Free-Living Pathogenic Amoebae

- FLA are ubiquitous/WW distribution
- Opportunistic amoebae
- **Habitat**:
  - soil, dust, air, seawater, drinking water, swimming pools,…
- Many genera, only **four** have medical importance
  - *Naegleria*
  - *Acanthamoeba*
  - *Balamuthia*
  - *Sappinia (S. diploidea)*

Pathogenic free-living amoebae cont'd

- *Naegleria fowleri*: PAM
- *Acanthamoeba* spp
  - Primary disease: Amoebic keratitis
  - Opportunistic infection:
    - Granulomatous amoebic encephalitis
    - Cutaneous infection
    - Nasopharyngeal & pulmonary infection
- *Balamuthia mandrillaris*: immunocompetent children
  - Skin & fatal BAE

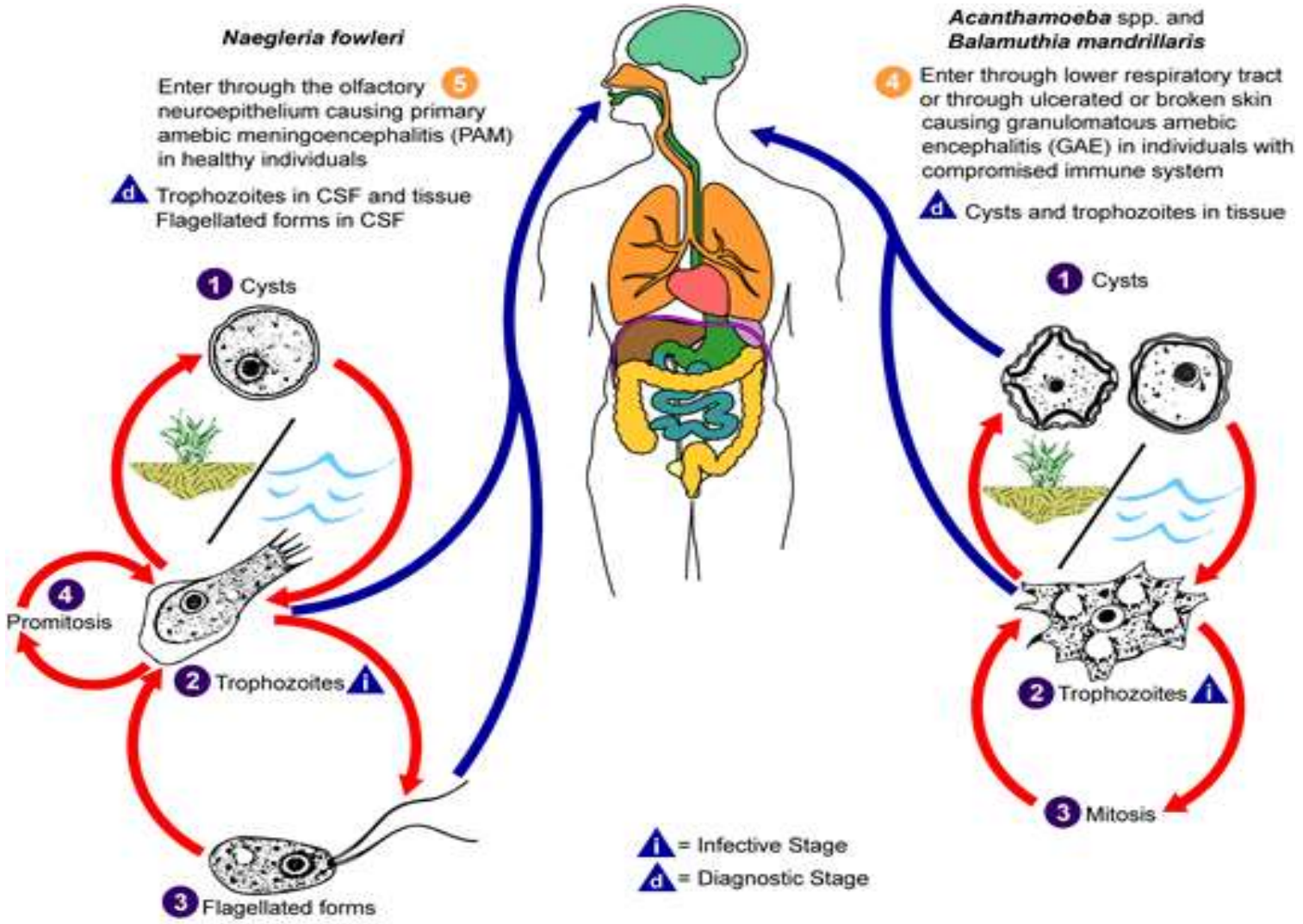
Pathogenic free-living amoebae cont'd

- Differ from intestinal amoeba by:
  - Naturally found freely outside the host in the environment
  - Possesses plenty of mitochondria
  - Nuclear membrane is distinct (not lined by peripheral chromatin granules) and nucleolus is large, deep stained
    - Intestinal amoeba has a delicate nuclear membrane, small, pale stained nucleolus
  - Cause opportunistic infection affecting CNS

General Life Cycle of free-living amoeba

- *N. fowleri* has cysts and trophozoites (amoeboid & flagellated)
  - trophozoites infect humans or animals by entering the olfactory neuroepithelium and reaching the brain
- *Acanthamoeba* spp has cyst and trophozoites, no flagellated form
  - trophozoites are the infective forms and are believed to gain entry into the body through:
    - the lower respiratory tract, ulcerated or broken skin and invade the central nervous system by haematogenous dissemination

Life cycle cont'd



Naegleria fowleri

- Class: **Heterolobosea,** family: **Vahlkampfiidae**, order: **Schizopyrenida**
- Only one species, *N. fowleri*, is known to cause infection, although two other species; *N. australiensis* and *N. italica*, can cause infection in mice
- Also known as "the brain eating amoeba"
- First described by two physicians:
    - Malcom Fowler and R.F. Carter in Australia in 1965

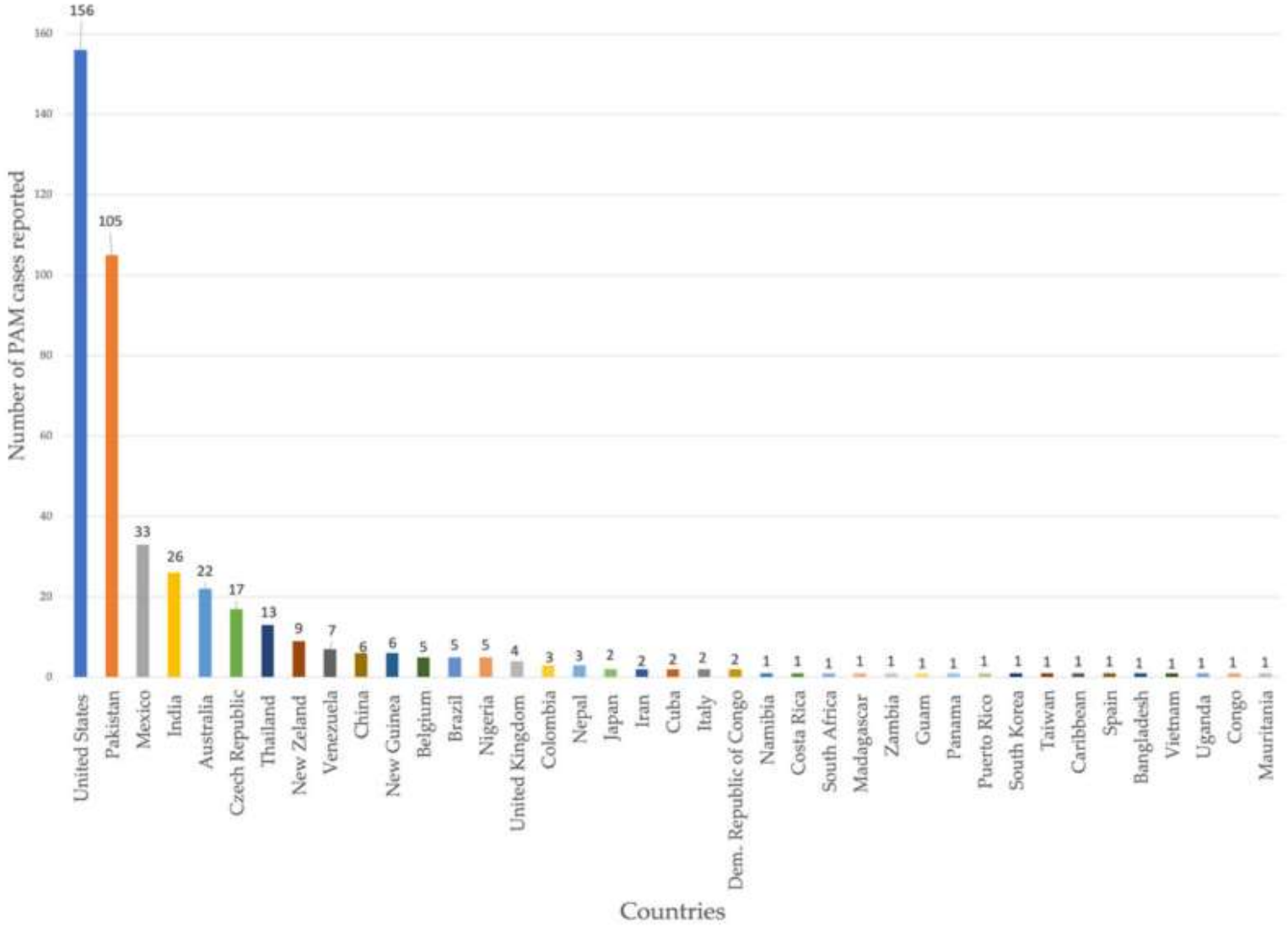
Naegleria fowleri cont'd

- sensitive to **drying, pH extremes & salinity**
- thermophilic (grow at temperature of 45^{o}C)
- feeds predominantly on bacteria living in natural bodies

Epidemiology: WW distribution: **39 countries**

- >400 cases of PAM have been reported mainly from USA and also from other parts of the world
  - Pakistan, Mexica, India, Australia, Czech Republic, etc

Epidemiology cont'd, until 2018



Naegleria fowleri cont'd

Habitat:

- Free-living: lakes, fresh water, swimming pools, aquaria, sewage, irrigation canals, ponds, hot springs, thermally polluted streams and rivers
- Parasitic:
  - Trophozoites: in CSF and tissue
    - also isolated from the nasal mucosa of healthy asymptomatic children

N. fowleri: morphology

- Exist in nature as cyst and trophozoite
- All stages are characterized by:-

  -Single nucleus, single central karyosome

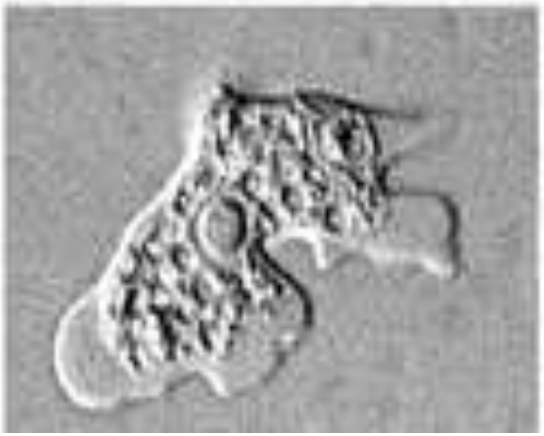
  -No peripheral chromatin granules

Trophozoites: two forms; amoeboid and flagellate, both measure 8–15 µm

- **Amoeboid**:
- Motility: actively motile with broad pseudopodia, lobopodia

N. fowleri: morphology cont'd

- Inclusion: doesn't ingest RBCs
- Cytoplasm: well differentiated in to ectoplasm & endoplasm
  - Endoplasm posses numerous **mitochondria**, food vacuoles & ribosomes
- The only replicating form and it divides by binary fission
- appropriate temperature for the best growth is 35–46°C



