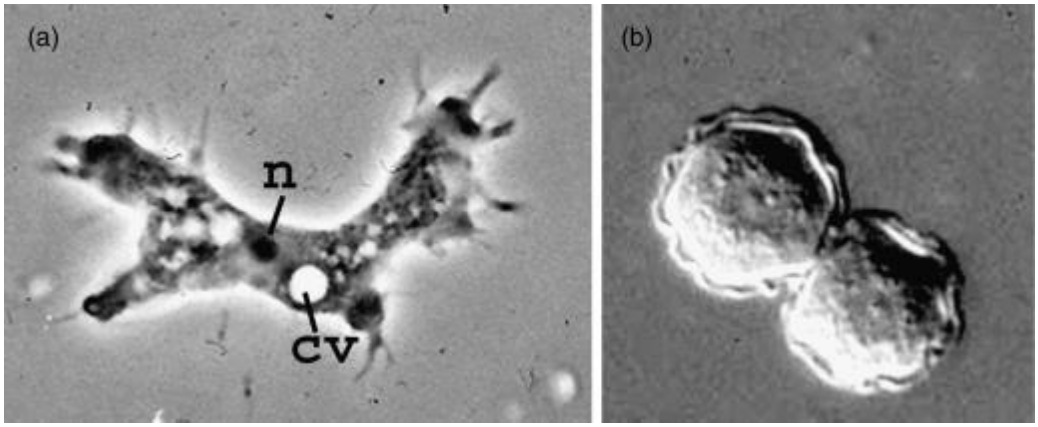
Free living Amoebae

Free living amebae (FLA) of the genera *Acanthamoeba*and *Naegleria*are ubiquitous in nature and can be found in nearly all environments worldwide. *Acanthamoeba* cause Acanthamoeba keratitis (AK) which is reported in 1-2 cases per million contact lens wearers annually, and is capable of causing skin lesions and granulomatous amebic encephalitis (GAE) in individuals with compromised or competent immune systems, who inhale infective cysts or develop indolent, granulomatous skin lesions in soil-contaminated wounds. Unlike *Acanthamoeba*, only one species of *Naegleria*, *Naegleriafowleri*, is known to infect humans by causing an acute, fulminant, usually lethal, central nervous system (CNS) infection, known as primary amebic meningoencephalitis (PAM).

Acanthamoeba spp.

Acanthamoeba has two stages in its life cycle: a vegetative or trophozoite stage that feeds voraciously on bacteria and detritus present in the environment and reproduces by binary fission, and a dormant but resistant cyst stage. A unique and characteristic feature of Acanthamoeba spp. Is the presence of fine, tapering, thorn-like acanthopodia that arise from the surface of the body. The trophozoites range in size from 15 to 50 mm depending upon the species. They are uninucleate, and the nucleus has a centrally placed, large,densely staining nucleolus. The cytoplasm is finely granular and contains numerous mitochondria, ribosomes, food vacuoles, and a contractile vacuole. When food becomes scarce, or when it is facing desiccation or other environmental stresses, the amoebae round up and encyst. Cysts are double-walled and range in size from 10 to 25 mm. The outer cyst wall, the ectocyst, is wrinkled with folds and ripples and contains protein and lipid. The inner cyst wall, the endocyst, contains cellulose and hence is Periodic acid-Schiff (PAS)- positive. The endocyst varies in shape: it may be stellate, polygonal, oval, or spherical. Cysts are uninucleate and possess a centrally placed dense nucleolus. Upon return to favourable growth conditions, the dormant amoeba is activated to leave the cyst by dislodging the operculum and reverting to a trophic form. In either the trophic or the cyst stage these organisms have a wide distribution in nature. Acanthamoebaspp. are ubiquitous and occur worldwide. They have been isolated from soil, fresh and brackish waters, bottled mineral water, air conditioning units, dental irrigation units, dialysis machines, dust in the air, bacterial, fungal and mammalian cell cultures, contact-lens paraphernalia, ear discharge, pulmonary secretions, swabs obtained from nasopharyngeal mucosa of patients with respiratory complaints. In addition, several Acanthamoebaspecies have been isolated from the brain, lungs, skin, and

cornea of infected individuals. Acanthamoebaspp. are tolerant of a wide range of osmolarity, enabling them to survive in distilled water, tissue culture media, mammalian body fluids, and sea water.



