Protozoa
Free-Living Amoeba

- *Naegleria fowleri*, *Acanthamoeba spp*, *and* Balamuthia mandrillaris

Free-Living Amebic Infections

- Opportunistic parasites of humans
- Thermophilic: found in warm fresh water, moist soil or decaying vegetation; swimming -> enters nose and migrates to the brain
- Three notable potential pathogens :
  - Naegleria fowleri
  - Acanthamoeba spp
  - Balamuthia mandrillaris

Free-Living Amebic Infections

- Naegleria fowleri
  - Acute Primary Amebic Meningoencephalits (PAM) -- RARE
  - Usually in children and young adults WITHOUT predisposing conditions
  - WHY?
    - Swimming in warm stagnant lakes, brackish or muddy water

Naegleria fowleri

- Organism is highly thermophilic, survives water temp up to 45℃
- Less likely in room temperature or colder water
- Tolerates chlorinated water
  - infections have been acquired by drinking unfiltered chlorinated water
- Amebic encephalitis should be added to differential diagnosis if spinal fluid is purulent
  - neutrophil 200 to 20,000 cells/µl with no bacterial in culture workup
- Brain biopsy at autopsy show inflammatory lesions with segmented neutrophils, eosinophils and trophozoites
  - Motile trophozoites may be seen in unstained samples

Naegleria fowleri

- Cysts are not seen in clinical or biopsy specimens but are 10 µm and have round smooth double wall
- A drop of unrefrigerated CSF should be examined for motility since the organism resembles WBC when observed on a counting chamber
- Warming the slide will promote motility

Free-Living Amebic Infections

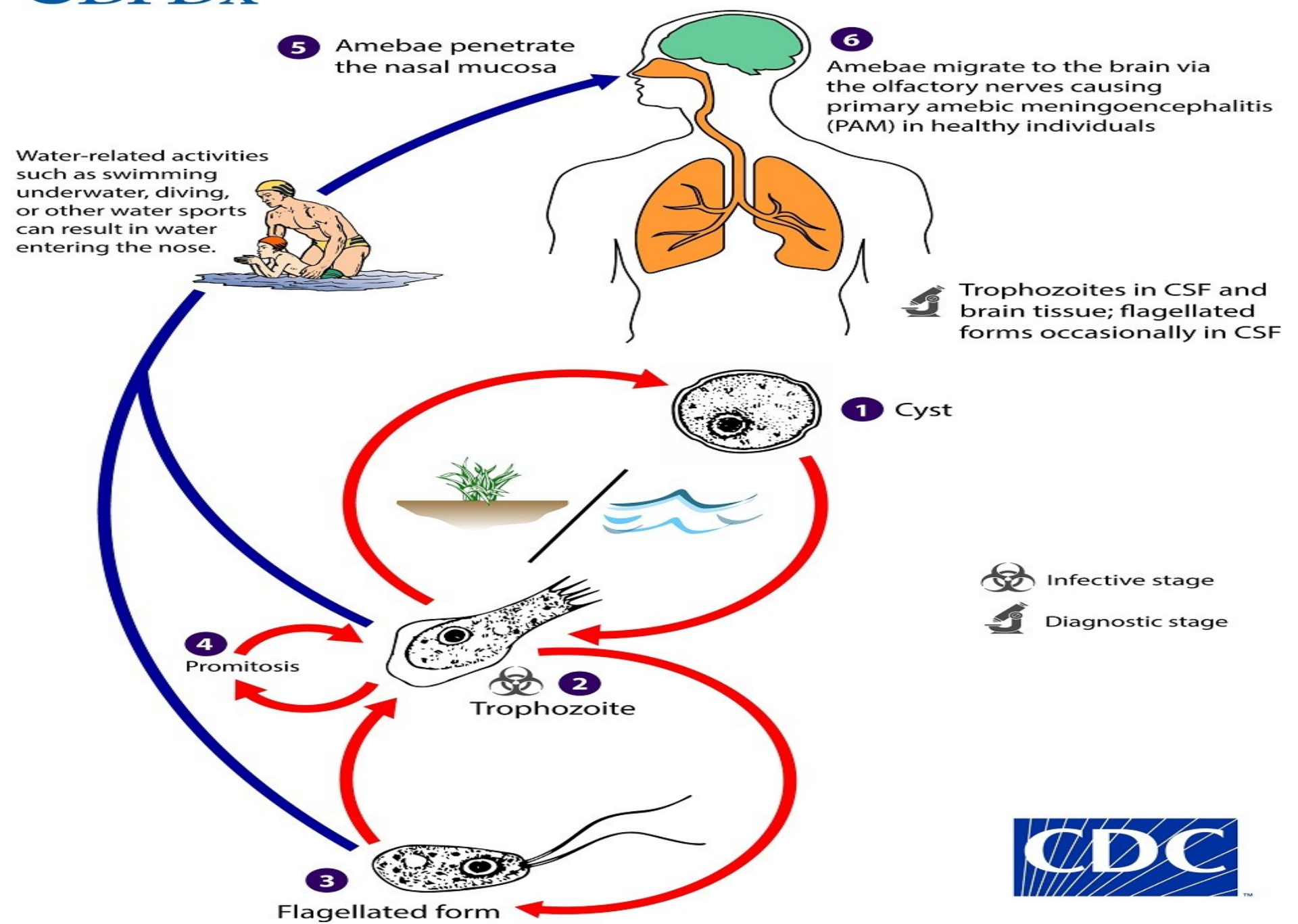
- Naegleria fowleri - Pathogenesis
  - PAM is characterized by severe CNS dysfunction with rapid degeneration caused by hemorrhagic-necrotizing meningoencephalitis
  - Onset of PAM symptoms occurs quickly following infection (1-9 days; median 5 days after swimming or other nasal exposure to *Naegleria*-containing water)
  - Symptoms are like bacterial meningitis, for which it is often mistaken, with deteriorating neurological function and complications
  - The case fatality rate is extremely high
  - Rare possibility of cure depends upon early diagnosis and aggressive therapy w/ Amphotericin B + fluconazole + azithromycin