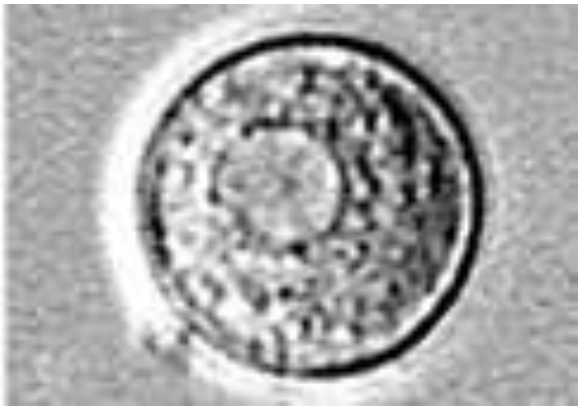
N. fowleri: morphology cont'd

- **Flagellate**:
- Amoeboid trophozoites transform to pear shaped flagellated form, exposed to a change in ionic concentration
  - placement in distilled water at 27–37°C
- Has two terminal flagella with equal length
- They show typical jerky or spinning motility
- When the flagella are lost, they revert back to amoeboid form

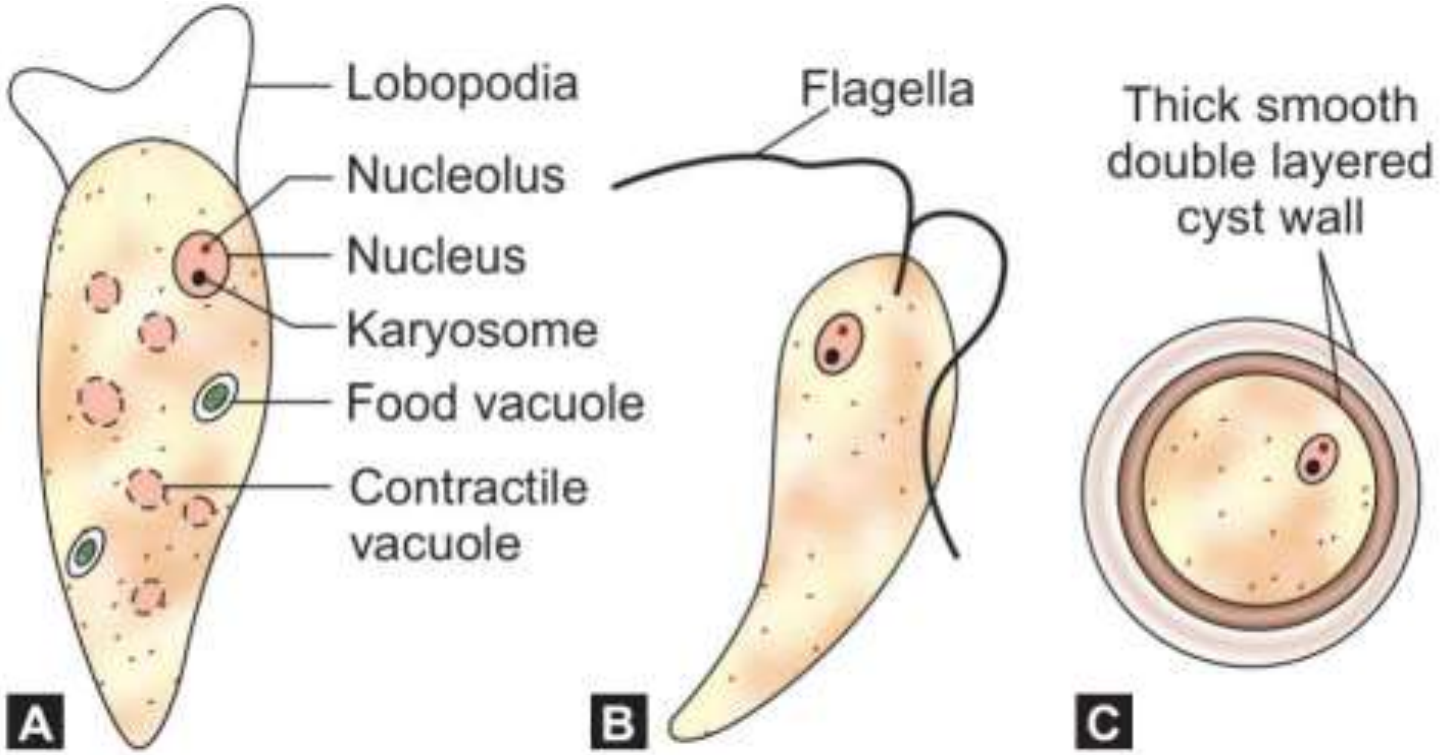
N. fowleri: morphology cont'd

Cyst Stage

- Cysts measure 7–15 µm in size and is surrounded by a thick, smooth double wall
- Nucleus is identical to that found in the trophozoite
- Cysts are not found in tissue (humans) but can be grown in culture



N. fowleri: morphology cont'd



Figs 1: A to C: *N. fowleri* (schematic diagram) (A) amoeboid form; (B) flagellated form; (C) cyst stage

N. fowleri: transmission

- **Infection:-** through nasal cavity by aspiration of water contaminated with trophozoites (amoeboid form) while:
  - Swimming or diving in warm freshwater river or ponds and poorly maintained swimming pools or
  - Nasal irrigation using contaminated tap water
- Risk groups: healthy young adults or children

Life cycle

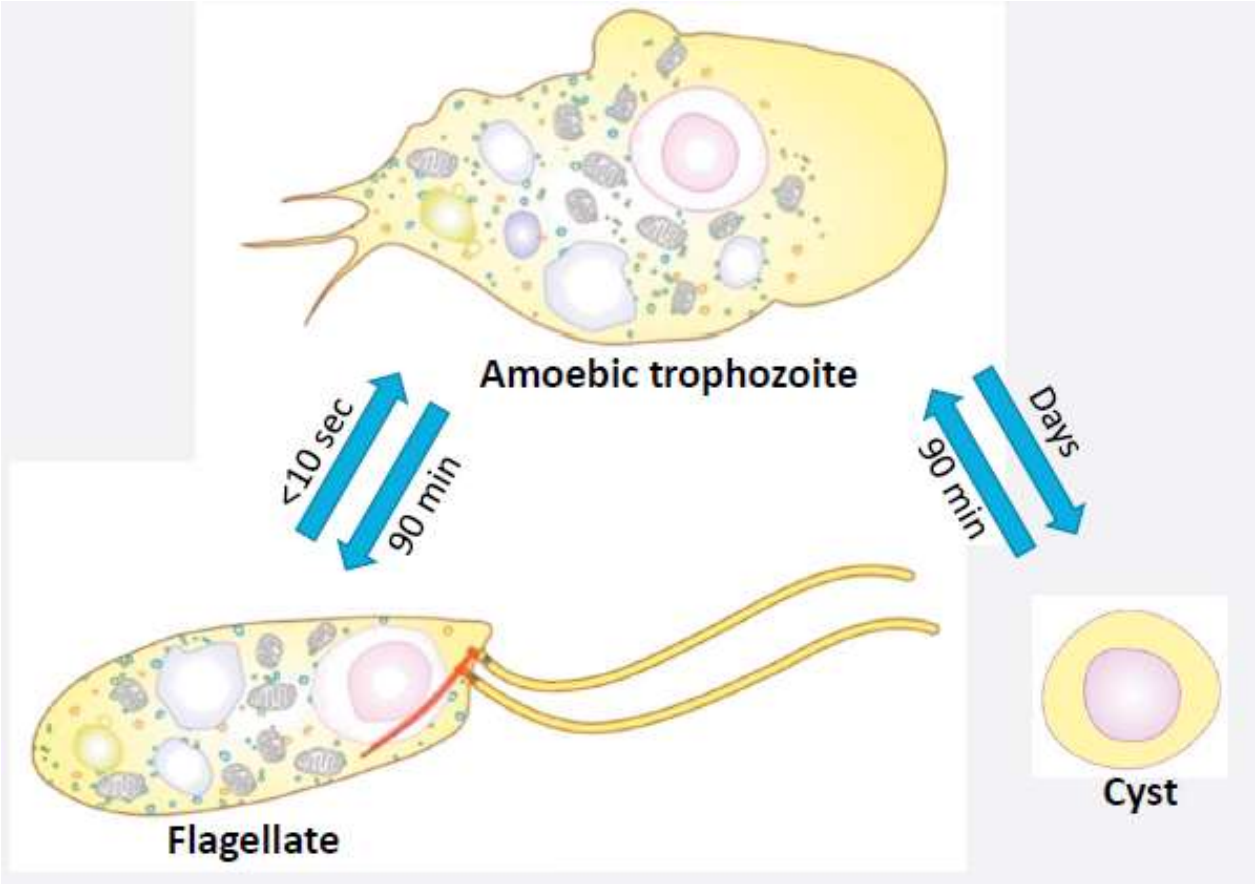
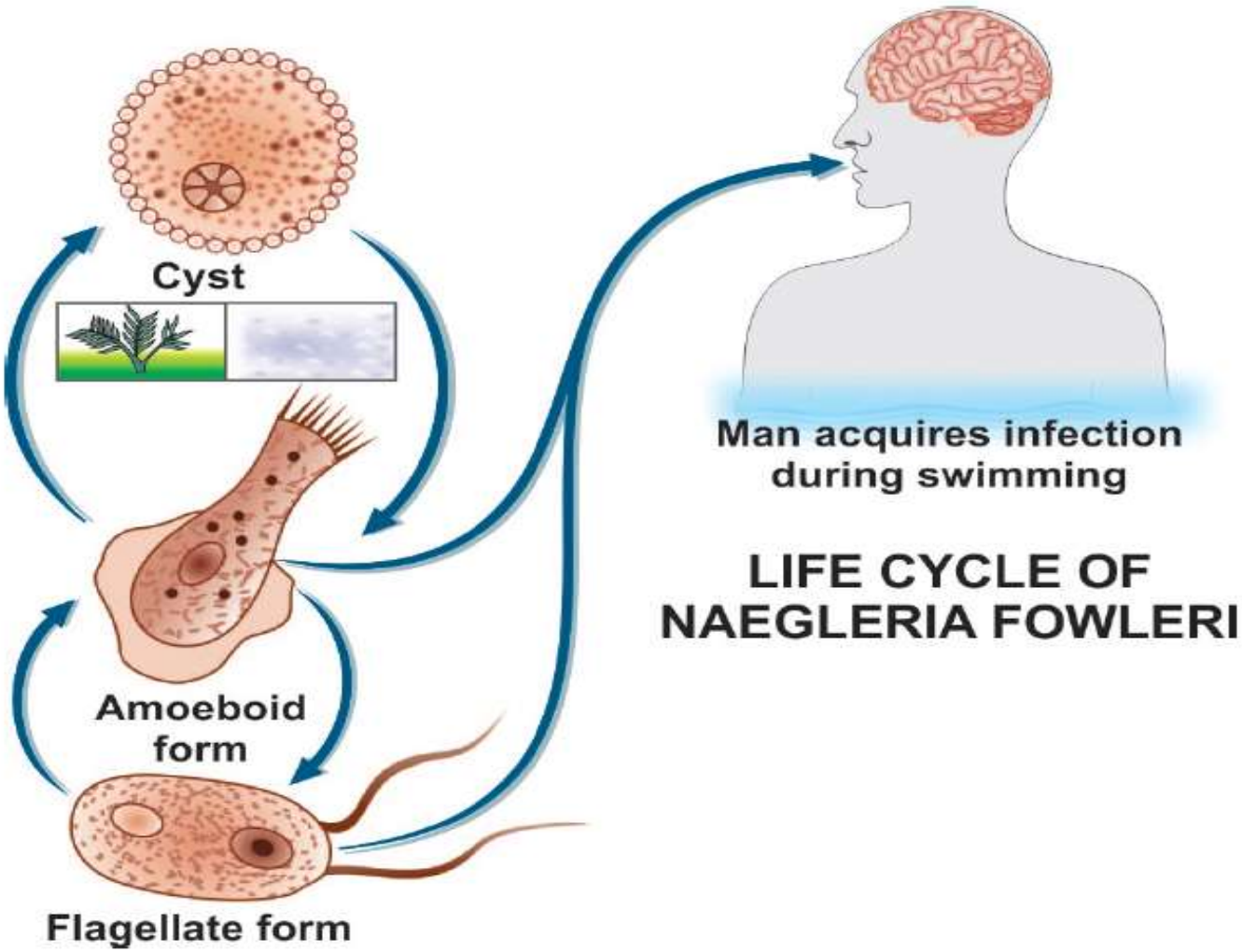


