

Free-living Amebae as agents of CNS infections in humans.

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Three genera of Free-Living Amebae are associated with fatal CNS infections.

***Naegleria fowleri* – causative agent of PAM.
Primary Amebic Meningoencephalitis**

***Acanthamoeba spp.* – causative agent of GAE
(Granulomatous amebic encephalitis), amebic
keratitis, & cutaneous acanthamoebiasis.**

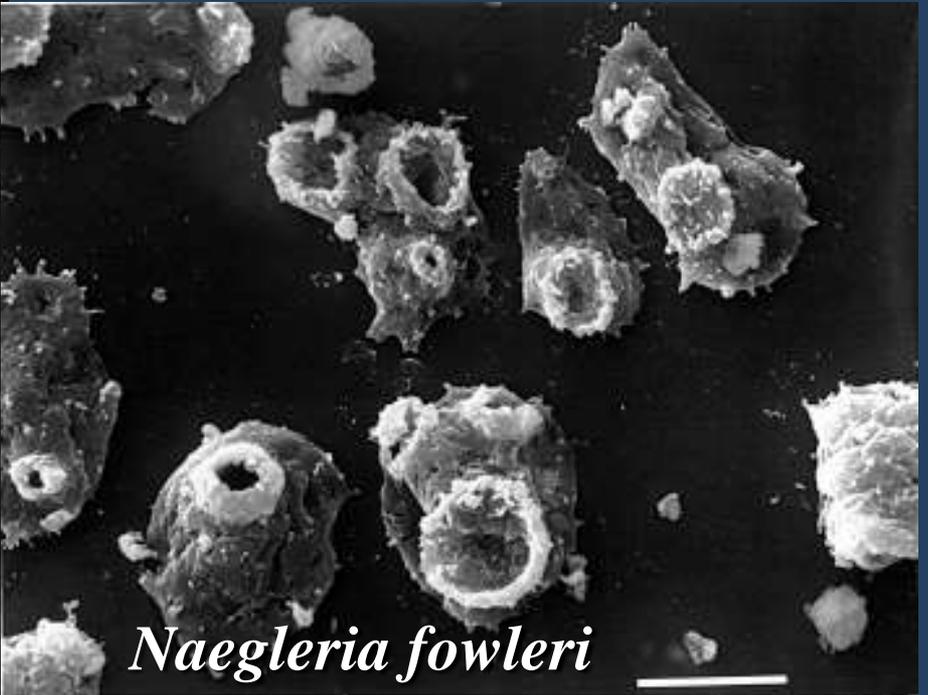
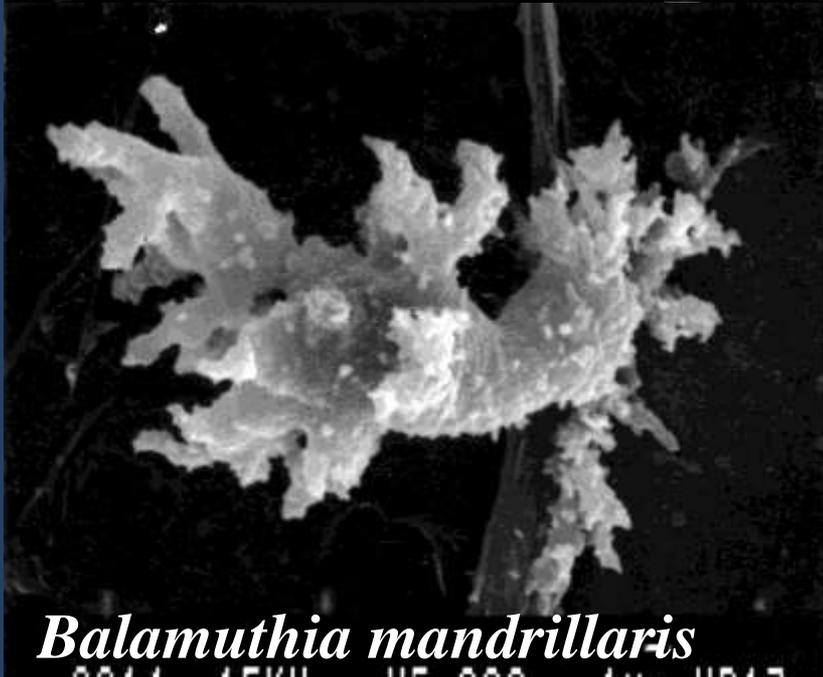
***Balamuthia mandrillaris* – causative agent of
BAE (Balamuthia amebic encephalitis).**

Free-living organisms

Free-living organisms can exist in the environment. Do not need to infect a host to complete their life cycle.

Parasitic organisms need a host to complete their life cycle. Parasite dependent on host to survive.