Naegleria fowleri Life Cycle

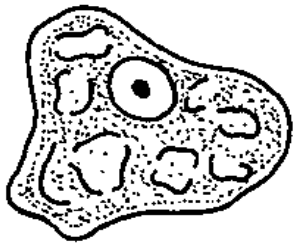
- *Naegleria fowleri* has three stages in its life cycle: cysts, trophozoites , and flagellated forms
- *N. fowleri* is found in fresh water, soil, thermal discharges of power plants, geothermal wells, and poorly-chlorinated recreational and tap water
  - Trophozoites can reversibly & temporary non-feeding flagellated forms during stressful events
  - *Naegleria fowleri* has three stages in its life cycle: cysts, trophozoites , and flagellated forms
- Trophozoites infect humans or animals by penetrating the nasal mucosa, usually during swimming or sinus irrigation , and migrating to the brain via the olfactory nerves
  - Primary amebic meningoencephalitis (PAM)
- Trophozoites are found in cerebrospinal fluid (CSF) and tissue, flagellated forms are occasionally seen in CSF, cysts are not seen in brain tissue.

DPDx

Naegleria fowleri



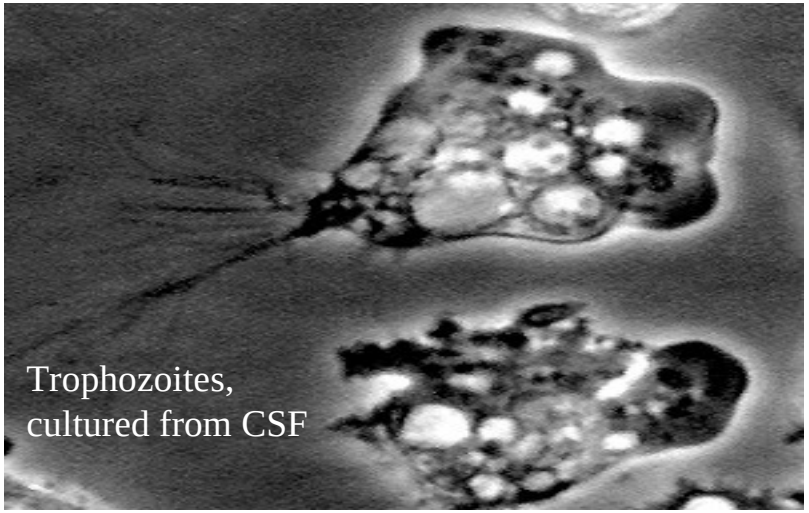
CDC

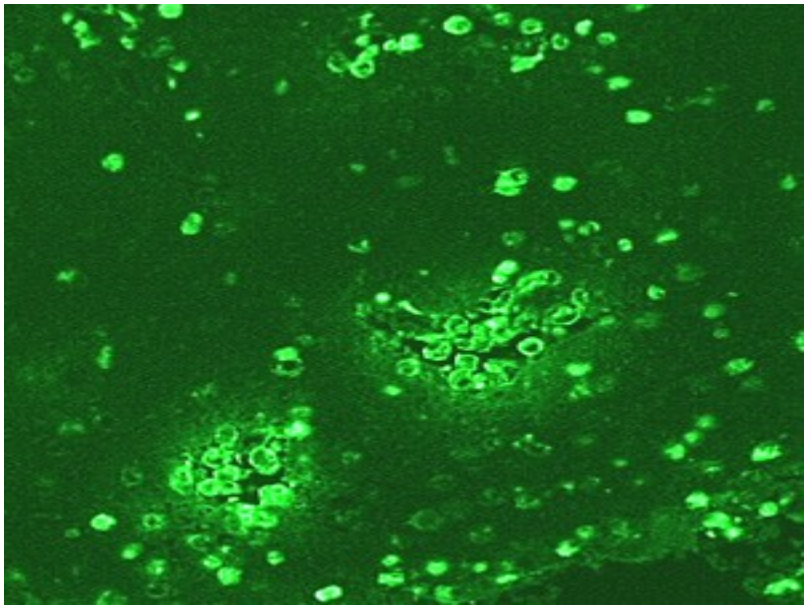


Naegleria fowleri

- Trophozoite: ranges from 7 to 20 µm
  - large central karyosome and a granular vacuolated cytoplasm
  - motile trophozoites are seen in CSF w/ many WBC and no bacteria seen
- Isolation method
  - Overlay non-nutrient agar w/ E. coli (bacteria)
  - Inoculate w/ a drop of CSF sediment from patient
    - Examine for clearing of agar, indicating that trophozoites are feeding on bacteria!
- Trophozoite can be converted to flagellate stage
  - How? Adding a drop of CSF sediment to 1 ml of distilled water and incubate at 37° for 2 – 20 hours
- PCR + molecular testing
- IFA