Figure 2. The Life Cycle of *N. fowleri*., Trends in Parasitology, 2019

Life cycle of *N. fowleri*



Pathogenesis of *N. fowleri*

- the exact pathogenesis is still not known
- causes PAM, acute suppurative fulminant infection
- usually occurs in healthy children or young adults
- **Incubation period:** 1–2 days to 2 weeks
- Clinical course is acute and fulminant
- Trophozoite ➔nasal cavity ➔attach to nasal mucosa ➔olfactory nerves (through cribriform plate) ➔olfactory bulb ➔brain (through olfactory nerve bundles) = severe cerebral damage and inflammation

Pathogenesis of *N. fowleri* cont'd

- Initially, penetration results in significant necrosis and hemorrhages in the nasal mucosa and olfactory bulbs
- Gradually, it produces an acute suppurative meningoencephalitis, which becomes hemorrhagic and necrotic later

Mechanisms of pathogenesis:

- Contact-dependent mechanisms
  - adhesion and phagocytic food-cups (**amoebostome**)
- Contact-independent mechanisms
  - cytolytic molecules secreted by the amoeba (Matrix metalloproteinases, phospholipase,)

Clinical features

1° symptoms:

- Anosmia, headache, anorexia, nausea, vomiting, high fever (38.5–41°C) and
- Signs of meningeal involvement; nuchal rigidity with Kerning and Brudzinski signs
- Photophobia
- Raised intracranial pressure (≥600 mm Hg)

Clinical features cont'd

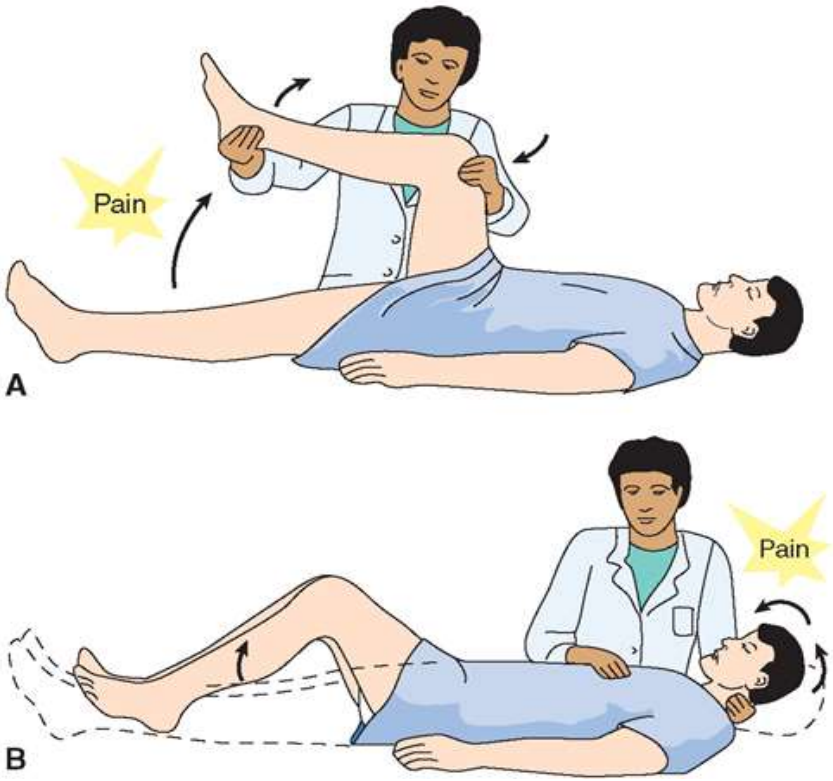
2° symptoms include:

- Confusion, hallucinations, lack of attention, ataxia, and seizures
- Brain edema and herniation
  - cause of death
  - mortality rate is nearly 98%, death occurs within 7–14 days after exposure

Clinical features cont'd

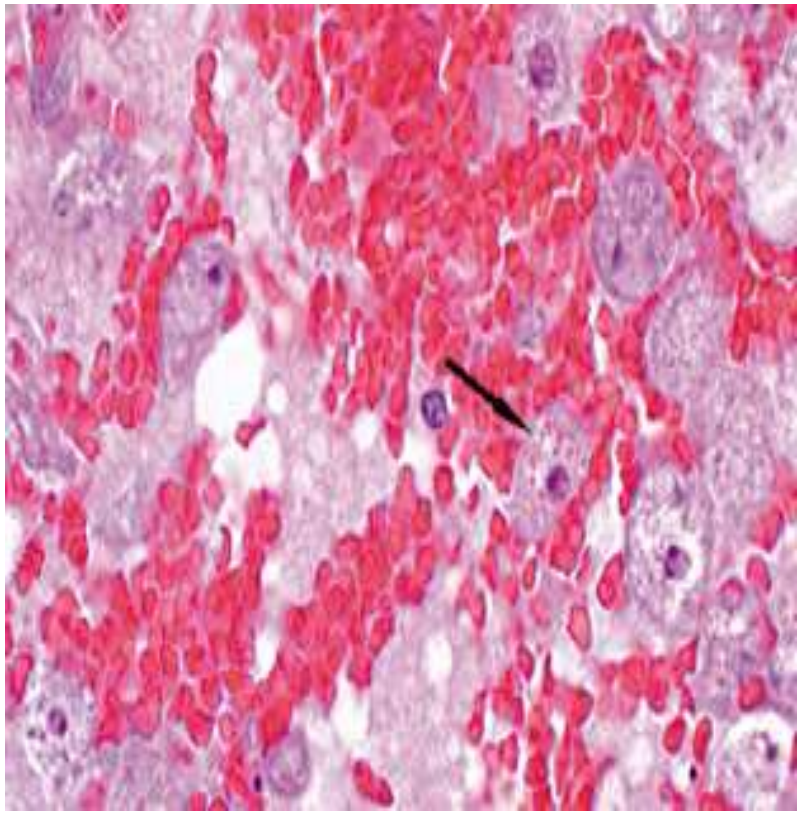
Nuchal rigidity

Kernig sign



Brudzinski signs

Clinical features cont'd



Clinical features cont'd

- **Advanced stage**
  - CSF color
    - greyish to yellowish-white, and may be tinged red with red cells
  - CSF cell count
    - RBC count: ≥24,600 cells/µl
    - WBC count: 300-26,000 cells/µl, predominantly PMN
  - CSF chemistry
    - Protein: 100-1000 mg/100 ml
    - Glucose: ≤10 mg per 100 mL

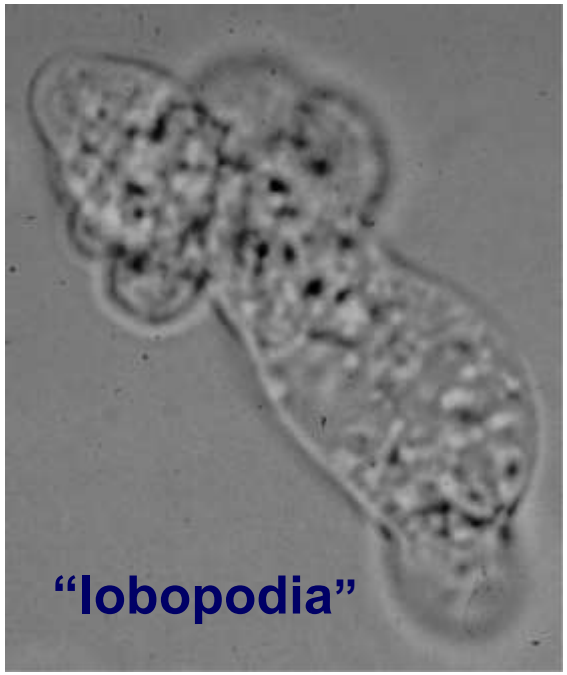
Laboratory Diagnosis

- Finding trophozoites in CSF by microscopy
  - **CSF**: purulent & may contain eosinophils, RBC with reduced glucose and raised protein
  - Indications of Naelgeria infection:
    - elevated white cell count in CSF without the successful recovery of bacteria
- Differential diagnosis: bacterial meningitis

Laboratory Diagnosis cont'd

Naegleria trophozoites

- Elongate in form, 10-22x7 µm
- Rapidly motile (>2 body length/minute
- Psuedopodia are lobulate or explosive protrution
- No RBC ingested
- Remain motile for several hrs
- Can be stained by Giemsa or acridine orange



Laboratory Diagnosis cont'd

Culture

- Nelson's growth medium, most recommended medium
- **Comprises**: Page's amoeba saline (0.4 mg of $MgSO_4$, 0.4 mg $CaCl_2$, 14.2 mg Na_2HPO_4, 13.6 mg KH_2PO_4, 12 mg NaCl in 100 mL of distilled water)
  - 0.17 g liver infusion and 0.17 g glucose
  - Then, autoclave for 25 min at 121°C
  - Incorporate sterile, heat-inactivated fetal calf serum
  - Supplement with 1% peptone, improve the amoeba's growth
  - Incubate at 37°C and monitor every day for a week

Treatment

- Early aggressive treatment with intravenous and intrathecal with Amphotericin B
  - Side effects: renal toxicity, anemia, fever, chills, vomiting, nausea, and headache
- **Other drugs** like rifampicin, azithromycin and antifungals; miconazole and voriconazole are also found to be effective