**Three genera of
Free-Living
Amoebae
associated with
fatal infections.**



The genus *Naegleria*

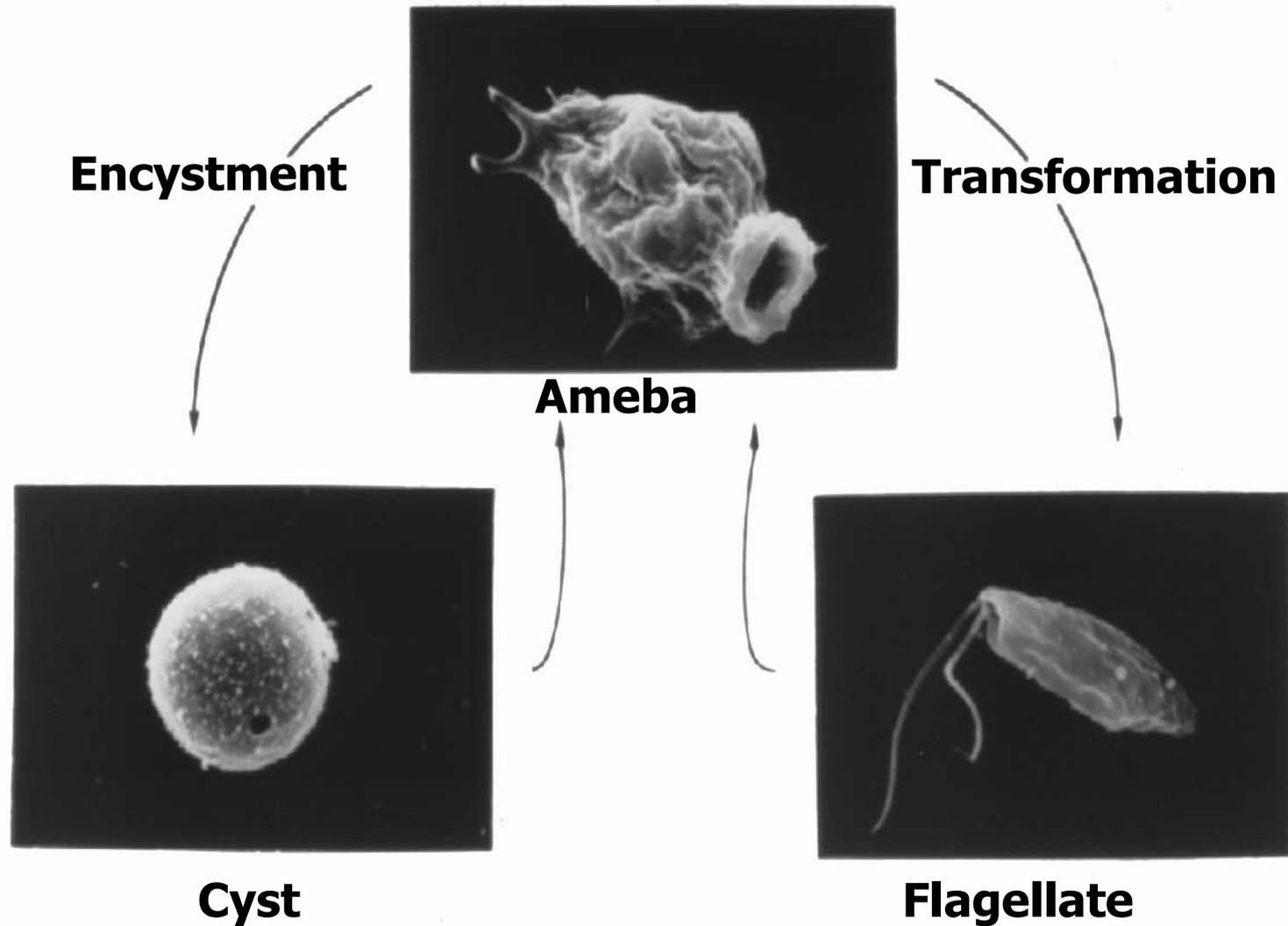
Free-Living Ameboflagellates

Of more than 40 species of *Naegleria* only *N. fowleri* associated with human infection.

Causative agent of Primary Amoebic Meningoencephalitis.



LIFE CYCLE OF *NAEGLERIA* spp.



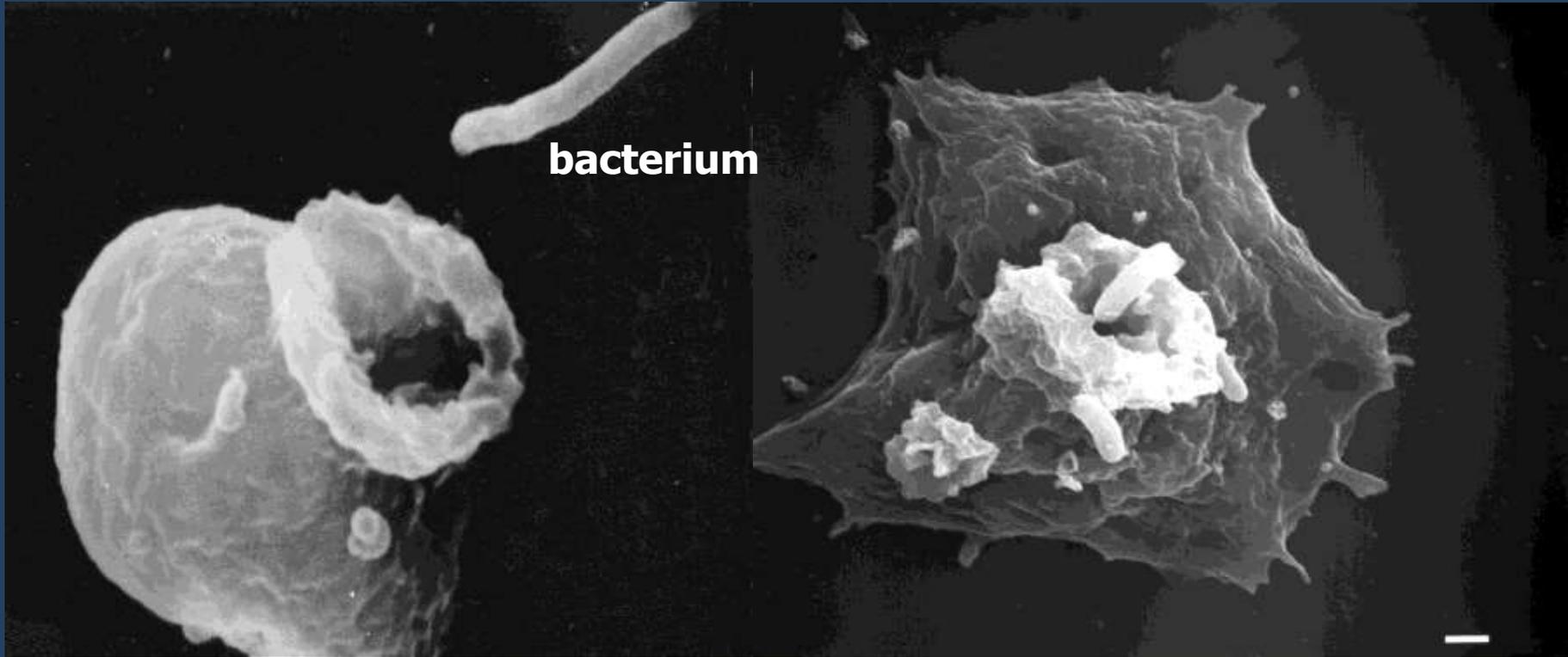
Ecology of *N. fowleri*

Isolation of *Naegleria spp.* has been reported from every continent.

Naegleria spp. have been isolated from:

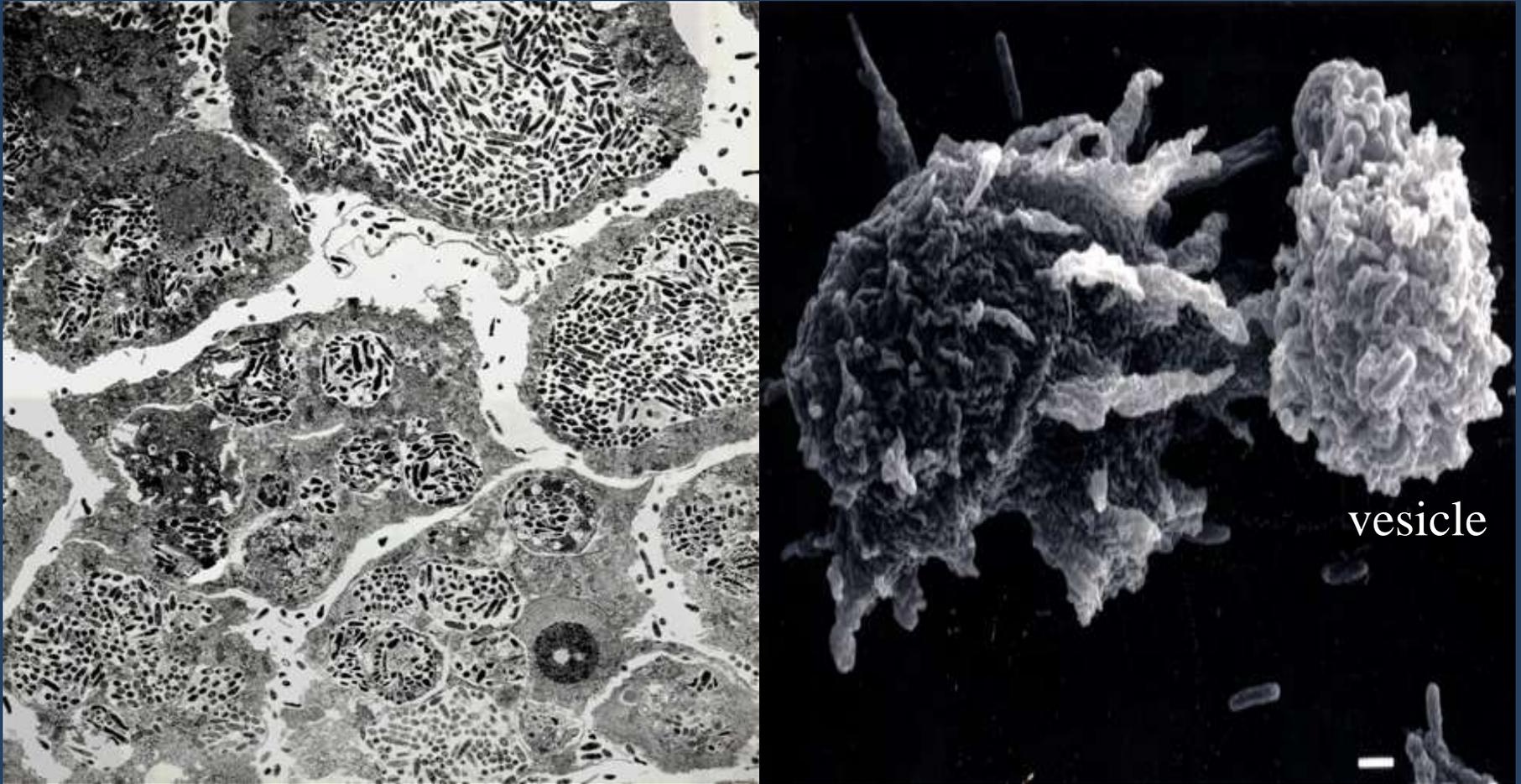
- air
 - sewage
 - chlorinated swimming pools
 - domestic waters supplies
 - humidifier systems
 - bottled drinking water
 - thermal springs
 - dialysis units
 - heated effluents from power plants, cooling towers and other industrial facilities
 - lakes
 - ponds
 - soils
- The majority of isolates come from artificially heated water systems