Trophozoite and cyst stage of Acanthameoba spp. n , nucleus CV , contractile vacuoles

Granulomatous amoebic encephalitis (GAE): clinical and laboratory diagnosis

GAE, as an opportunistic disease, affects hosts whose metabolic, physiological, or immunological integrity are compromised, and hence cases may occur at any time of the year with no pattern of seasonal occurrence. Several species of Acanthamoeba (A. culbertsoni, A. castellanii, A. polyphaga,A. astronyxis, A. healyi and A. divionensis) have been known to cause GAE, primarily in patients with HIV/AIDS or whoare chronically ill, diabetic, have undergone organ transplantation or are otherwise debilitated with no recent history of exposure to recreational freshwater. A few cases, have been described from individuals with no obvious signs of immunodepression. Onset of GAE is slow and insidious and develops as a chronic disease spanning from several weeks to months.

The usual features of GAE consist of headache, stiff neck, and mental-state abnormalities, as well as nausea, vomiting, low-grade fever, lethargy, cerebellar ataxia, visual disturbances, hemiparesis, seizures and coma. Facial palsy with numbness resulting in facial asymmetry is often seen. Cerebral hemispheres are usually the most heavily affected CNS tissue. They are often edematous, with extensive hemorrhagic necrosisinvolving the temporal, parietal, and occipital lobes. Computerized tomography (CT) scans of the brain show large, low-density abnormalities mimicking a single or multiple space-occupying mass. Magnetic resonance imaging (MRI) with enhancements shows multiple, ring-enhancing lesions in the brain.

Acanthamoeba spp. infecting the CNS are not readily found in the cerebrospinal fluid (CSF), A biopsy may demonstrate amoebictrophozoites and cysts. Amoebic trophozoites and cysts are found within the CNS tissues,

pulmonaryparenchyma, prostate, and skin lesions. The amoebae can be distinguished from host cells by their prominent central nucleolus and by their location in the perivascular areas in brain tissue. Definitive identification of the amoebic genus is based on the visualization of trophic or cystic stages in immunofluorescence- stained brain tissue sections.

Mechanisms of pathogenesis

Much of the damage done by Acanthamoebatrophozoites in the course of corneal or brain infections is probably the result of several different pathogenic mechanisms. Acanthamoeba undergoes certain morphological changes when associated with hamster cornea in vitro, and produces amoebastome-like (foodcup) surface structures that ingest detached epithelial cells and thus aid in phagocytosis. Several studies have described enzymes secreted by Acanthamoeba, which may facilitate the spread of amoebae by opening avenues for invasion, and providing nutrients in the form of lysed host cells. The pathogenic species A. culbertsoni excretes more phospholipase enzyme into the culture medium than does the nonpathogenic species.

Acanthamoeba keratitis: clinical and laboratory Diagnosis

Acanthamoeba keratitis (AK) is a painful vision-threatening infection caused by these amoebae. If the infection is not treated promptly, it may lead to ulceration of the cornea, loss of visual acuity, and eventually blindness. AK is associated with trauma to the cornea or contact-lens wear and the use of amoeba-contaminated saline. Minor erosion of the corneal epithelium may occur while wearing hard or soft contact lenses, and the subsequent use of contaminated saline solution is the major risk factor for Acanthamoeba keratitis. AK is characterized by inflammation of the cornea, severeocular pain and photophobia. Typically, only one eye is involved; however, bilateral keratitis has also been reported.

Acanthamoebatrophozoites and especially cysts can be recovered from corneal scrapings or biopsy. Definitive diagnosis is based on visualization of amoebae upon microscopic examination of corneal scrapings or biopsies or on their cultivation from affected tissues.