Prevention and Control

- Avoiding contact, swimming and sniffing in waters of ponds, lakes
- Treatment of water (chlorination; ≥ 0.5 mg/l for at least 30')
- Use of distilled, sterile and filtered (≤ 1µm pore size) water for nasal irrigation
- Proper sewage disposal
- Treatment of infected individuals and health education

Acanthamoeba species

- **Griffin** and **Sawyer** proposed the name in 1975
  - named because of the spine-like pseudopodia present in trophozoite (acanthopodia)
- At lest 31 species have been identified so far
  - Medically important ones include:
  - *A. culbertsoni (Hartmanella culbertsoni), A. castellanii, A. polyphaga, and A. astronyxis*

Acanthamoeba species cont'd

- worldwide distribution
- opportunistic & amphizoic
- **Habitat**
  - soil, air and water, fresh and sea water, beach sands, sewage, flower pot soils, home aquaria, humidifiers, heating, hospital environment, dental units, dialysis units, contact lens. . .
- can tolerate a wide range of **osmolarity, T°, salinity and pH** conditions

Acanthamoebaisis: Epidemiology

- In the US, AK cases are increased year by year
  - 22 cases were diagnosed in 1999
  - 43 cases were diagnosed in 2003 and
  - 170 cases were diagnosed in 2007
- In the UK, the numbers of AK cases diagnosed annually at the Moorfields Eye Hospital in London
  - 36–65 cases/year, from 2011 to 2014
- >200 cases of GAE have been reported so far, half of those from USA

Classification of Acanthamoeba

I. Morphological: size and shape of cyst

- **Group I**: cysts with average sizes of ≥18 µm
  - Inner layer: star shape
  - Outer layer: wrinkled or smooth
  - Ostioles present at the tips of rays
  - and the space between both layers is large
- **Group II:** cysts with average sizes of <18 µm
  - Inner layer: different forms (round, oval, triangle, polygon or star shape)
  - Outer layer: wrinkled and/or wavy

Classification of Acanthamoeba cont'd

- ostioles present at the angles of the rays
- and the space between both layers differs
- Most pathogenic spps attributed to this group (*A. polyphaga* and *A. castelanii*)

- **Group III**: cysts with average sizes of <18 µm
  - Inner layer: soft with three to five angles
  - Outer layer: may or may not present wrinkles
  - ostioles are single or obscure
  - Pathogenic spp *A. culbertsoni* belongs to this group

Classification: morphological cont'd

Group I	Group II	Group III
A. astronyxis –T7	*A. castellanii* –T4	*A. palestinensis* – T2
A. comandoni –T9	*A. mauritaniensis* –T4	*A. culbertsoni* – T10
A. echinulata –T4	*A. polyphaga* –T4	*A. lenticulata* –T5
A. tubiashi –T8	*A. lugdunensis* –T4	*A. pustulosa* –T2
A. byresii –T18	*A. quina* –T4	*A. royreba* –T4
	A. rhysodes –T4	*A. healyi* –T12
	A. divionensis –T4	*A. jacobsi* –T15
	A. paradivionensis –T4	*A. sohi* -
	A. griffini –T3	
	A. triangularis –T4	
	A. hatchetti -T11, etc	

Classification cont'd

II. Genotype:

- Currently the most conventional classification scheme
- To date, 23 different genotypes of *Acanthamoeba* (T1-T23) recognized
  - 18S rRNA genotyping
- Genotypes related to corneal infection; T2 –T11, T13, T15 & T16
  - genotype 7, 9 & 16, rarely cause AK
- Genotypes cause GAE: T1, T2, T4, T5, T10, T12 & T18

Acanthamoeba species cont'd

Reservoir for bacteria:

- ~20–24% of clinical and environmental isolates of *Acanthamoeba* harbor bacterial pathogens;
  - *Legionella* species, *Mycobacterium avium* and *Listeria monocytogenes, Vibrio cholerae, Escherichia coli O157*, *Mycobacterium bovis, etc*
- may serve as a potential reservoir and act as **Trojan horse** of the microbial world

Acanthamoeba spp: morphology

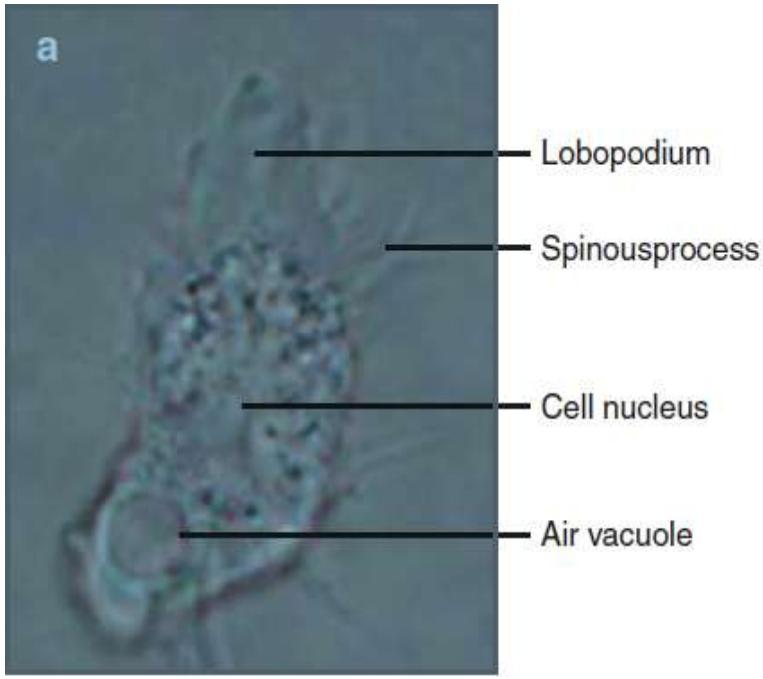
- Has trophozoite and cyst forms

❑**Trophozoite**

- Active form
- Reproduce by binary fission
- Produce human infection
- Often round or oval in shape with a diameter of 15–50 µm (average 20 µm)
- Possess fine, tapering, thorn-like pseudopodia = acanthopodia

Acanthamoeba spp: morphology cont'd

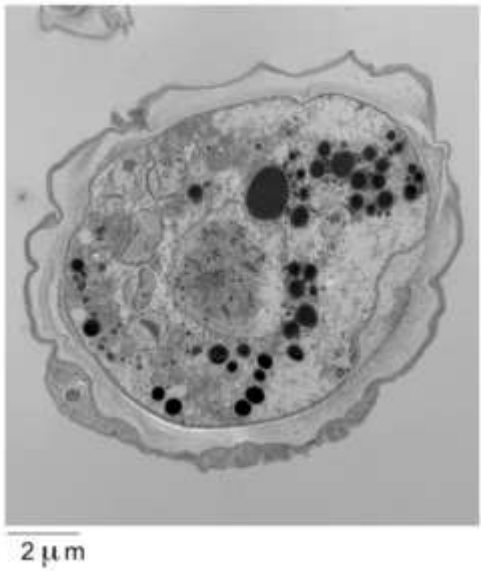
- finely granular cytoplasm with numerous **mitochondria**, ribosomes and vacuoles
- Spinousprocess (spiculate protuberances stretching from the surface of the cell membrane) -used to distinguish it from Naegleria spps

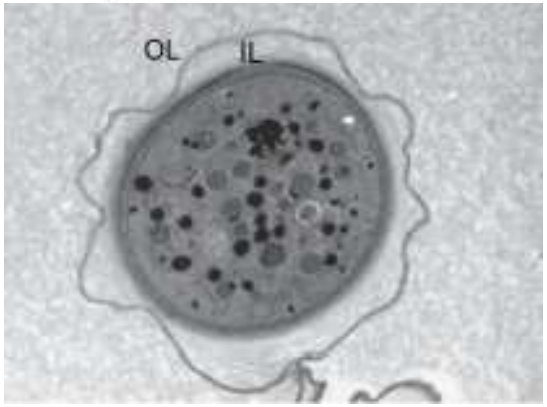


Acanthamoeba spp: morphology cont'd

❑ **Cyst**:

- a cellulose double wall with a fibrous laminar exocyst and a granular smooth endocyst
- The size varies depending on the genotypes
  - generally between 5–30 µm
- The morphology also vary between isolates
  - round, ovoid, triangular, hexagonal or polygonal





Acanthamoeba spp: morphology cont'd

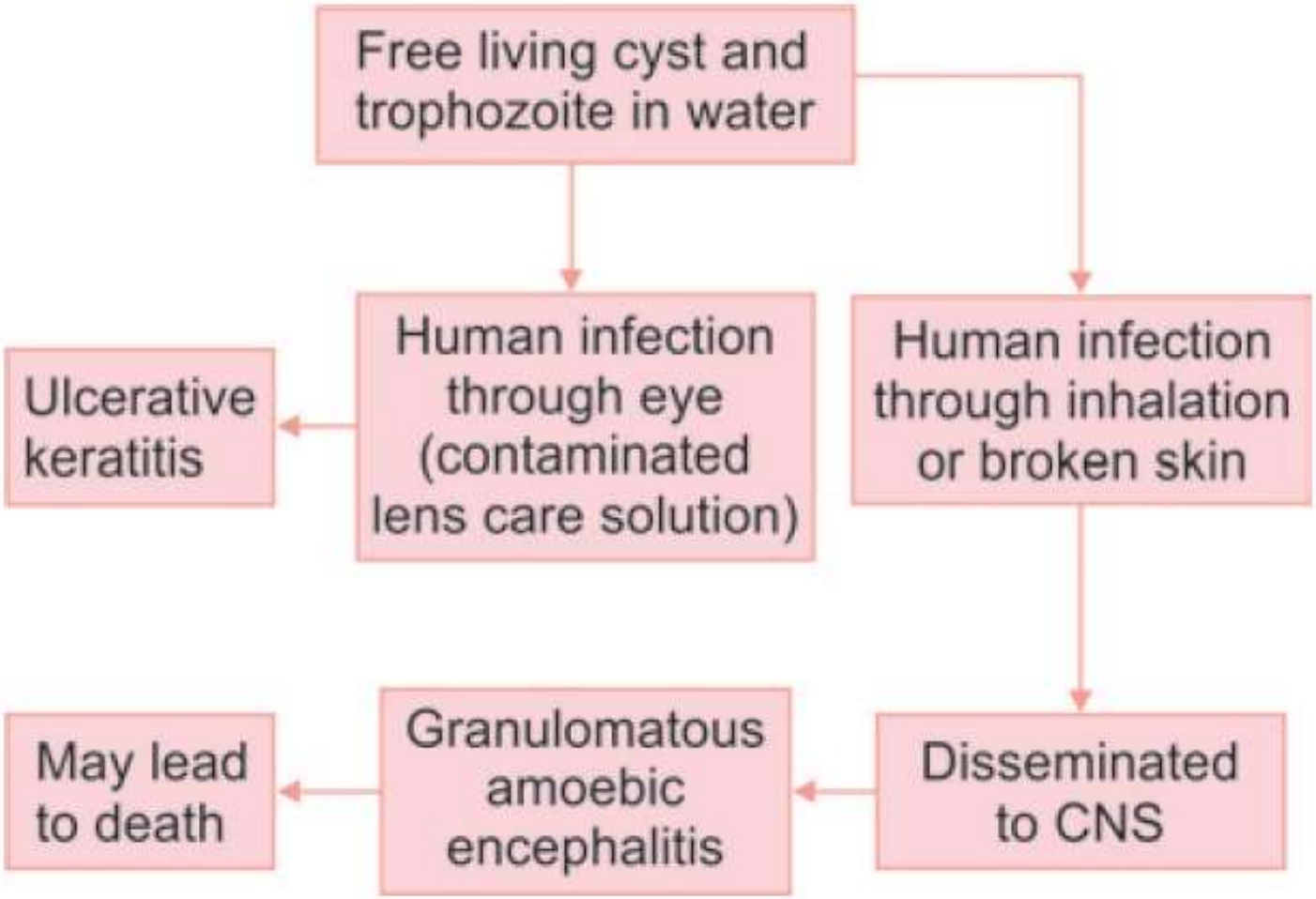
- withstand various physical and chemical factors in the environment, i.e., cold, heat, desiccation, a variety of disinfectants containing chlorine, and antibacterial drugs
- survive in conditions with pH 3.9 – pH 9.75 and temperature from −20°C to 56°C
  - *A. castellanii*, can tolerate the T° of 65°C for 5 min or remain alive after being frozen and dissolved repeatedly for five times
- In the natural environment, cysts generally can remain viable for several years while maintaining their pathogenicity