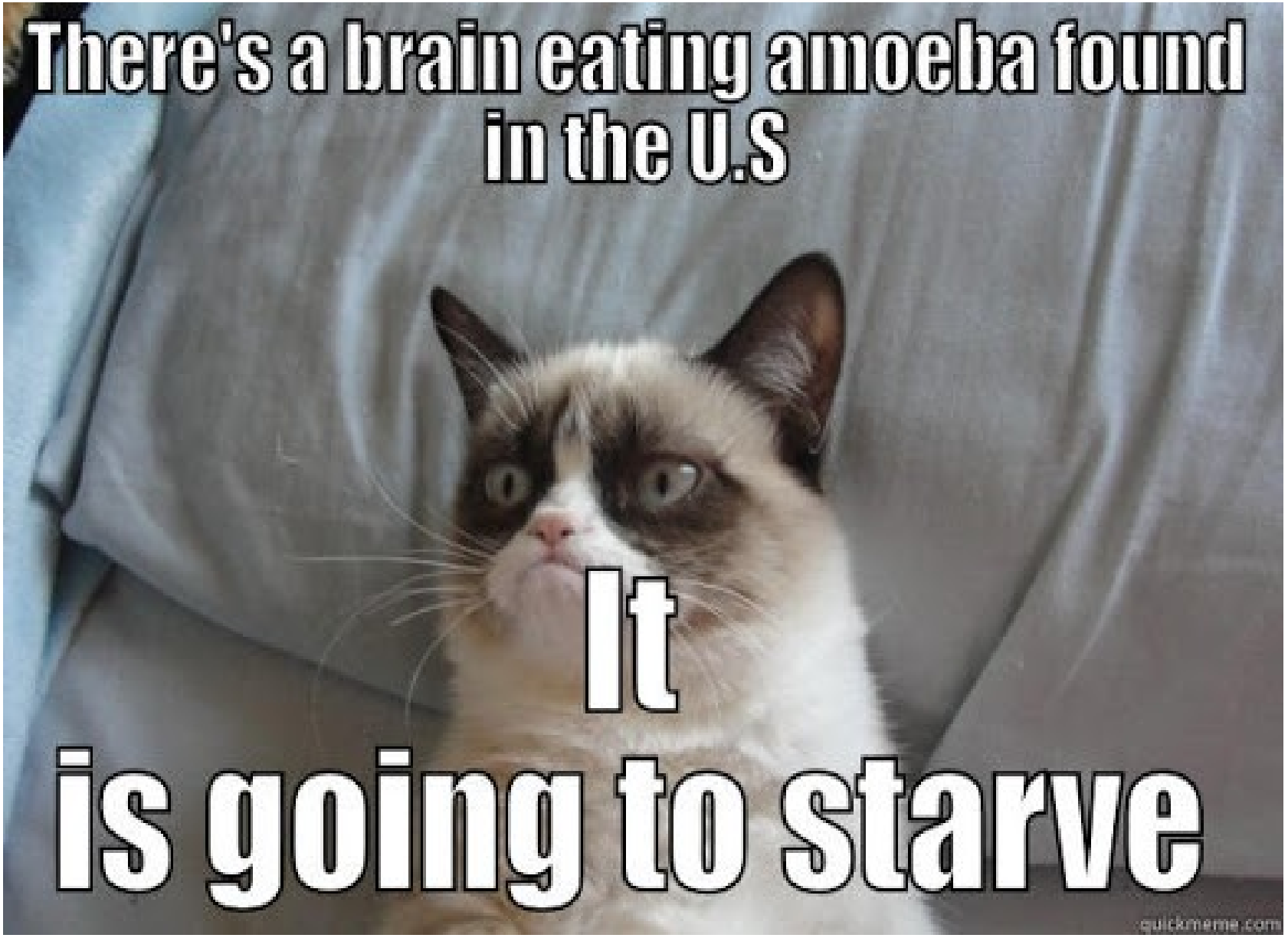
Naegleria fowleri





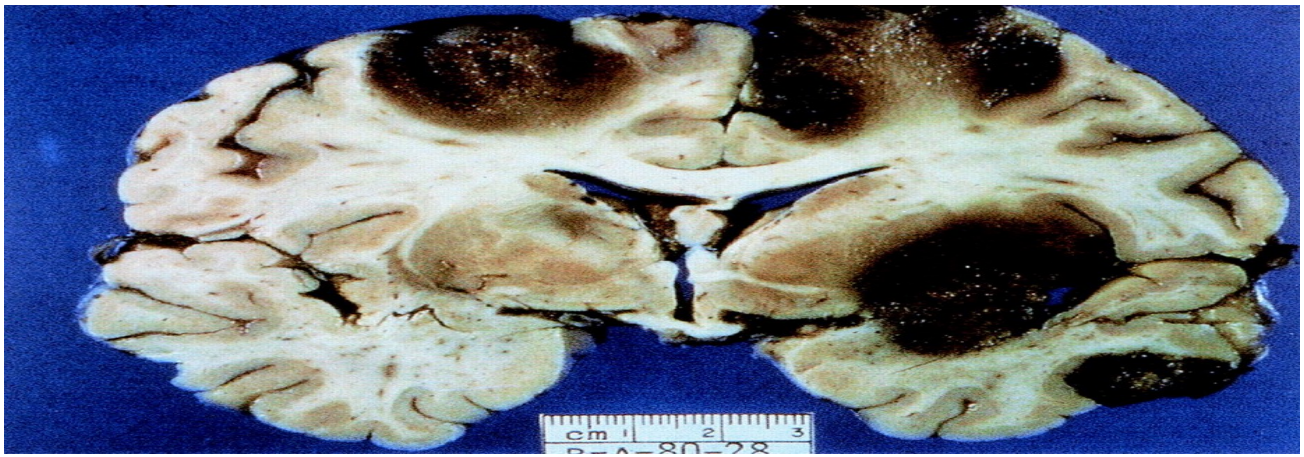
brain biopsy IFA

brain biopsy


There's a brain eating amoeba found in the U.S
It
is going to starve
quickmeme.com