Treatment and prevention

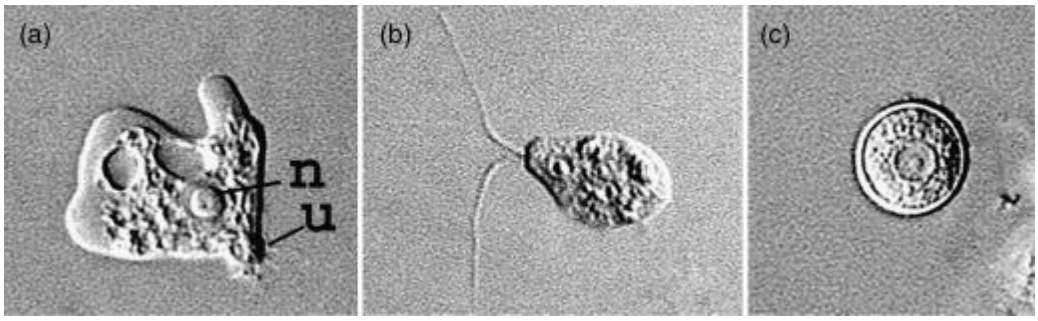
GAE caused by Acanthamoebaspp., as well as some with Acanthamoebacutaneous infection without CNS involvement, have been successfully treated with a combination of pentamidineisethionate, sulfadiazine,flucytosine, and fluconazole or itraconazole. For Acanthamoebacutaneous infection without CNS involvement, topical applications of chlorhexidinegluconate and ketoconazole cream in addition to

the above-noted antimicrobials have resulted in therapeutic success.Treatment ofAcanthamoebakeratitis has been fairly successful. A variety of drugs have been used, including chlorhexidine, polyhexamethylenebiguanide, propamidineisethionate, dibromopropamidineisethionate, neomycin,paromomycin, polymyxin B, clotrimazole, ketoconazole, miconazole, and itraconazole.
Because GAE caused by Acanthamoebaspp. occurs in hosts with weakened immune functions, no clearly defined methods exist for the prevention of infection with these amoebae. In the case of AK, however, because contact lenses and lenscare solutions are well-known risk factors, educating lens wearers regarding the proper care of contact lenses (and contact-lens cases) is important for the prevention of infection.

Naegleriafowleri

Naegleriafowleri is an amoeboflagellate, as it has a transitory, pear-shaped flagellate stage along with amoeboid trophozoite and resistant cyst stages in its life cycle. The trophozoite moves rapidly by producing hemispherical bulges, lobopodia, at the anterior end and exhibits active locomotion. It measures from 10 to 25 mm, reproduces by binary fission. It has a single vesicular nucleus with a prominent, centrally placed nucleolus that stains densely with chromatic dyes. The cytoplasm shows a sharp distinction between ectoplasms and endoplasms; the latter contains numerous mitochondria, ribosomes, food vacuoles, and, defining the posterior end (uroid), the contractile vacuole and trailing protoplasmic
filaments. The trophozoite transforms into a flagellate stage, usually with two flagella, when the ionic concentration was changed. The temporary, pear-shaped flagellate stage neither divides nor feeds, ranges in length from 10 to 16 mm, and usually reverts to the amoeboid form within an hour or less. The trophozoite transforms into the resistant cyst when the food supply diminishes and/or growth conditions become adverse. The cyst is usually spherical and double-walled with a thick endocyst and a closely apposed thin ectocyst. Both the flagellate and cyst stages also possess a single nucleus with a prominent nucleolus. Naegleriafowleri occurs worldwide and has been isolatedfrom soil and fresh water.

Naegleriafowleri is thermophilic and can tolerate temperatures of up to 45 1C. Therefore, these amoebae proliferate during warmer months of the year when the ambient temperature is likely to be high. Infections occur in children and young adults – age groups that are more energetic in aquatic activities and thus are likely to come into contact with amoebae in water.



Naegleriafowleri: (a) trophozoite, (b) a flagellate, and (c) a cyst.

Primary amoebic meningoencephalitis (PAM)

Naegleriafowleri causes an acute, fulminating hemorrhagic meningoencephalitis principally in healthy children andyoung adults with a history of recent exposure to warm fresh water. Because PAM occurs primarily in healthy individuals, the organism is not regarded as an opportunistic amoeba, as Acanthamoeba , but as Pathogen. The striking feature of PAM is the rapid onset of symptoms following exposure. The disease progresses rapidly, and, without prompt diagnosis and intervention, death usually occurs within a week or less.

