acanthamoeba keratitis should be considered in the differential diagnosis of uveitis in AIDS patients. Initially this diagnosis is often missed and the lesion is considered to be a herpetic or fungal keratitis. Infection can follow corneal trauma (e.g. corpus alienum). The number of cases has grown in recent years as the result of increased use of contact lenses and the practice of rinsing these with tap water, as a result this is a cosmopolitan infection. It is likely that bacteria in the biofilm on dirty contact lenses constitute a good source of nutrition for the amoebae. The amoebae are often scarce in corneal smears. Culture is possible on nonnutrient agar plates

with an overlay growth of Esch. coli or Pseudomonas aeruginosa bacteria on which the trophozoites feed. Sometimes the diagnosis is made purely on anatomopathological grounds, e.g. during a cornea transplantation.

**3. Other locations.** Abscesses in other locations and granulomatous skin lesions in which histological investigations show amoebae, have also been observed. Skin lesions are more common in AIDS patients. Hard erythematous papulonodular lesions or non-healing indurated ulcers may be the first sign.

The optimal approach for GAE management is uncertain; therefore, combination regimens are preferred over single-drug regimens. An empiric treatment could be a combination of miltefosine, fluconazole, and pentamidine isethionate. Trimethoprim-sulfamethoxazole, metronidazole and a macrolide (azithromycin or clarithromycin) can be added to this regimen as well. Single cerebral lesions should be resected if possible.

Treatment of Acanthamoeba keratitis employs a combination of propamidine isethionate eye drops (Brolene®), topical neomycin, polyhexamethylene biguanide collyre (Lavasept®) and/or topical chlorhexidine (Hibitane®). Brolene® available in Great Britain is an antiseptic which is moderately toxic for the corneal epithelium. The use of topical steroids is controversial but probably beneficial. Oral itraconazole is probably also active. Topical miconazole is sometimes also used. Pentamidine (a diamidine related to propamidine) is being evaluated. Chronic refractory cases may require corneal transplantation. Unresponsive cases may require enucleation.

| **COMPARISON** | |
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| **Naegleria fowleri** | **Acanthamoeba sp** |
| Amoebic form with lobate pseudopodia;<br>Flagellate form (two flagella) | No flagella, filiform sharp pseudopodia |
| Cysts not present in tissue; they are small and smooth | Cysts can be found in tissues;<br>large and wrinkled with a double wall |

| | |
|---|---|
| Culture requires living cells (bacteria or cell culture)<br>No growth if NaCl concentration > 0.4% | May grow without bacteria; not affected by NaCl 0.85% |
| Smaller than Acanthamoeba; dense endoplasm;<br>less distinct nuclear staining | Large round, less endoplasm; more distinct nucleus |

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