Acanthamoeba spp: transmission

Man acquires infection:

- by inhalational route by aerosol contaminated with cyst or trophozoite
    - from lungs, trophozoites reach CNS by hematogenous route
- rarely by direct spread through broken skin or infected eye

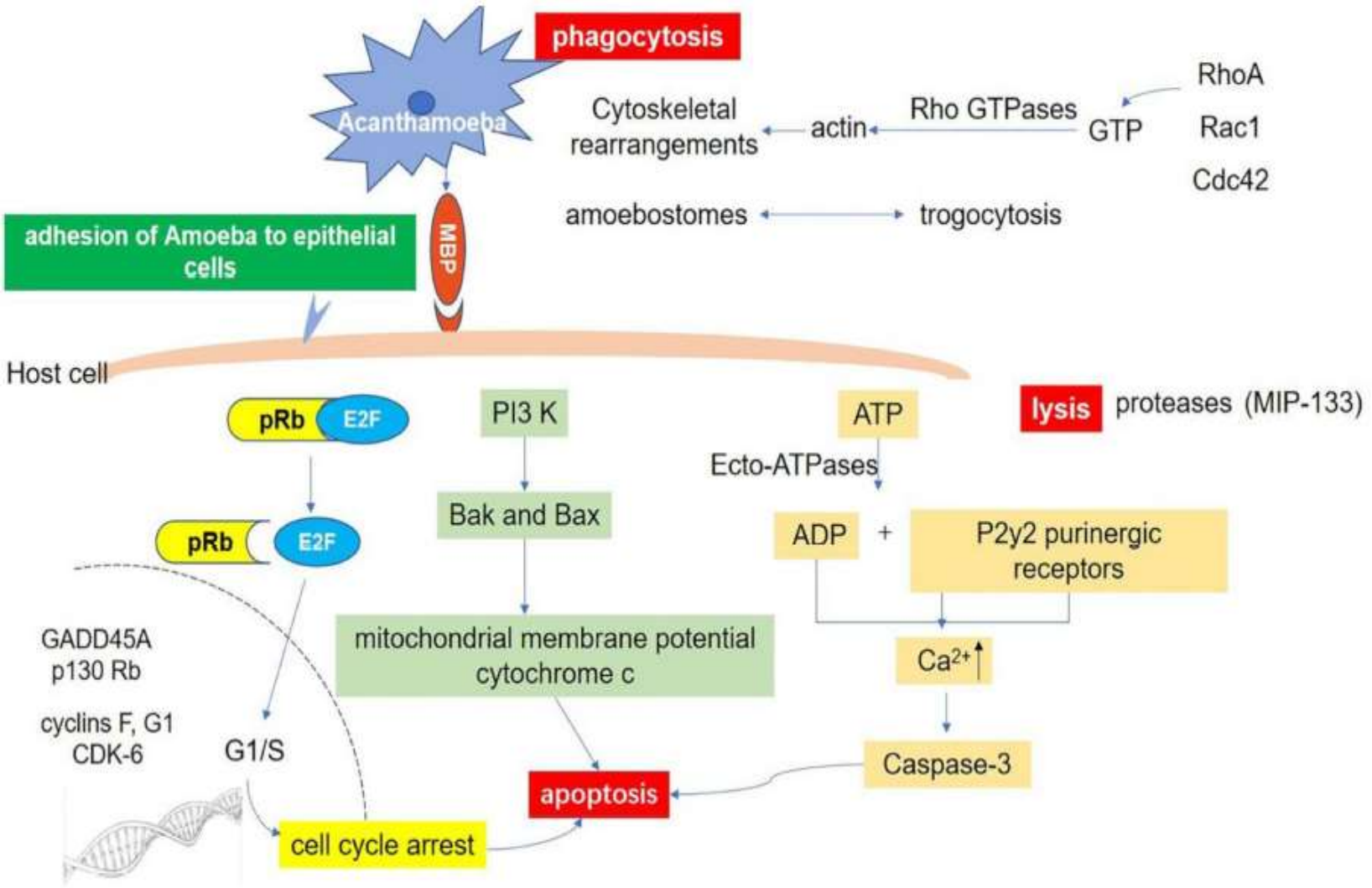
Acanthamoeba spps: Life cycle



Pathogenesis

- Acanthamoeba, causes:
  - Acanthamoeba keratitis, the most common
  - GAE, rare central nervous system disease
    - highly fatal with mortality rate >90%
  - Cutaneous acanthamoebiasis
  - Pulmonary infection
- **Pathogenic mechanism:**
  - Pathogenic mechanism of the rare GAE, CA, and pulmonary infection is not apparent
  - But in AK: adherence, phagocytosis of target cells, host cell lysis and induction of apoptosis

Pathogenesis cont'd



Clinical features & Pathology

- Mainly causes:
  - Amebic Keratitis in healthy individuals
  - Chronic granulomatous encephalitis in immunocompromised pts

❑**Granulomatous amoebic encephalitis (GAE)**

- is an opportunistic & fatal disease
- Affects immunocompromised or debilitated people
  - HIV/AIDS patients, diabetics
  - Patients with history of organ transplantation

GAE cont'd

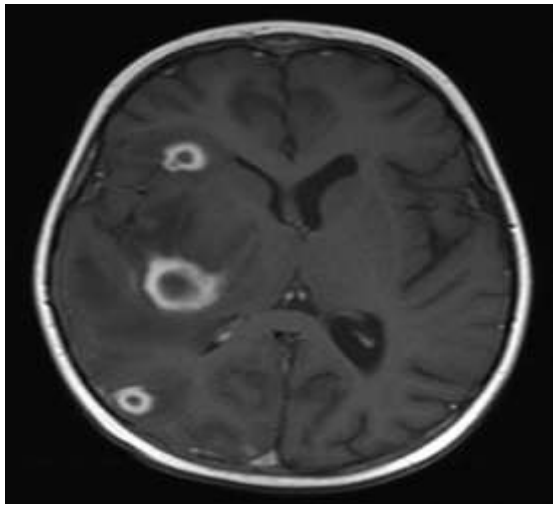
- **Route of entry**
  - lower respiratory tract & skin lesions followed by hematogenous spread
  - entry into the CNS: through the BBB (via endothelial lining of cerebral capillaries)
  - Other organs are also affected:
    - skin, liver, lungs, kidneys, adrenal glands, pancreas, prostate, lymph nodes & BM

GAE cont'd

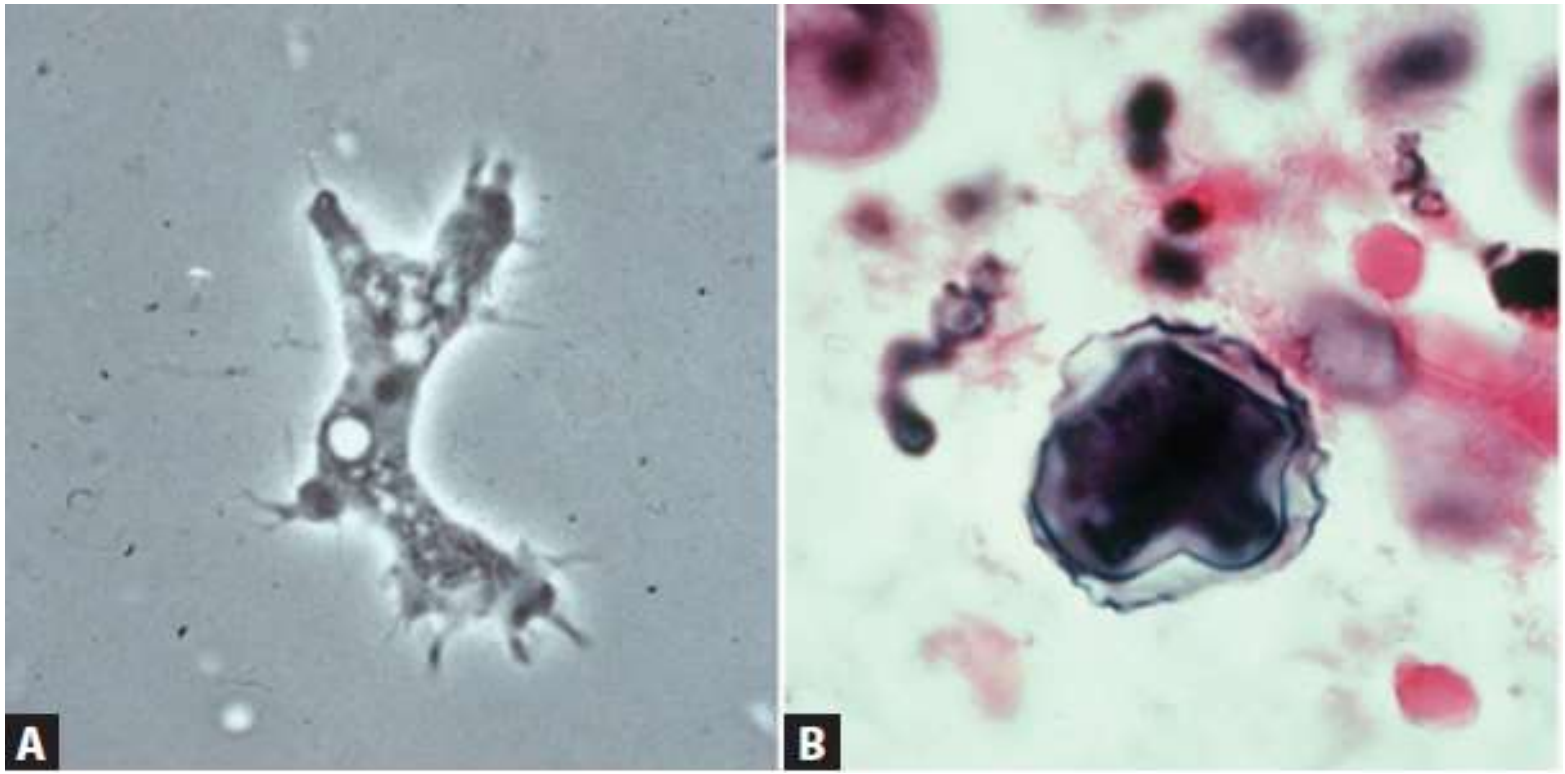
- **GAE**, focal granulomatous lesions in brain
- **Insidious onset**; several weeks – months
- **Chronic course**; months – yrs
- Clinical symptoms:
  - headache, fever, hemiparesis, lethargy
  - Stiff nick, aphasia, vomiting, nausea, visual disturbance
- Later stages:
  - loss of consciousness
  - palsy with numbness resulting in facial asymmetry
  - seizures, coma and death