The time from initial contact (swimming, diving, water skiing, or simply immersing head in water) to onset of illness is usually 5–7 days, and may even be as short at 24 h. Because there are no distinctive clinical features that differentiate PAM from acute pyogenic or bacterial meningoencephalitis it is imperative that the attending physician obtains information regarding the patient's contact with fresh water, including hot springs, during the past week. The earliest symptoms are sudden onset of bifrontal or bitemporal headaches, high fever, nuchal rigidity, followed by nausea, vomiting, irritability and restlessness. Photophobia may occur late in the clinical course, followed by neurological abnormalities, including lethargy, seizures, confusion, coma, diplopia or bizarrebehaviour, leading to death within a week.

CSF may vary in color from greyish to yellowish-white, and may be tinged red with a few red cells in the early stages of disease. However, as the disease progresses the red blood cell number increases to as high as 24600mm^3. The white blood cell count, predominantly polymorphonuclear leukocytes (PMN), No bacteria areseen.

A wet-mount of the CSF should be examined immediately after collection under a microscope,preferably equipped with phase-contrast optics, for the presence of actively moving trophozoites. Smears of CSF should be stained with Giemsa or Wright stains to identify the trophozoite, if present. The amoeba can be clearly differentiated from host cells by the nucleus with its centrally placed large nucleolus.

Mechanisms of Pathogenesis

The portal of entry into the CNS is the olfactory neuroepithelium. It is believed that the sustentacular cells lining the olfactory neuroepitheliumphagocytose the amoebae that enter the nasal passages of the victims while indulging in aquatic activities. The amoebic trophozoites pass through the sieve-like cribriform plate and penetrate into the subarachnoid space and continue on to the brain parenchyma.

The incubation period of PAM varies depending on the size of the inoculum, and on the virulence of the particular strain of infecting amoebae. Naegleriafowleri when inoculated into tissue culture cells destroy the cell monolayer by causing a cytopathic effect or CPE. The amoebae produce sucker-like appendages or amebostomes that 'nibble' away at the tissue culture cells. Other possible factors involve the production of: (i) phospholipase A and B activity or a cytolytic factor causing destruction of cell membranes; (ii)neuraminidase or elastase activity facilitating destruction of tissue culture cells; (iii) a perforin-like, pore-forming protein that lyses target cells; and (iv) the presence within Naegleria amoebae of a cytopathic protein that triggers the apoptosis pathway in susceptible tissue culture cells.

Prevention and control

Because N. fowleri is susceptible to chlorine in water (one part per million), amoeba proliferation can be controlled by adequate chlorination of heavily used swimming pools, especially during summer months. However, it is not possible to chlorinate natural bodies of water such as lakes,ponds and streams, where N. fowleri may proliferate. Sunlight and the presence of organic matter in swimming pools can reduce the efficacy of chlorine. In high-risk areas, monitoring of recreational waters for N. fowleri amoebae should be considered by local public health authorities and appropriate warnings posted, particularly during the hotsummer months.

Treatment

Few patients have survived PAM. the survivor were aggressively treated with intravenous and intrathecalamphotericin B, intravenous and intrathecalmiconazole, and oral rifampin.

Non pathogenic amoebae

EmtamoebaDispar

Non invasive protozoan , has recently been separated from *E. histolytica* . The two parasite are morphologically identical species but genetically distinct species . Is predominant cause of colonization in many asymptomatic (cyst passers) in developing countries ,as well as in sexually active male homosexuals in developed countries .

Morphology :

The are two species of *Entamoeba* only one of these caused disease in human , Other non pathogenic , species E. dispar . The inability to differentiate between these two species by morphological or biological means until recently led to significant debate on this topic . Isoenzymes typing could be used to distinguish the pathogenic fromnon – pathogenic species of EntamoebaToday the two species are classified as *E.histolytica* and *E . Dispar*

Pathogenesis:

E. dipar has never been documented to cause colitis or liver abscess Because*E .dispar* colonization is more common than *E. histolytica* infection and not need treated . an important clinical advance has been the
development of Ag detection test that differentiate between themStating that the *E . Dispar* infection doesnot need to be treated ,and *E. dispar* is capable of killing target cells such as neutrophils

Clinical features:

Although colonization with E . Dispar is known to occur , the organismhas never been known to cause disease . In patients with HIV infection no correlation has been established between the presence of E. dispar and gastrointestinal symptoms.