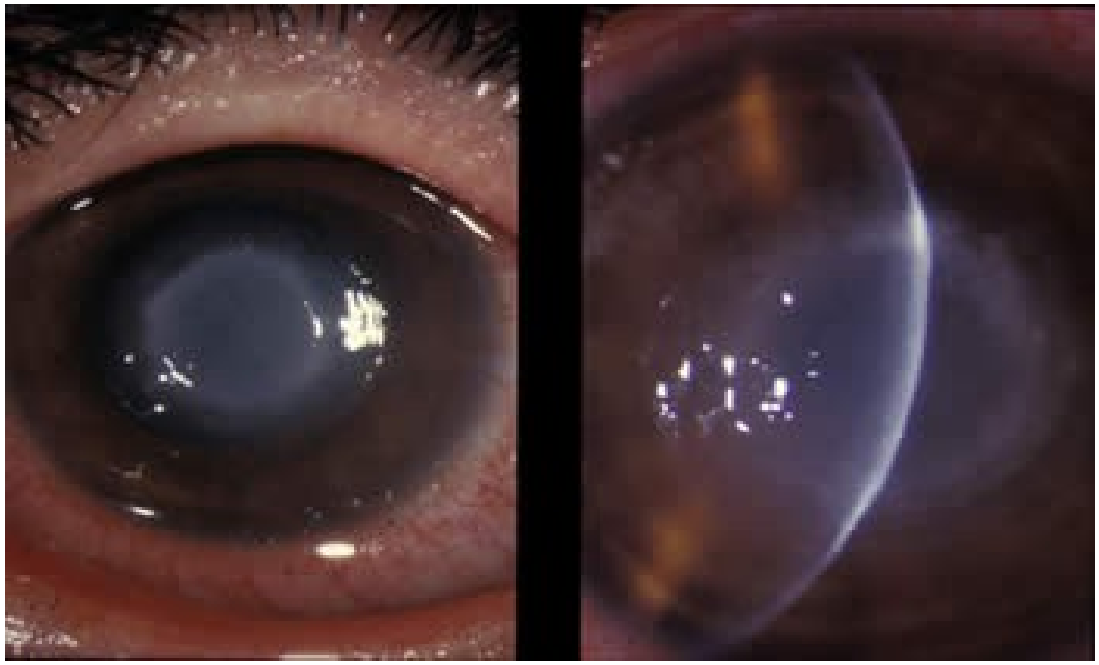
Acanthamoeba keratitis and other spp.

- Subacute or Chronic Granulomatous Amebic Encephalitis (GAE)
  - at least 6 species known to cause GAE
  - infected patients often have compromised immunologic systems
  - incubation period is unknown (range from months to years)
  - infections occur either through inhalation of contaminated dust or aerosols or through invasion of broken skin or mucous membrane followed by hematogenous spread to CNS
  - symptoms are headache, drowsiness, seizures, stiff neck, hemiparesis and personality disorders
  - no therapeutic agents established since diagnosis are done at autopsy



Acanthamoeba Keratitis and other spp.