In the Environment Free-Living Amebae Feed on Bacteria and Yeast.



Bacteria are a food source, endosymbionts, or pathogens of amoebae.

Amebae serve as reservoirs of pathogenic bacteria

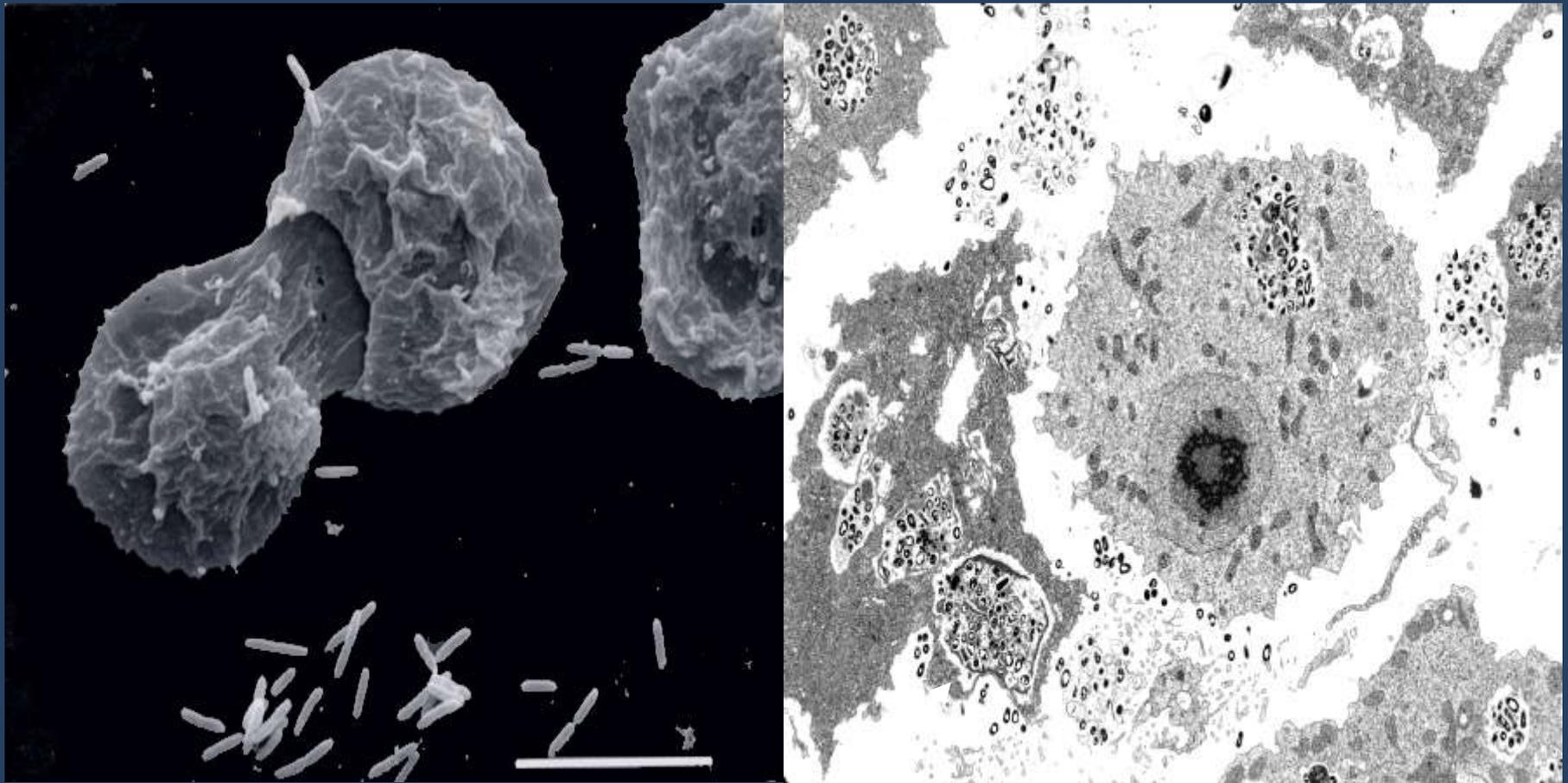


Amebae cocultured with *Legionella pneumophila*.

Dispersion of bacteria into the environment.