Diagnosis:

*Entamoebadispar*a non-pathogen is indistinguishable bymicroscopy and is a much more common intestinal protozoan than *Entamoebahistolytica*.

Antigen capture and PCR tests can distinguish *E. dispar*from *E. histolytic*a in heavier infections

EntamoebaGingivalis

General characterized :

1- was the first amoeba ofman to be described .
2- it is global in distribution .
3- this type is seen in tooth .
4- no cyst in stool .

morphology:

This species of amoeba is no cyst stage is seen Only troph of this parasite are seen . The troph is 10 -20mm in diameter , Activelymotile with multiple pseudopodia .The cytoplasm contains food vacuoles with ingested bacteria . ingested Leukocytes and epithelial cell . The nucleus is round , with central

karyosome and nuclear membrane is lined with evenly chromatin granules . Only E . gingivalis ingestW.B.Cs.

Clinical Disease

It is commensal organism , and not considered to cause any disease ,It lives in the gingival tissues . It has been misdiagnosed in various condition .

A- in periodontal disease where it is just accidently present and has no pathogenic role .

B- itmultiplies in bronchialmucosa and appears in sputum where itmight be mistaken for E . histolytica from a pulmonary abscess .

C- it has been recovered fromvaginal and cervical smears of woman using intrauterine devices but has on pathogenic role.

Entamoeba Coli

Non pathogenic amoeba (can not produce virulent factor). Found in healthy stool . Normally present in intestine . That very closely resembles Entamoebahistolytica .

Morphology:

There are three stages in this type: Trophozoites. Pre cyst. Cyst.

Characterized of trophozoites

The troph range in size from15 – 50mm . There is single nucleus with enteric karyosome and unevenly distributed peripheral chromatin . The cytoplasm is granular . The trophmove by short multiple pseudopodia(multi– directional). No RBC are present. Ability to ingested multiple type of bacteria can be causesmany disease.

Characterized of pre cyst and cyst

1- pre cyst :

This stage is very transitory

2- cyst

measure 10 – 35mm in diameter . The average diameter is definitely greater than the cysts of thenucleus vary in number from 1 – 8 pathogenic amoeba .The karyosome can be frequently distinguish even in unstained amoebae . Peripheral Chromatin are unevenly distributed.

Life cycle

Human infected occurs through ingestion of food or water contaminated by cyst bearing faeces . Eight nucleatedmetacyst is excycted in intestine , After a series of cytoplasmic division eight to fewer metacystictrophozoites are formed and develop in to mature troph in caecum, Trophmultiply by binary fission .

Clinical features : Entamoeba coli is non – pathogenic No clinical manifestation results.

Endolimax nana

Non pathogenic amoeba , classified within intestinal amoeba , They are found only in lumen cavity of the intestinal tract, These nonpathogenic protozoa do not causes any disease.

Morphology

The troph exhibit non progressive motility which is achieved by blunt hyaline pseudopodia . The single nucleusmay or not be visible in stained preparation , The karyosome is typically large and irregularly shaped , and is often described as "blot like " in appearance . Absence of peripheral chromatin is key in trophs identification. The cytoplasm is granular , vacuolated and usually contains bacteria . Non pathogenic amoeba , classified within intestinal amoeba , They are found only in lumen cavity of the intestinal tract. These nonpathogenic protozoa do not

causes any disease . Infection with this parasite by ingesting food or water that is contaminated with feces. This is called fecal-oral transmission.