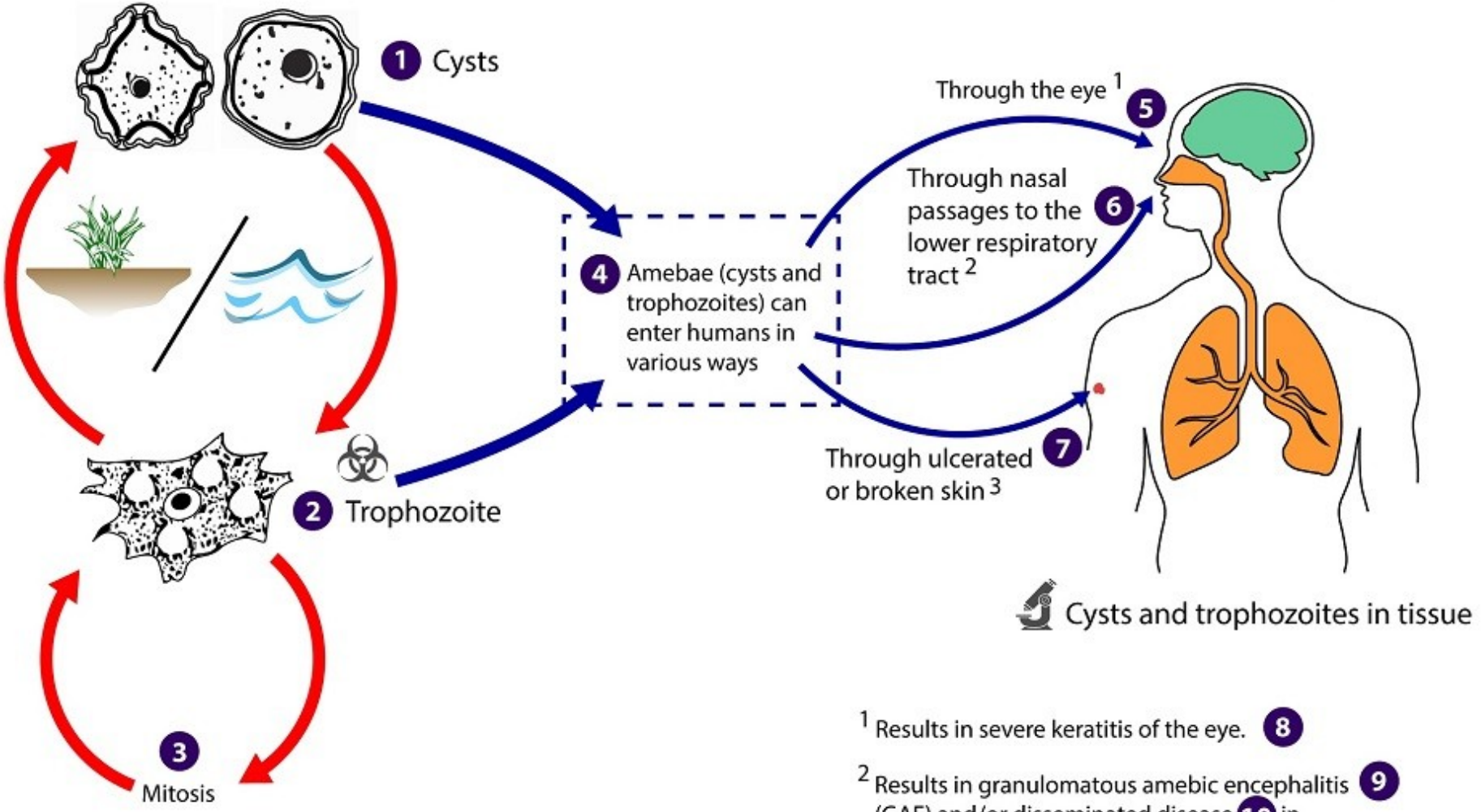
- Amebic keratitis
  - group at risk are contact lens users
  - use of homemade saline solutions
  - history of corneal trauma
  - patients experience photophobia, blurred vision , inflammation and pain



Acanthamoeba Keratitis and other spp. Life Cycle

- *Acanthamoeba* spp. are ubiquitous in the environment:
  - soil; water (potable, fresh, brackish, water); vegetables; sewage; swimming pools; contact lens supplies; dental treatment units; dialysis machines; HVAC units
- *Acanthamoeba* has two stages; cysts and trophozoites with no flagellate stage
- The trophozoites are the infective forms, although both cysts and trophozoites can enter the body through the eye, the nasal passages, or ulcerated or broken skin
  - Enters the eye, it can cause severe keratitis in otherwise healthy individuals, particularly contact lens users
  - Enters the respiratory system or the skin, it can invade the CNS by hematogenous dissemination causing granulomatous amebic encephalitis (GAE)
- Cysts and trophozoites are found in tissue

Acanthamoeba spp.