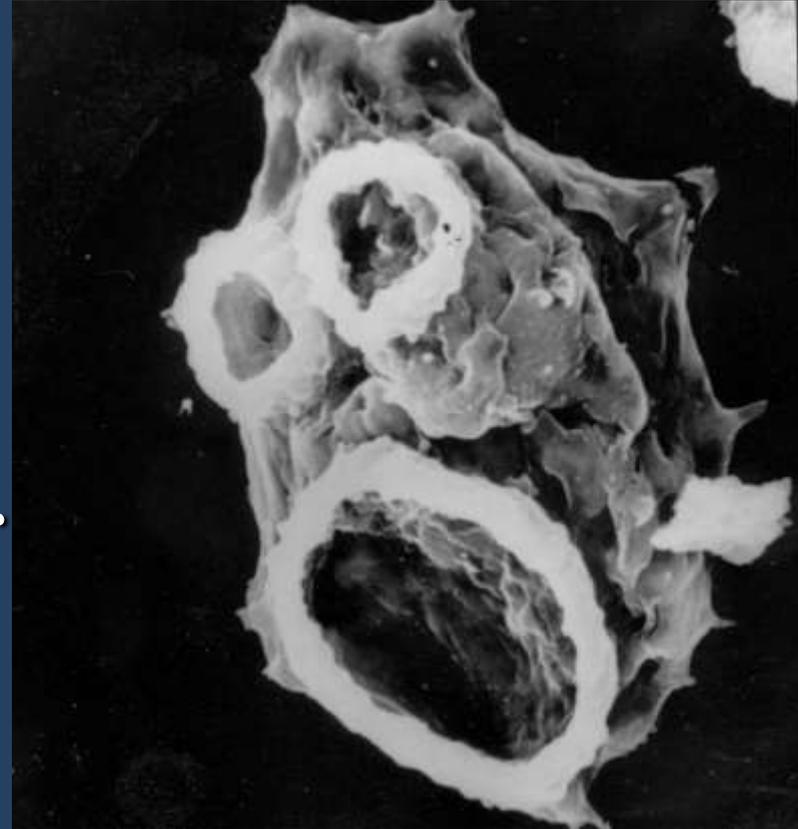
Excystment of ameba

Release of bacteria-filled vesicles

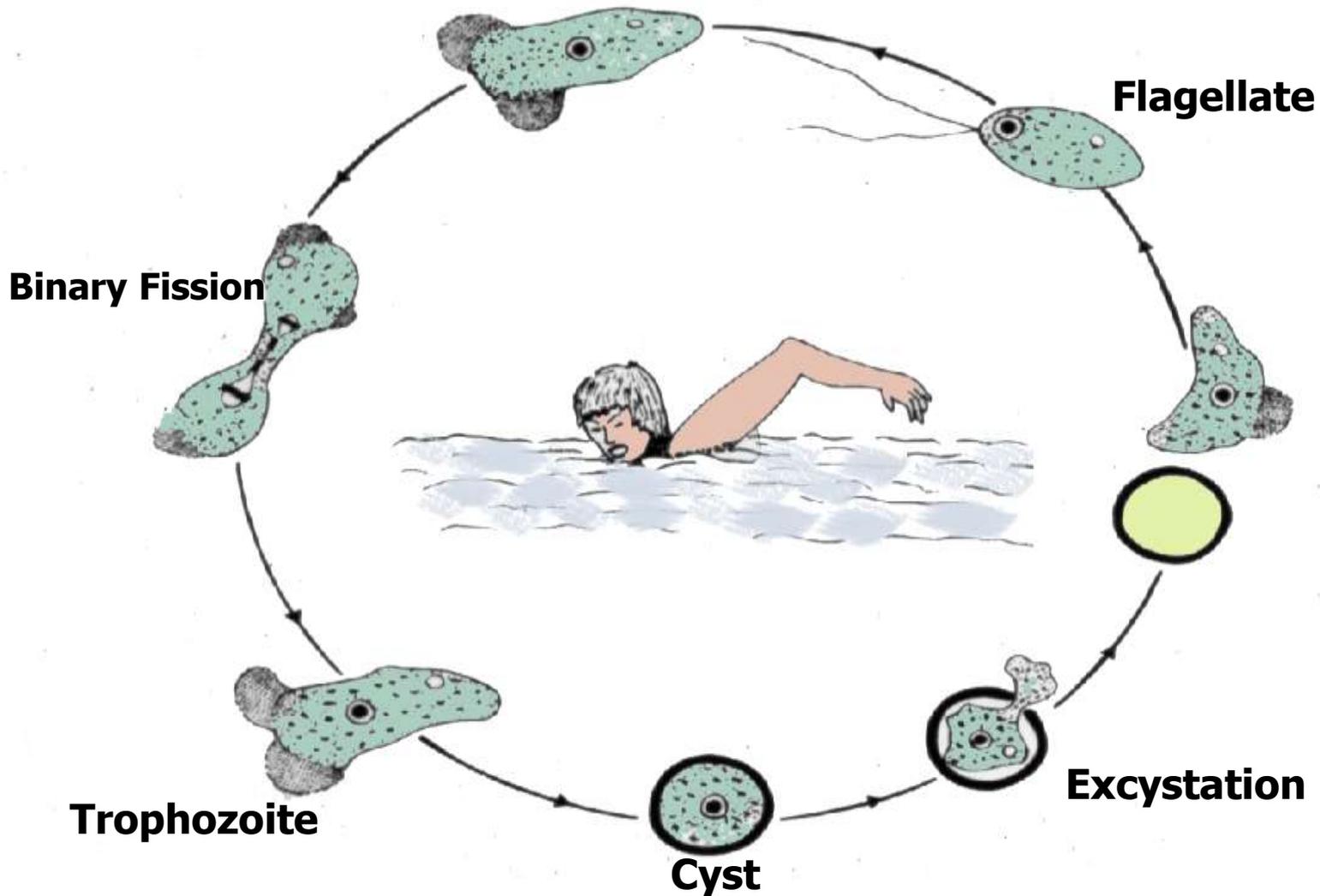


Naegleria fowleri

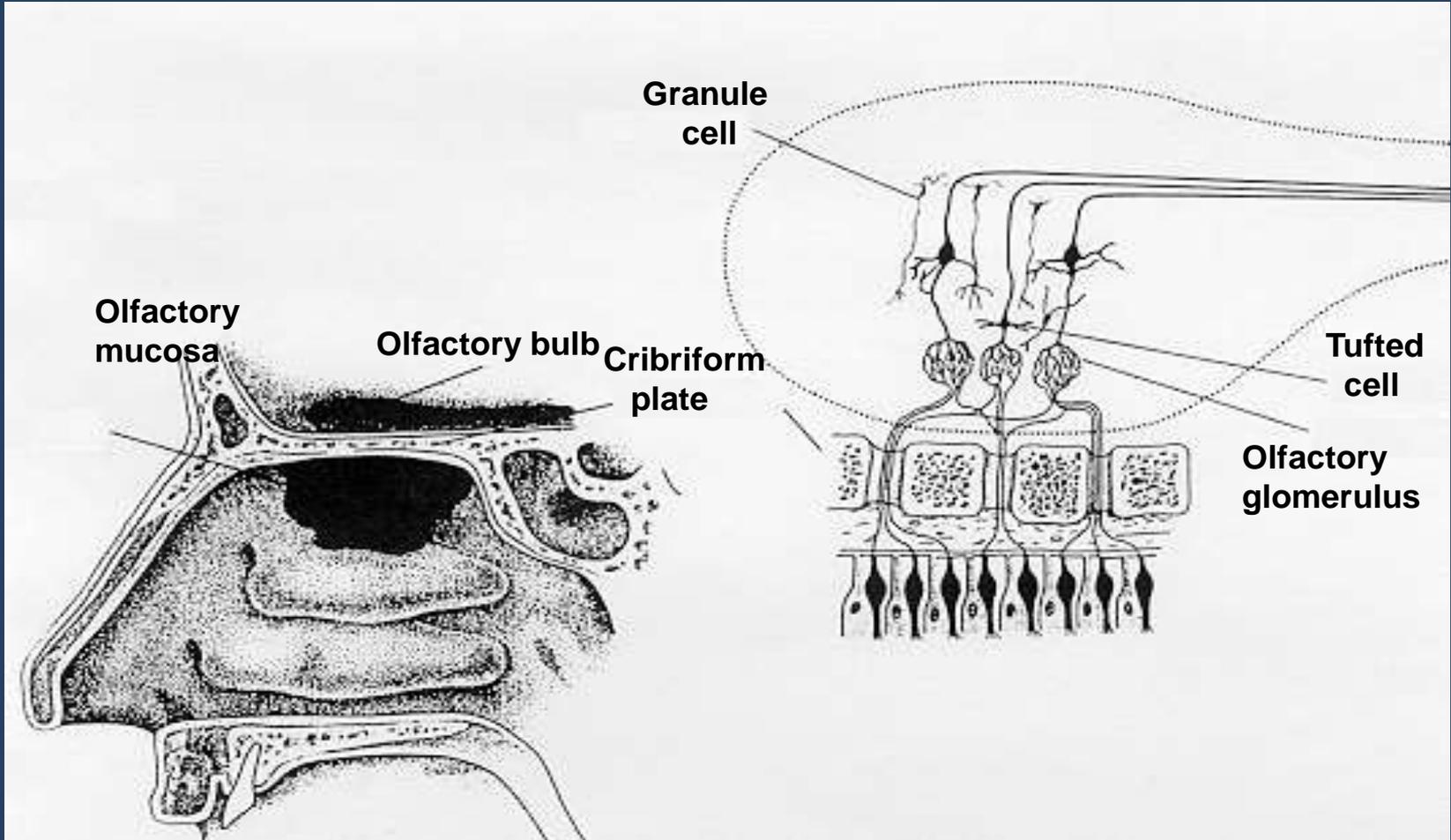
- PAM: rapidly fatal disease of CNS
- rare but almost always fatal
- occurs during hot summer months
- acquired while swimming or diving in fresh water