E. nana

DientamoebaFragilis

Amoebic – flagellated parasite This parasite is classified as an amoeba because this organismmoves by pseudopodia and does not have external flagella**.**

Morphology

Only troph is present in the life cycle . The troph is irregular and roundish in shape . Motility is progressive and accomplished by broad hyaline pseudopodia , The topical troph has 2 nuclei each consisting of 4-8 centrally located massed chromatin granules , No peripheral chromatin is present . most troph are binucleated , therefore this parasite named Dientamoeba . Vacuoles containing bacteriamaybe present in the cytoplasm of these troph . No cyst is seen The specimen of choice for recovery Dientamoeba is the stool . It is known that this parasite resides in themucosal crypts of the large intestine . There is no evidence to suggest that D . Fragilistrophinvade their surrounding tissues, and has only rarely been known to ingest R.B.Cs . It is estimated that the majority of persons with D. fragilis infectious remain asymptomatic

Life cycle

the cyst stage has not been identified in *D. fragilis*life cycle, and the trophozoite is the only stage found in stools of infected individuals . *D. fragilis*is transmitted by fecal-oral route and transmission via helminthes eggs (e.g., *Ascaris*, *Enterobius*spp.)

Clinical feature

Trophozoites of *D. fragilis*have characteristically one or two nuclei , and it is found in children complaining of intestinal (diarrhea, abdominal pain) and other symptoms (nausea, anorexia, fatigue, malaise, poor weight gain). Other documented symptoms that may occur include bloody or mucoid stools Some patients experience diarrhea with constipation .

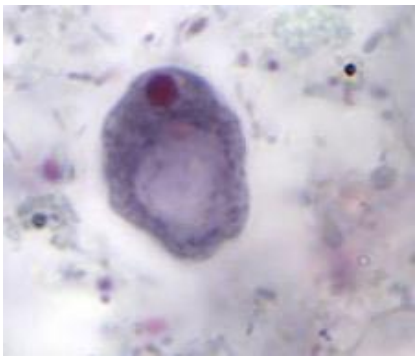
Treatment

The drug are used in this infection is Iodoquinol.

Iodamoebabutchlii

The trophozoites are 9–14 micrometres in diameter. Trophozoites are one of the two forms of *I.bütschlii*. This form has a pseudopodia for locomotion. The pseudopodia is short and blunt. It moves in a slow manner. The trophozoite has a single nucleus, prominent for nuclear endosome and many cytoplasmic vacuoles. The ectoplasm and the granular endoplasm are often hard to distinguish. The nucleus is fairly large and vesicular, containing a large endosome, surrounding by light staining granules about midway between it and the nuclear membrane. Achromatic strands stretch between the endosome and nuclear membrane without any peripheral granules. Food vacuoles are commonly filled with bacteria and yeast. Trophozoites are often identified by a stool smear, found in loose stools.

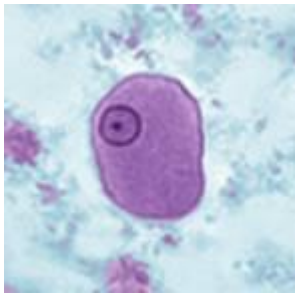
The cysts are 8–10 micrometres in diameter, with a thick wall and a large glycogen vacuole that stains darkly with iodine. Usually harmless, it may cause amebiasis in immunologically compromised individuals. As the second form of *I. butschlii*, cysts have an oval shaped- single nucleus with a prominent nuclear endosome. This form is also large, single, glycogen-filled vacuole called iodinophilous vacuole (glycogen stains with iodine). Cysts are the infective stage of *I. bütschlii*. Unlike trophozoites, cysts are often found in formedstool

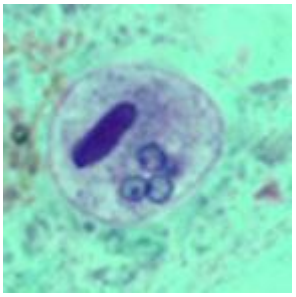


cyst of I. butchlii

Entamoebahartmanii

This organism belongs to the amebae, is a nonpathogen, and causes no disease. Both the trophozoite (usual size, 4-12 µm) and cyst forms (usual size, 5-10 µm) can be found in clinical specimens.





Trophozoite<12 microns Cyst <10 microns

Life Cycle:

Large bowel, organisms passed in feces

Acquired:

Fecal-oral transmission via cyst form; contaminated food and water

Epidemiology:

Worldwide, primarily human-to-human transmission

Clinical Features:

None