[1] Results in severe keratitis of the eye. 8

[2] Results in granulomatous amebic encephalitis (GAE) and/or disseminated disease 10 in individuals with compromised immune systems. 9

[3] Results granulomatous amebic encephalitis (GAE), disseminated disease 10 or skin lesions 11 in individuals with compromised immune systems. 9

Acanthamoeba spp

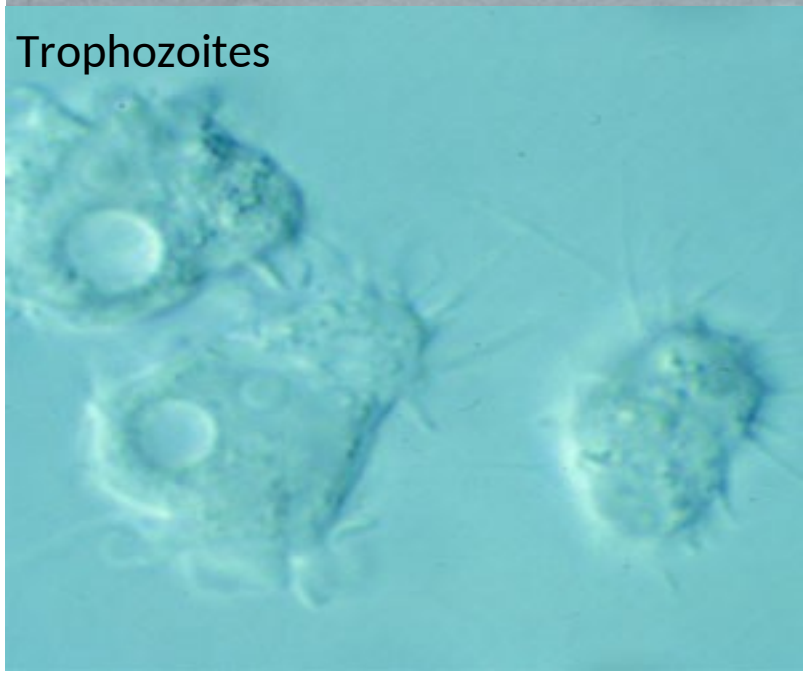
- Soil and water organisms
- Cyst: double-walled wrinkled appearance in tissues
  - size: 15 to 20 µm
  - Number of nuclei: 1
- Trophozoite
  - size : 10 to 45 µm
  - Number of nuclei: 1
  - blunt pseudopods and spinelike projections may be seen in wet preparations
- Nuclear appearance of both cyst and trophozoite:
  - distinct nucleus with a central prominent endosome, no chromatin

Acanthamoeba spp

Cyst

Trophozoites

Trophozoites



Diagnosis of Acanthamoeba spp

- Trophozoite is rarely seen in CSF
- Both cysts and trophozoites are seen in brain biopsy
- Histologic preparations of brain at autopsy show:
  - inflammatory lesions w/ neutrophils, eosinophils and trophozoites
- Amebic keratitis
  - Tissue damage is like that of HSV infection
  - Direct wet mount preparations of corneal scrapings
    - may show cysts
  - Trophozoites may be demonstrated by permanent stains (trichrome or Giemsa)
  - Isolation is done using corneal scraping to inoculate a lawn of E. coli in a non-nutrient agar
    - Look for clearing

Balamuthia mandrillaris

- The cysts of *Balamuthia mandrillaris* are highly like *Acanthamoeba* spp
  - typically, 10—25 µm (mean 15 µm) in diameter
- Generally, these cannot be reliably distinguished from *Acanthamoeba* spp. without either molecular confirmation or electron microscopy
- The cysts have two walls apparent in light microscopy, and do not have pores:
  - a wrinkled fibrous outer wall (exocyst)
  - an inner wall (endocyst) that may be variable in shape
- Cysts contain only one nucleus with a large karyosome
- Cysts may be found in the brain, skin, lungs and other organs