GAE: Diagnosis

- MRI & CT scans of the brain
  - Multifocal, granulomatous, space-occupying lesions
- **CSF examination**
  - Pleocytosis with lymphocytic predominance
  - Increased protein concentration
  - Decreased glucose concentration
  - Acanthamoeba trophozoites and cysts can be detected in CSF



GAE: Diagnosis cont'd



Acanthamoeba species; (A) trophozoite in CSF saline mount, (B) cyst in brain tissue (hematoxylin and eosin stain)

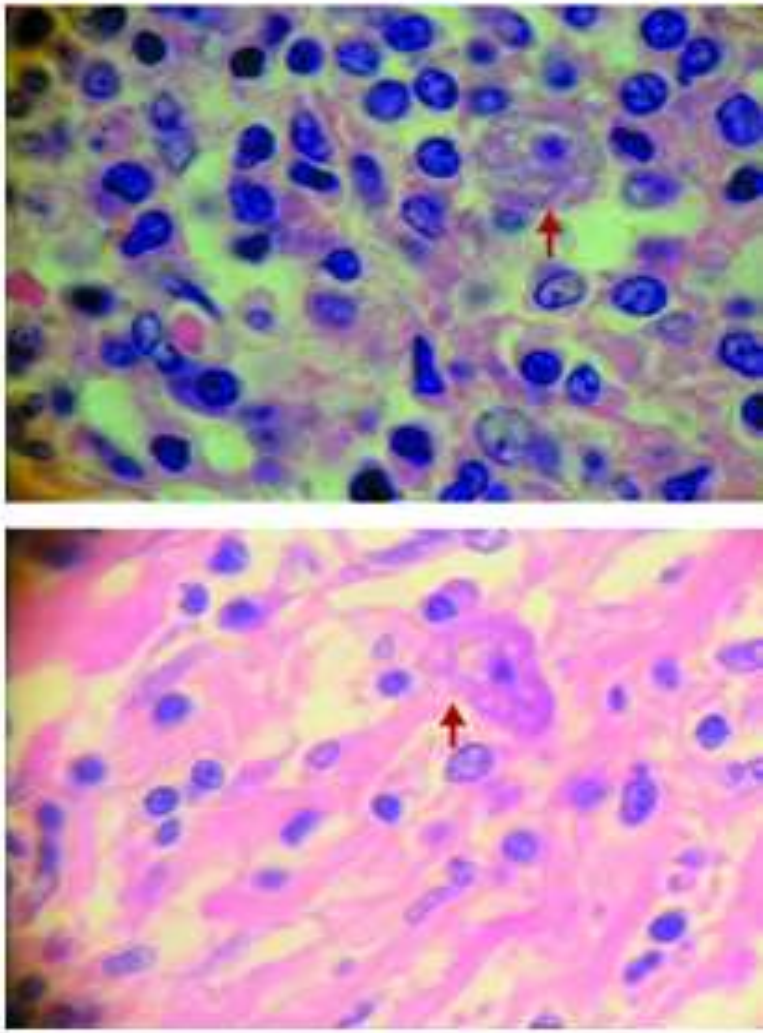
Cutaneous acanthamoebiasis

- is a rare opportunistic infection
- Mostly lesions occur on the face, trunk and extremities
- they are a route of entry into the blood stream & hematogenous spread to different tissues

Ulceronecrotic cutaneous lesions caused by *Acanthamoeba* spp in patients with HIV

Cutaneous acanthamoebiasis cont'd

(b)



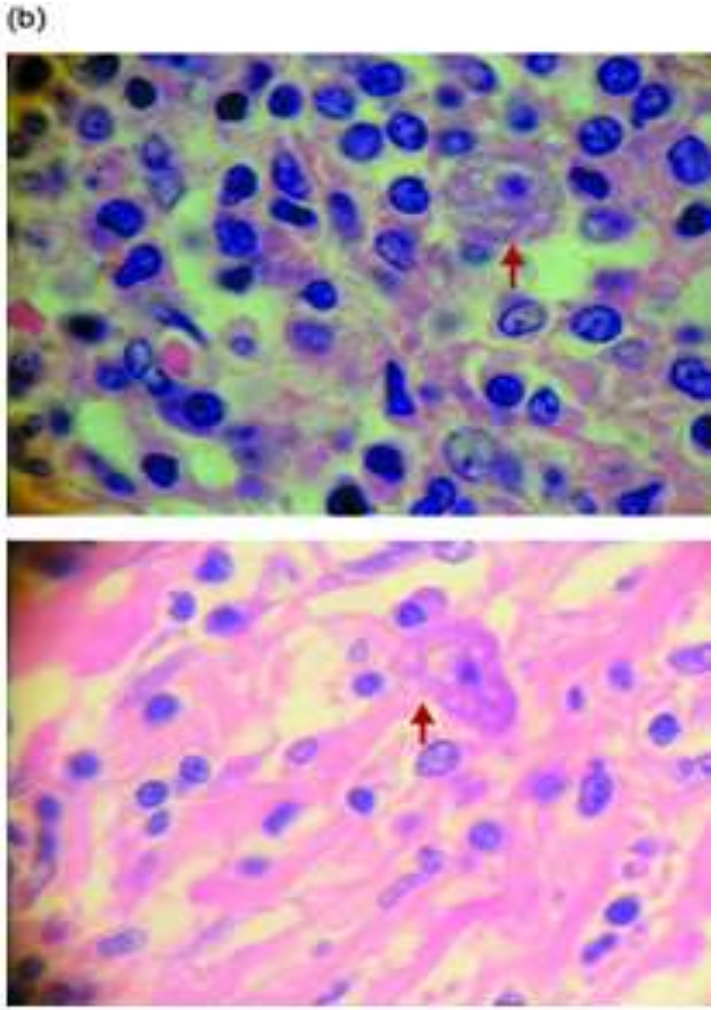
Histologic description:

- ulcerated epidermis, the dermis shows an intense inflammatory reaction with a predominance of histiocytes & neutrophils
- Presence of *Acanthamoeba* trophozoite & cyst

Cutaneous acanthamoebiasis cont'd

Diagnosis:

- **Biopsy-** visualizing numerous amoebic trophozoites and cysts in a necrotic ulceration with mixed inflammatory cell infiltrates



Acanthamoeba keratitis

- A vision threatening chronic inflammation of the cornea, rare but severe
- Corneal spread of *Acanthamoeba* occurs following:
  - trauma (onset is rapid)
  - contact lens use; especially present in the lens cleaning solution (onset is slow)
  - contaminated water (onset is slow)
- **Mechanism of adhesion:** MBP adheres to glycoprotein receptors on corneal epithelium

Acanthamoeba keratitis cont'd

- *Acanthamoeba* species causing AK:
- *A. culbertsoni*, *A. castelanii*, *A. polyphaga*, *A. hatchetti*, *A. griffin*, *A. rhysodes*, *A. lugdunensis* and *A. quina*
  - *A. culbertsoni, A. castellanii and A. polyphaga* show the strongest pathogenic potential to AK;
    - their adhesion ability to the corneal tissues
    - Extracellular protease production
    - Reproduction speed
    - and specific enzymatic activity

Acanthamoeba keratitis cont'd

- **Clinical manifestations**:
  - Redness, epiphora, lacrimation, ptosis
  - Conjunctival hyperaemia, foreign body sensation, pain and photophobia, blurred vision
  - Opacities can be early observed
- **Advanced stages**
  - stromal abnormalities; e.g. nummular infiltrates and radial keratoneuritis
  - ring infiltrate, central inflammation (causes increased vision loss)

Clinical manifestations cont'd

Clinical symptom	Time	Special properties
Chameleon-like epithelial changes ("dirty epithelium") (Fig. 1A)	Within the first 2 weeks (in 50% of the pts)	Grey epithelial opacities, pseudodendritiformic epitheliopathy, epithelial microerosions or microcysts
Multifocal stromal infiltrates (Fig. 2A)	Within the first 2 weeks	Mostly central and paracentral
Ring infiltrate/Wessely immune ring (Figs. 1B and 2A)	In the first month (in 20% of the Pts)	From polymorphonuclear leukocytes, antigen-antibody complex and complement; incidence increases with time

Clinical manifestations cont'd

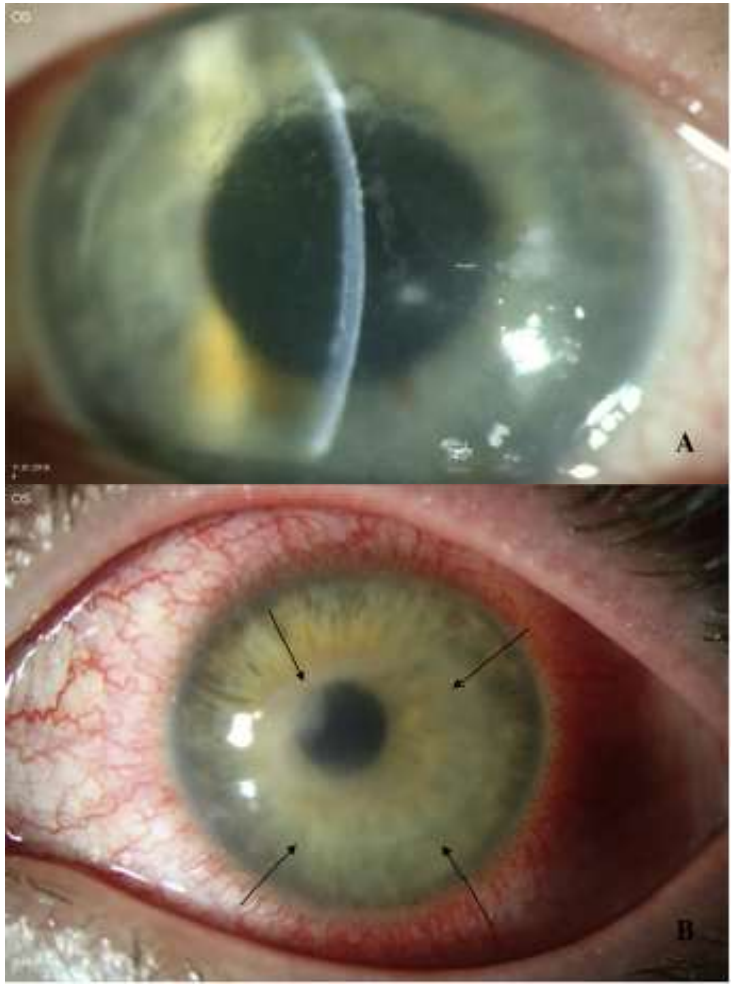


Fig. 1. "Dirty epithelium" (A), ring infiltrate (arrows) (B

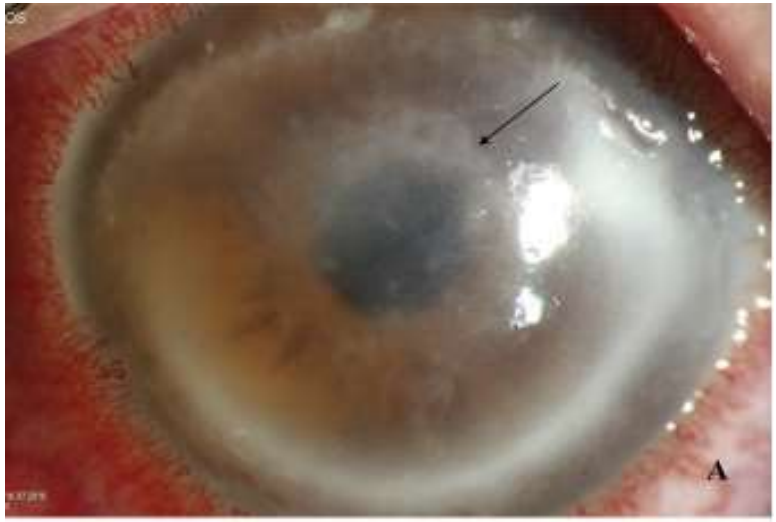


Fig. 2. Incomplete ring infiltrate (arrow) and multifocal stromal infiltrates (A)