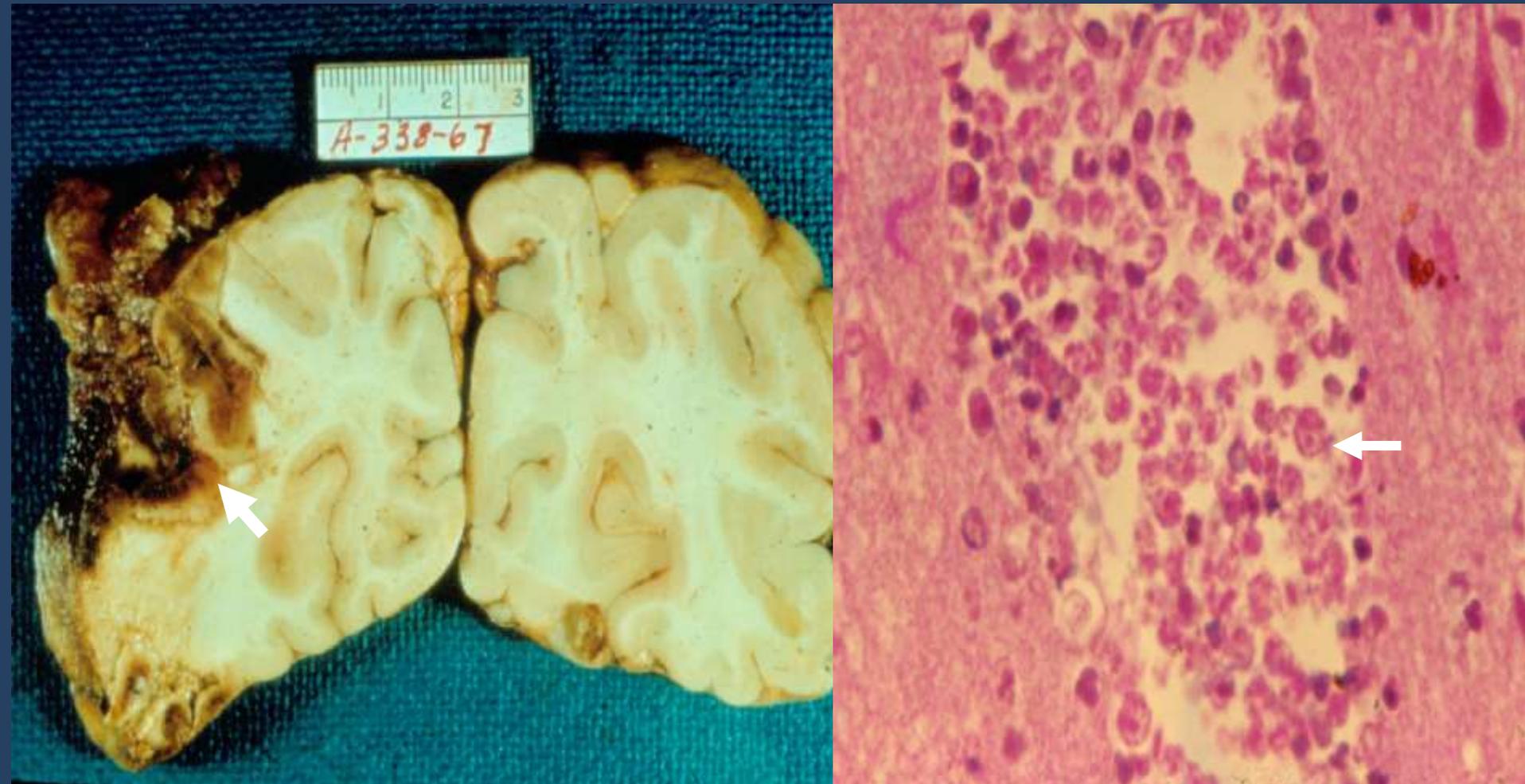
Cases of Primary Amebic Meningoencephalitis have been acquired by swimming and diving in freshwater lakes and ponds