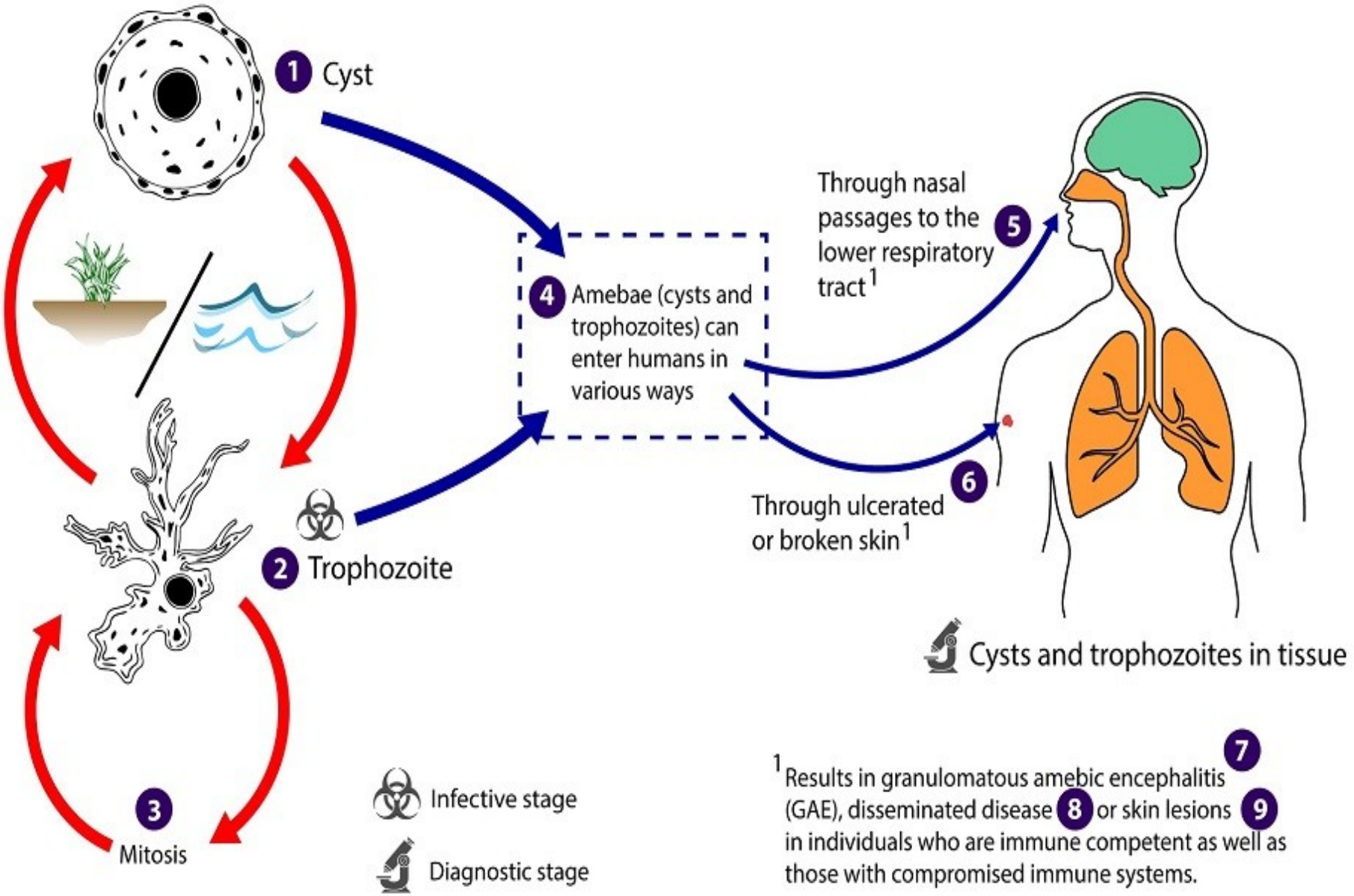
Balamuthia mandrillaris

- *B. mandrillaris* involves a similar course to *Acanthamoeba*-associated GAE
- The disease might appear mild at first but can become more severe over weeks to several months
- Often the disease is fatal with a case fatality rate of more than 89%
- Plaque-like skin lesions, most often on the face, particularly in the cheek or nose area
  - Sometimes on the torso or limbs may precede the development of neurological symptoms by weeks or months

Balamuthia mandrillaris Life Cycle

- *Balamuthia mandrillaris* has been isolated from soil and dust, and from autopsy specimens of infected humans and animals
- *B. mandrillaris* has two stages:
  - cysts and trophozoites without a flagellate stage
- The trophozoites are the infective forms, although both cysts and trophozoites enter the body through the nasal passages to the lower respiratory tract or ulcerated or broken skin
- Respiratory system or through the skin, it can invade the central nervous system by hematogenous dissemination causing granulomatous amebic encephalitis (GAE)
- *B. mandrillaris* cysts and trophozoites are found in tissue; a few cases have been associated with solid organ transplantation from an infected donor.

Balamuthia mandrillaris



Balamuthia mandrillaris