Clinical manifestations cont'd

Peripheral perineurial infiltrate (Fig. 3)	In the first month (in 2.5-63% of the Pts)	Radial, from limbus to middle stroma, results in loss of corneal nerve fibers
Broad-based anterior synechiae, secondary glaucoma, iris atrophy, mature cataract (Fig. 4), persistent endothelial defect	Late symptoms, following months	Common complications
Sterile anterior uveitis, scleritis (Fig. 4)		Rare
Chorioretinitis and retinal vasculitis		Very rare complications

Clinical manifestations cont'd

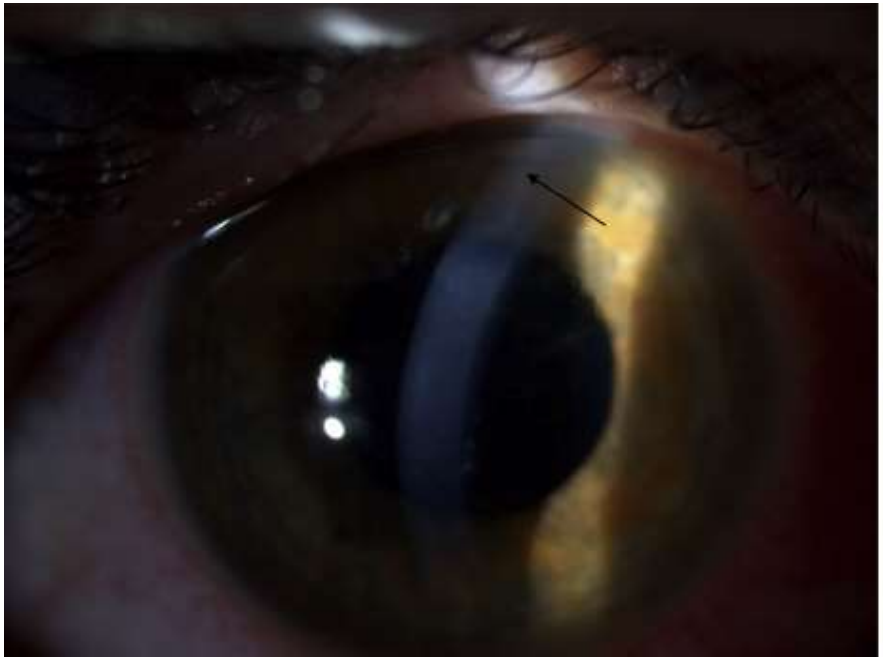


Fig. 3. Perineuritis (arrow), 4 weeks after first symptoms (contact lens wearer)

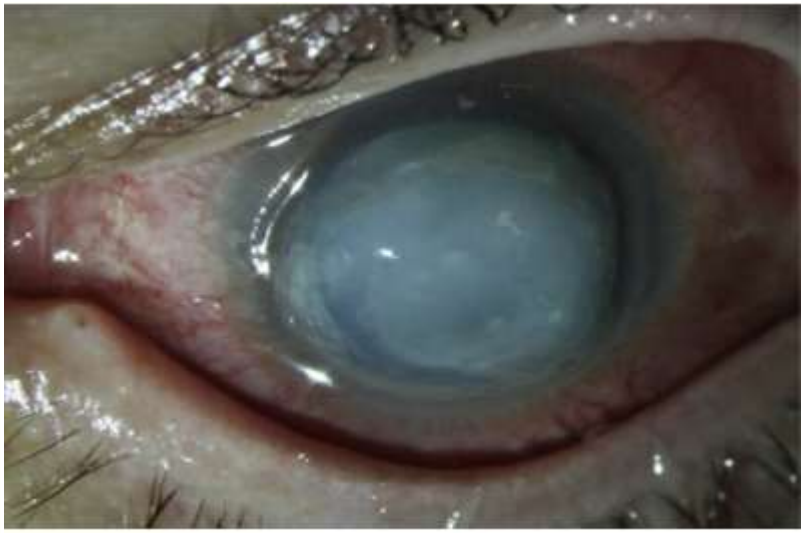


Fig. 4. Scleritis, corneal ulcer, iris atrophy, persistent mydriasis, and mature cataract in severe cases

Acanthamoeba keratitis cont'd

- **Risk factors:**
  - wearing contact lens for an extended period of time
  - Corneal trauma
  - non-sterile contact lens rinsing
  - biofilm formation on contact lens
  - swimming in contaminated water while wearing a contact lens

Laboratory diagnosis

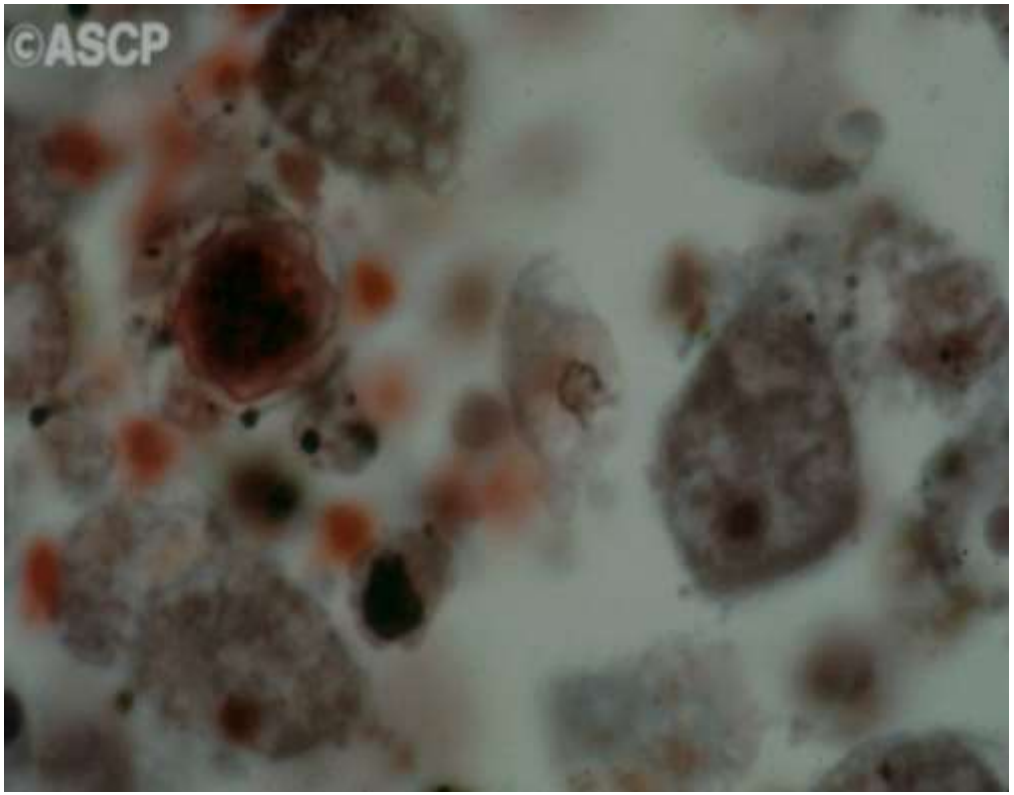
- AK is often misdiagnosed and treated as:
  - herpetic, bacterial, or mycotic keratitis, as many signs and symptoms may look similar
- It is challenging for an ophthalmologist to find the right diagnosis
- In about 23% of the cases, a mixed infection with virus, bacteria, or fungi is present

Laboratory diagnosis cont'd

- **Direct microscopy**
  - Finding of trophozoite and cyst in wet mount examination of corneal scrapping
- **Culture on non-nutrient agar**
  - Cultivating corneal scraping on non-nutritive agar cover with *E. coli*
  - Trophozoites appear first (slow and contractile movements) and cysts develop only after a few days, when the food is used up
- **Molecular methods –**PCR

Laboratory diagnosis cont’d

In histological examination, Naegleria & Acanthamoeba trophozoite are indistinguishable



Treatment

- **Granulomatous amoebic encephalitis**
  - Unfortunately, there are no therapies with proven efficacy against this disease
  - Only three cases survived so far
    - Treated with multidrug combinations: cotrimoxazole, ketoconazole, pentamidine, flucytosine and rifampin
- **Amoebic keratitis**
  - Topical antiseptic agents such as a biguanide or chlorhexidine are used
  - In severe cases of vision impairment may need penetrating keratoplasty

Prevention and control

- Avoid contact with sewage, proper sewage disposal
- Promoting education on wearing contact lenses
  - Avoid water as lens cleaner
  - Always wash hands before handling CLs
  - Use the sterile solutions recommended to clean and store CLs
- Treatment of infected individuals and health education

Balamuthia mandrillaris

- Formerly known as a **Leptomyxid amoeba**
- Free living, heterotrophic amoeba that causes BAE
- was first discovered, by Professor William Balamuth, in a pregnant mandrill (an old world monkey) at San Diego
- In USA, most commonly isolated from soil and dust sources