The route of CNS invasion by *Naegleria fowleri* amoebae

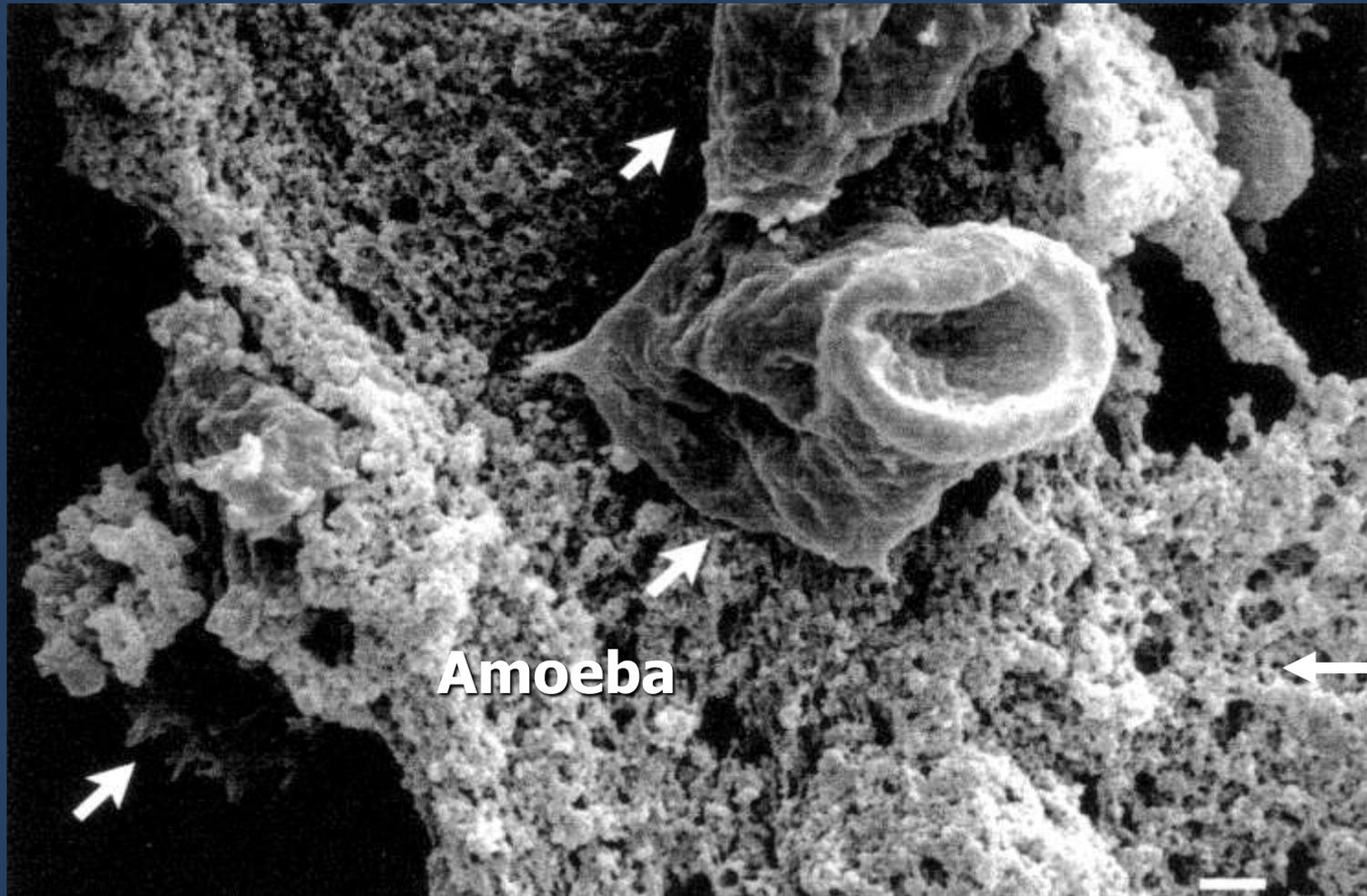


Human Case of Primary Amoebic Meningoencephalitis



Fatal 7-10 days post infection

Scanning Electron Micrograph of Amoeba in Brain



Amoeba

**Lysed
brain
tissue**

Water Park



Case of PAM in Oklahoma

Surveillance studies for waterborne disease outbreaks in the US indicate that the occurrence of PAM has increased in recent years (Lee et al. 2002 MMWR Surv.Sum.)

A high level of mortality associated with PAM indicates a need for a rapid and sensitive test, environmental testing and education programs.

Collection of small samples of water.



Water collected, centrifuged, and plated on NNA-*E.coli* plates.

Methods for Environmental Samples

Samples collected from water.



Plated onto Nonnutrient agar coated with *E. coli*.



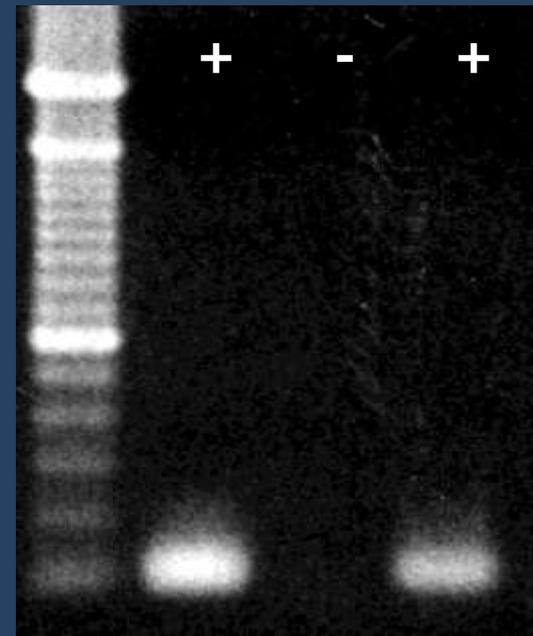
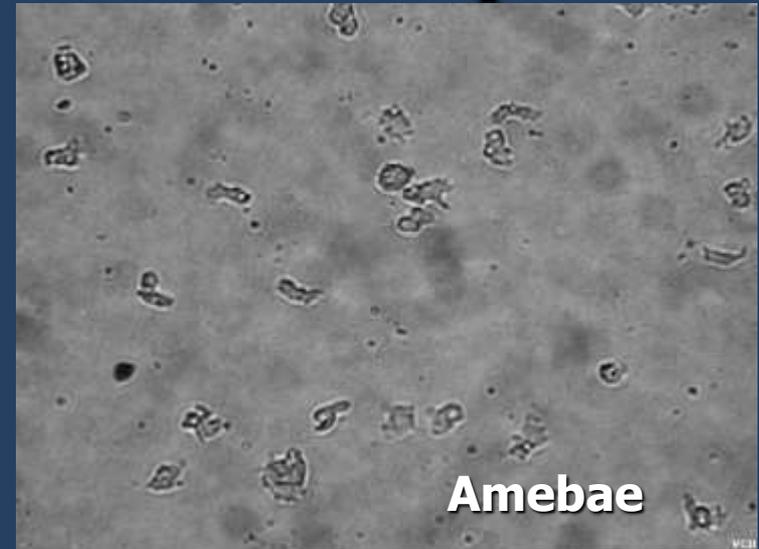
Amebae feed on bacteria and divide.



Amebae placed into Page Saline.



Nested PCR



Arizona Samples -domestic water

- **In October 2002, two previously healthy children died of PAM.
-no history of swimming or diving
-domestic water supply suspected source**
- **Samples were collected from both children's homes and a neighbor's home.**

Press releases in Arizona

Water company is closed

Deadly parasite found in Peoria

Lindsey Collom
The Arizona Republic
Nov. 3, 2002 12:00 AM

Peoria has urged customers of Rose Valley Water Co. to stop using their tap water for drinking, cooking or bathing for fear that it contains an organism that caused the death of two 5-year-olds last month.

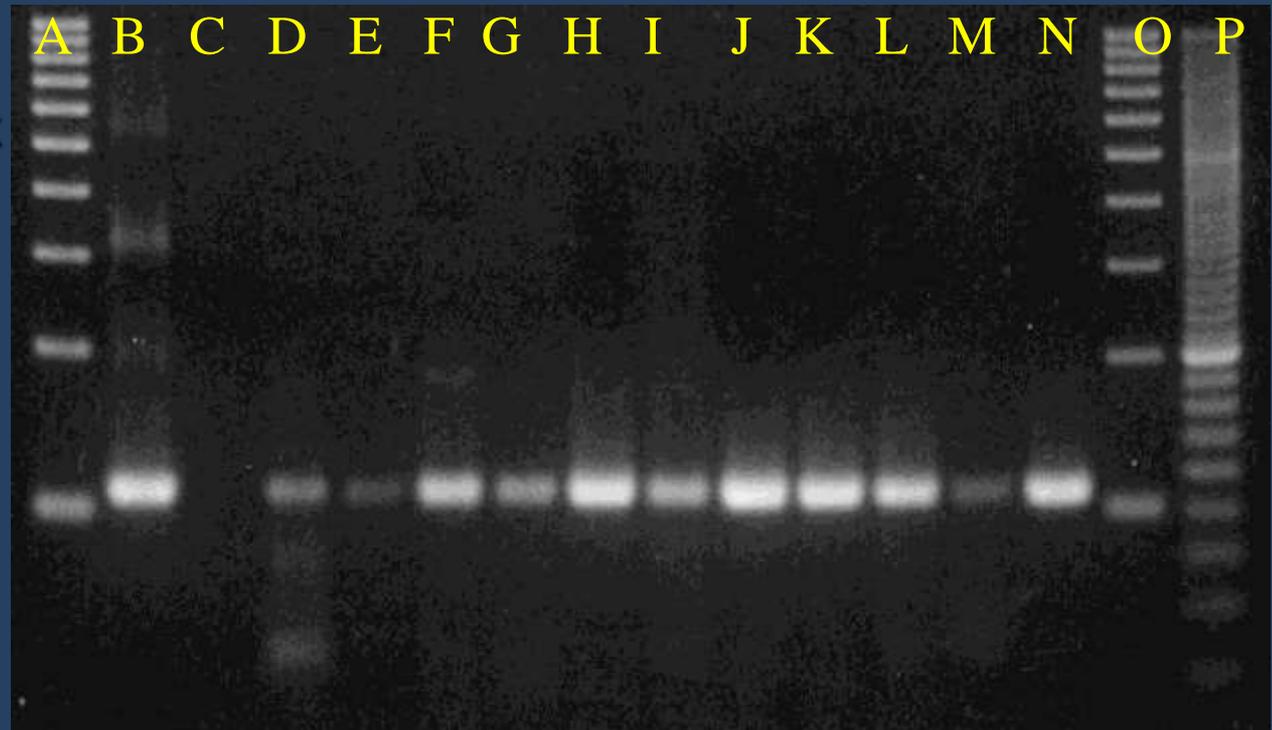
A statement from the Maricopa County Department of Public Health said that testing of Rose Valley's water supply gave an apparent positive result for *naegleria fowleri*, a parasite that causes primary amoebic meningitis.