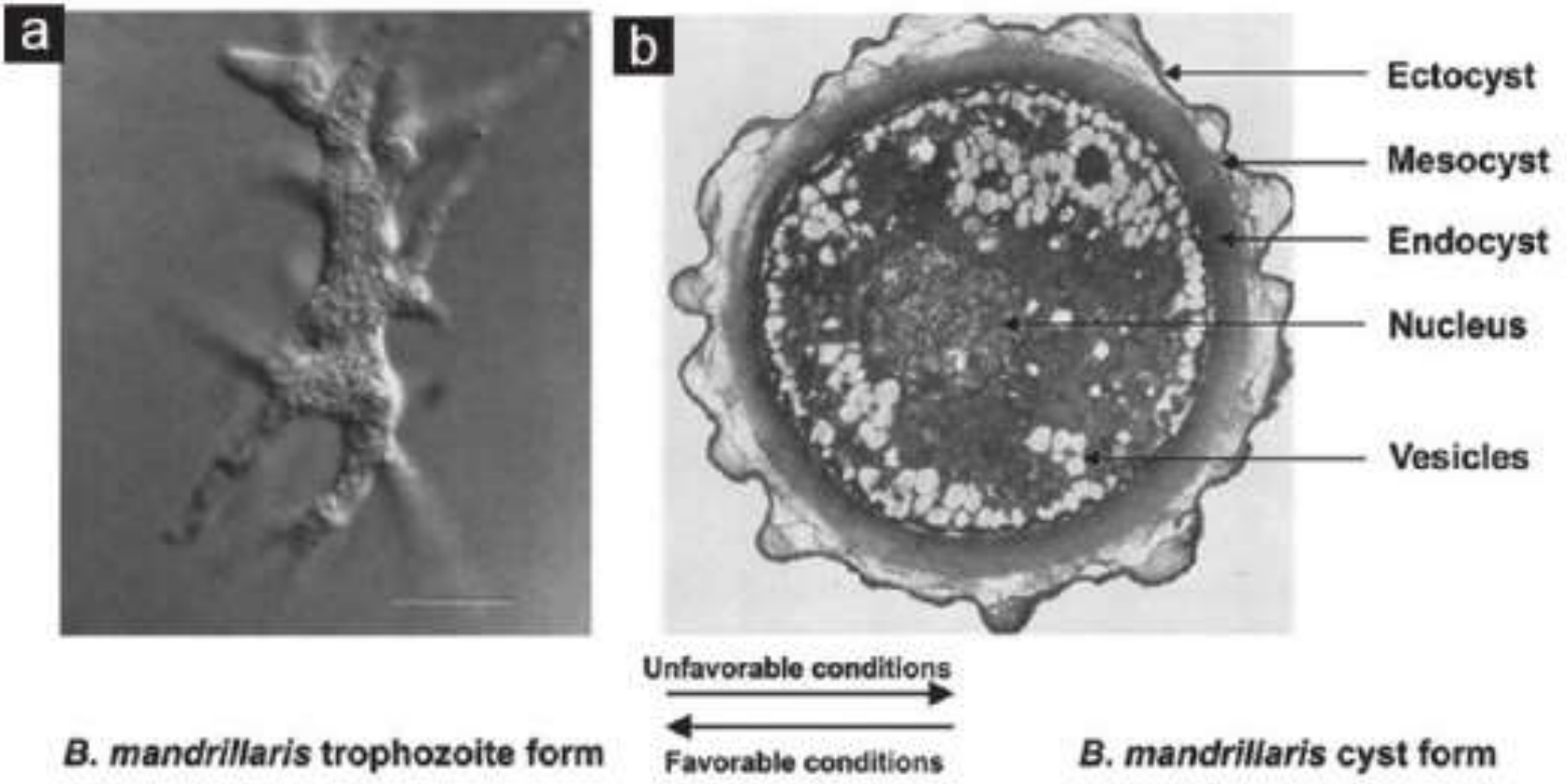
B. mandrillaris: epidemiology

- temperate regions of the world
- >200 cases are reported, half of them being from USA (North America & Latin America)
- few cases have also been reported in Asia, Australia, and Europe (UK, Portugal, and the Czech Republic)
- number of reported BAE cases is increasing worldwide
- a major concern because:
  - little is known about the pathogen
  - no standardized detection tools are available, and
  - most of the treatments are almost empirical

B. mandrillaris: morphology

- **Trophozoite**
  - Pleomorphic (12-60 µm)
  - Usually uninucleate, occasionally bi/tri nucleated (infected tissues)
  - irregular finger-like pseudopodia
- **Cyst**
  - Spherical & 10-30 µm
  - Three layered cyst wall
    - (ecto, meso, endo cyst)

B. mandrillaris: morphology cont'd



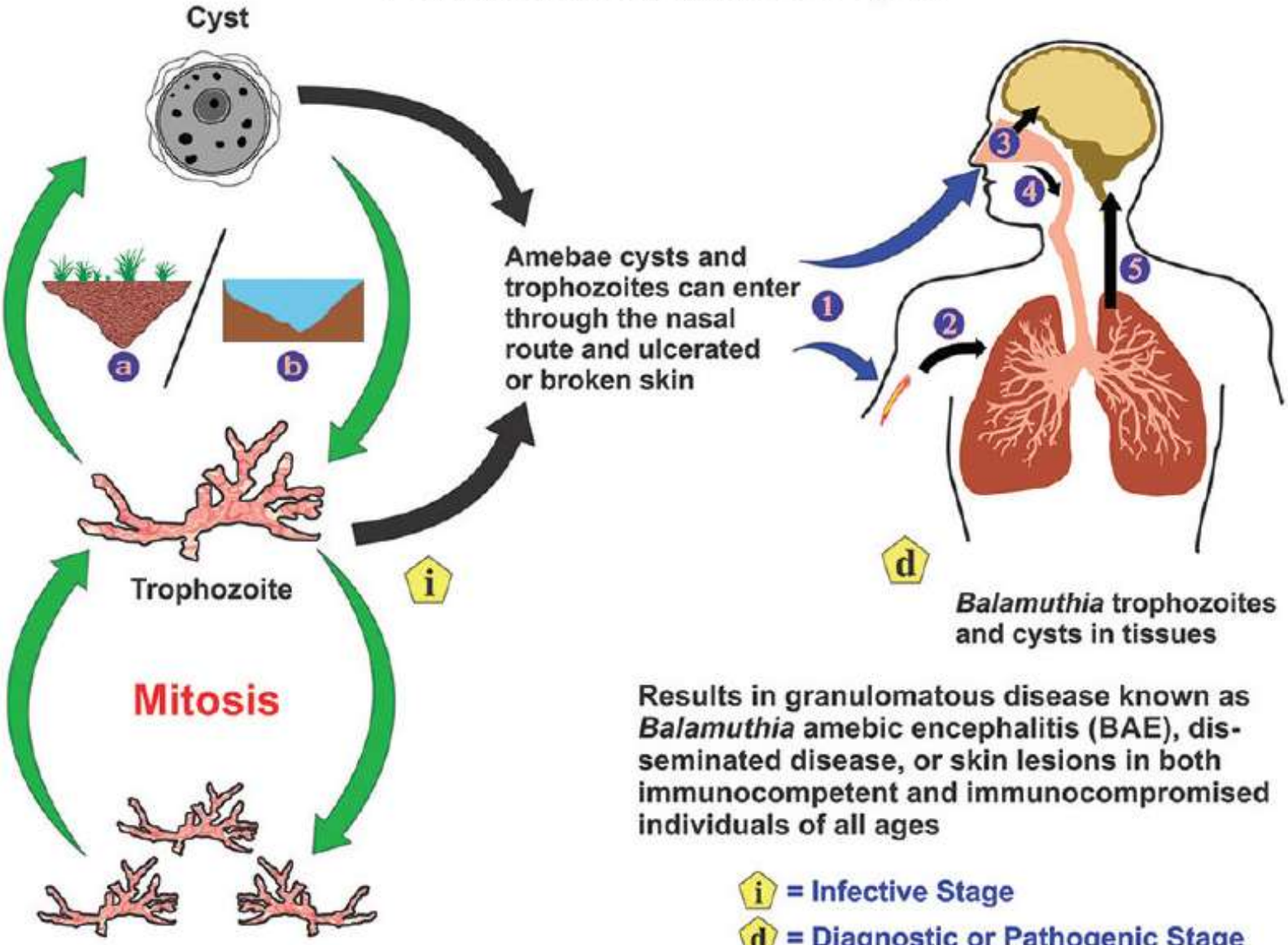
Transmission and life cycle

Transmission:

- Human gets infected by:
  - Inhalation of cysts or trophozoites or
  - Entry of cysts or trophozoite through a lesion in the skin
  - subsequently invade the bloodstream and spread hematogenously to the brain or through olfactory nerve structures

Life cycle: simple

Balamuthia mandrillaris life cycle



Results in granulomatous disease known as *Balamuthia* amebic encephalitis (BAE), disseminated disease, or skin lesions in both immunocompetent and immunocompromised individuals of all ages

i = Infective Stage

d = Diagnostic or Pathogenic Stage

Pathogenesis and clinical features

- Produces necrotizing hemorrhagic encephalitis

Mechanisms of pathogenesis:

- Contact-dependent mechanisms
  - adhesion and phagocytosis
- Contact-independent mechanisms
  - Protease & phospholipase

Clinical features

- Clinical symptoms are similar to viral or bacterial meningitis and include headache, stiff neck, fever, and photophobia
- As BAE progresses, other signs: nausea, vomiting, personality changes, aphasia, acute confusion, seizures, and lethargy
  - all are related to increased intracranial pressure
  - Lethargy progresses to coma and finally death

Laboratory Diagnosis

- Diagnosis of BAE is challenging and usually postmortem, because symptoms of the infection may be subtle or non-specific
- **Microscopy**: detection of amoebae in the skin and brain tissue, either by immunofluorescence staining or immunoperoxidase staining, both use polyclonal rabbit anti-Balamuthia serum in paraffin-fixed tissue
- **Imaging methods: MRI & CT scan**
- **PCR** also gives reliable diagnosis

Treatment, & prevention and control

Treatment: is not standardized;

- (i) non-specific clinical manifestations
- (ii) the lack of available diagnostic tools
- (iii) lack of in vitro (i.e., chemotherapy and colorimetric assays) and in vivo models for the evaluation of potential drugs

Prevention & control:

- Currently, no specific prevention & controls
  - not known why some individuals are affected while others are not

Take-home message

Free-Living Pathogenic Amoebae:

- *Naegleria fowleri & Acanthamoeba* spp
  - has Worldwide distribution
  - *N. fowleri* has cysts, trophozoites & flagellated forms
  - *Acanthamoeba* has only cysts and trophozoites
  - *Naegleria fowleri*: causes Primary Amoebic Meningoencephalitis (PAM)
  - *Acanthameoba* spp. causes amoebic keratitis or chronic granulomatous meningoencephalitis

Take-home message cont'd

Character	Naegleria	Acanthamoeba
Disease	PAM	AK, GAE, etc.
Clinical course	Acute	Sub-acute to chronic
Pathology	Diffuse suppurative changes	Focal granulomatous inflammation
Trophozoite	• Two form (amoeboid & flagellated) • broad pseudopods, lobopodia • Actively motile • 8–15 µm	• One form, no flagellated form • filamentous pseudopods, acanthopodia • sluggishly motile • 15–25 µm

Take-home message cont'd

Cyst	• Not present in tissue or CSF • Small (7–15 μm),	• Can be found in tissue or CSF • Larger (12–20 μm)
Spread to CNS	Direct neural spread	Hematogenous spread
CSF Leukocytes	Neutrophil	Lymphocyte
Culture	• Require bacterial supplement • Don't grow with >0.4% NaCl	• May grow without bacterial supplement • Not affected by NaCl

Review questions

- Describe the taxonomy and classification of free-living amoeba?
- List common *Acanthamoeba* species that affects human?
- Differentiate *N. fowleri* from *Acanthamoeba* species?