PCR after one week in culture.

(report of domestic water as source of infection)

- A, 0-100 bp ladder
- B-Pos. control
- C- Neg. control
- D- Bathroom¹ sink
- E- Residual water
- F- Bedroom¹ sink
- G- Residual water
- H- Bedroom² sink
- I- Residual water
- J- Bathroom² sink
- K- Sink Trap
- L-Water filter
- M-Bathroom sink
- N- Soil outside
- P-20bp ladder



17 of 19 samples were PCR+

2 samples negative - kitchen sink & garbage disposal

Local & State

B

Beware: Lethal amoeba lurks

☐ Deaths are rare, but the amoebas that killed an Orlando teen are common in area lakes. Here's how to protect yourself.

By Mike Oliver

OF THE SENTINEL STAFF

The chances of an amoeba's crawling up your nose and into your brain while you're swimming in a lake are about as small as those of an alligator's dragging you down for its dinner.

But if you saw a 10-foot gator glide by, you certainly wouldn't go in the water. Likewise, there are ways to lessen the risk of contracting the fatal brain inflammation caused by the *Naegleria fowleri* amoeba.

The death Thursday of a 14-year-old Orlando

boy after a visit to a neighborhood swimming hole has prompted health officials to re-emphasize these precautions to anyone who swims in fresh water during the warm summer months:

- Wear a nose clip when swimming in a lake, stream or river. Chlorinated pools and hot tubs are not a concern.

- Avoid stirring up the sediment on the bottom of the lake, where the microorganisms reside.

- Stay close to the surface of the water and in the deep part of the lake. The amoebas are typically found in the shallow areas.

Several years ago, officials tested 26 lakes in

Central Florida and found that 12 tested positive for the amoebas, Orange County epidemiologist Bill Toth said.

"It's there," Toth said. "They projected that virtually all of the lakes have or could have the amoebas."

But Toth said infection is rare, involving cases in which amoeba-laced water, usually from a stirred lake bottom, gets jammed up a swimmer's nose.

Richard Davis died Thursday — nearly two weeks after he went swimming with five friends

Please see AMOEBA, B-4

Teenager caught infection in canal

AMOEBA from B-1

in a drainage canal off of Forsyth Road. Richard got sick last Sunday, one week after his July 26 swim. No one else was infected.

His parents took him to the emergency room Monday with high fever. Doctors tested for meningitis and discovered the

infection after he went swimming in a Winter Park lake.

At the time, no one had heard of a lake-dwelling amoeba's causing infections in swimmers.

"He liked to snorkel dive and he liked to do flips off the dock," said Fred Compton, whose family lived on Lake Spier in Winter Park.

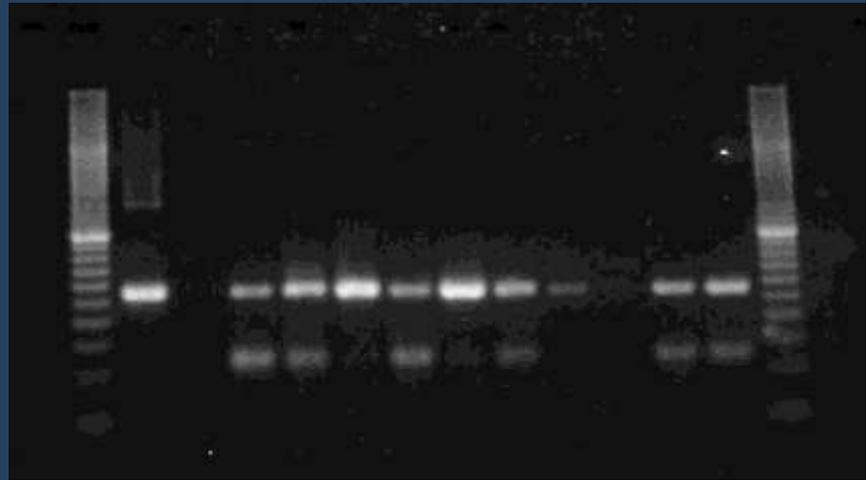
lake swimming, Compton said.

"We didn't even know how it got into the brain at that point," Compton said.

Another well-known Orlando area case occurred in 1985, when 8-year-old Billie Jo Nobles died after swimming in Starke Lake in Ocoee.

The little girl lapsed into a co-

Water samples from Florida (warm water)



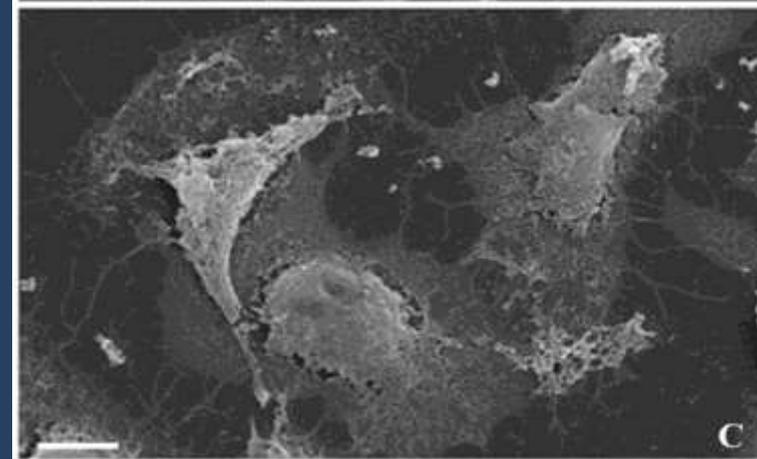
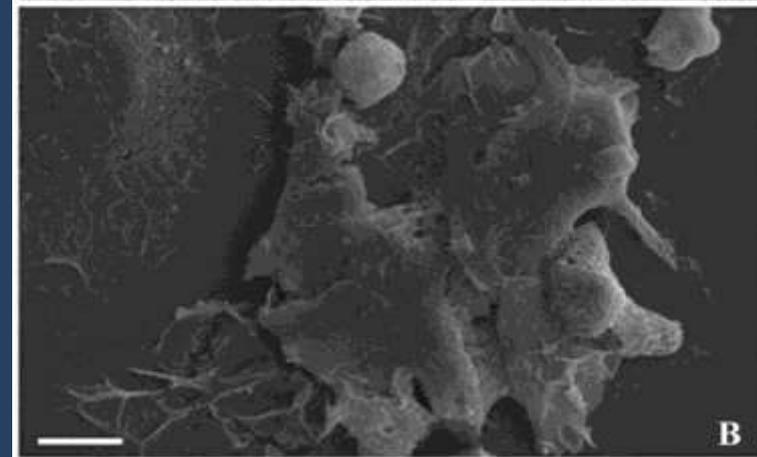
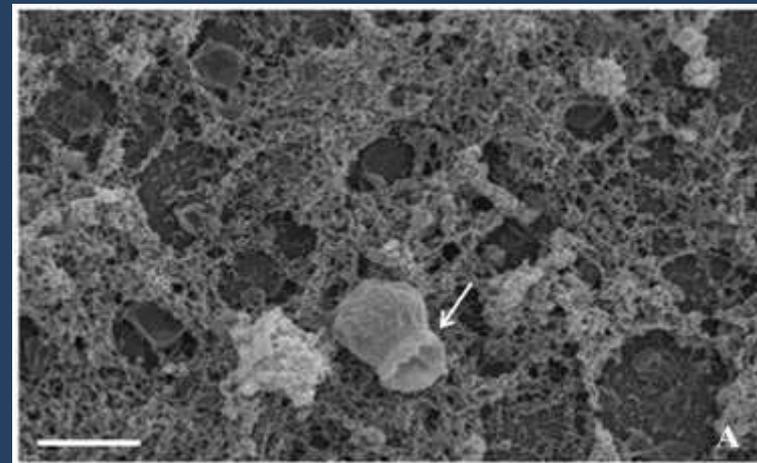
9 of 10 samples PCR positive (90%)

Virulence Factors

Virulence factors of the amebae are not well-known.

Invasion assays have been performed and indicate that pathogenic amebae secrete proteases that degrade ECM components.

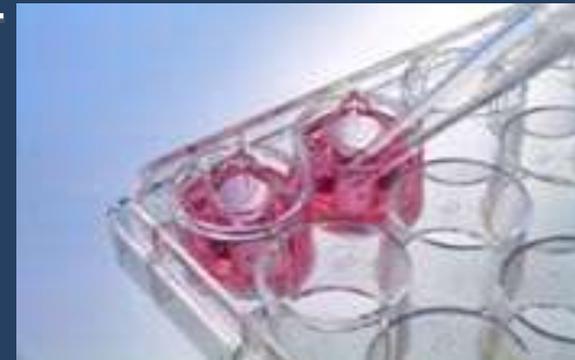
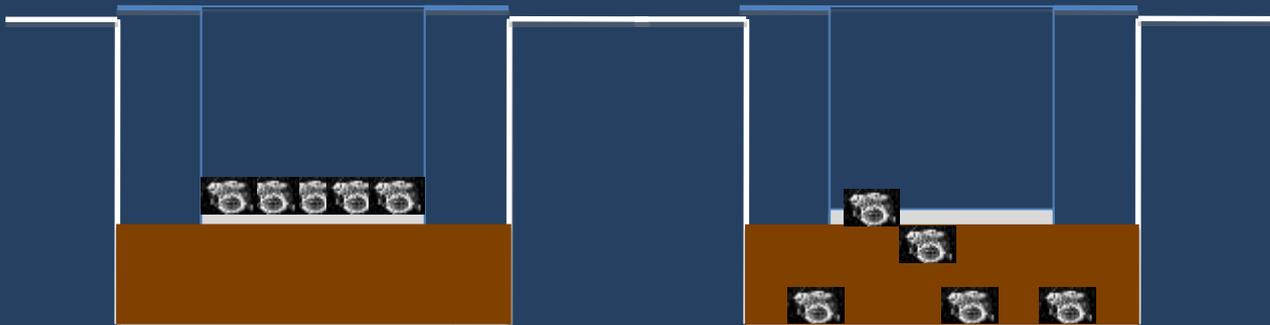
Proteases can destroy mixed glial cell cultures.



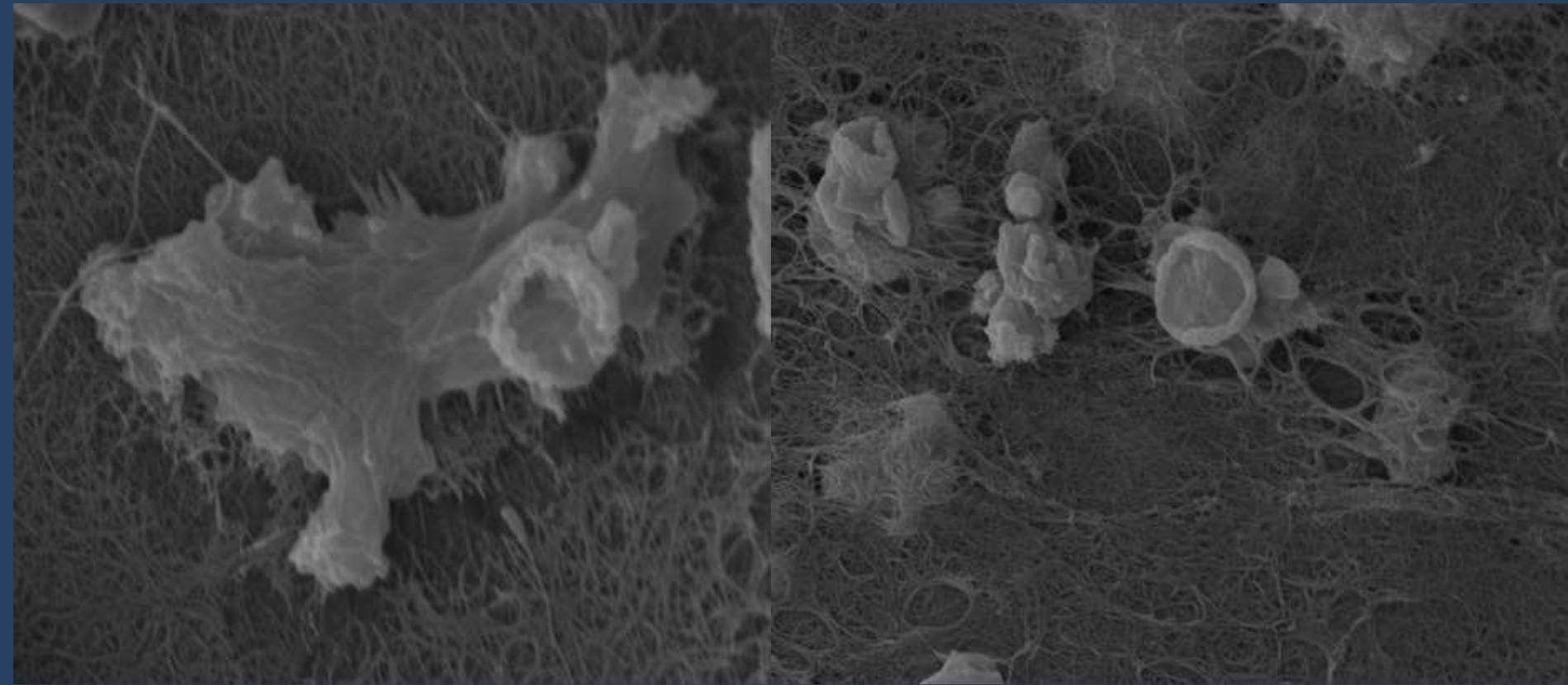
Virulence Factors - Invasion Assay

Invasion assays were performed to compare invasion rates of *N. fowleri* and *N. lovaniensis*

- Inserts (8um) were coated with Collagen or Matrigel
- Placed in wells with Media at the bottom
- Amebae added (10^5), incubated for 2 h and allowed to invade coated inserts.
- Amebae counted, Media collected

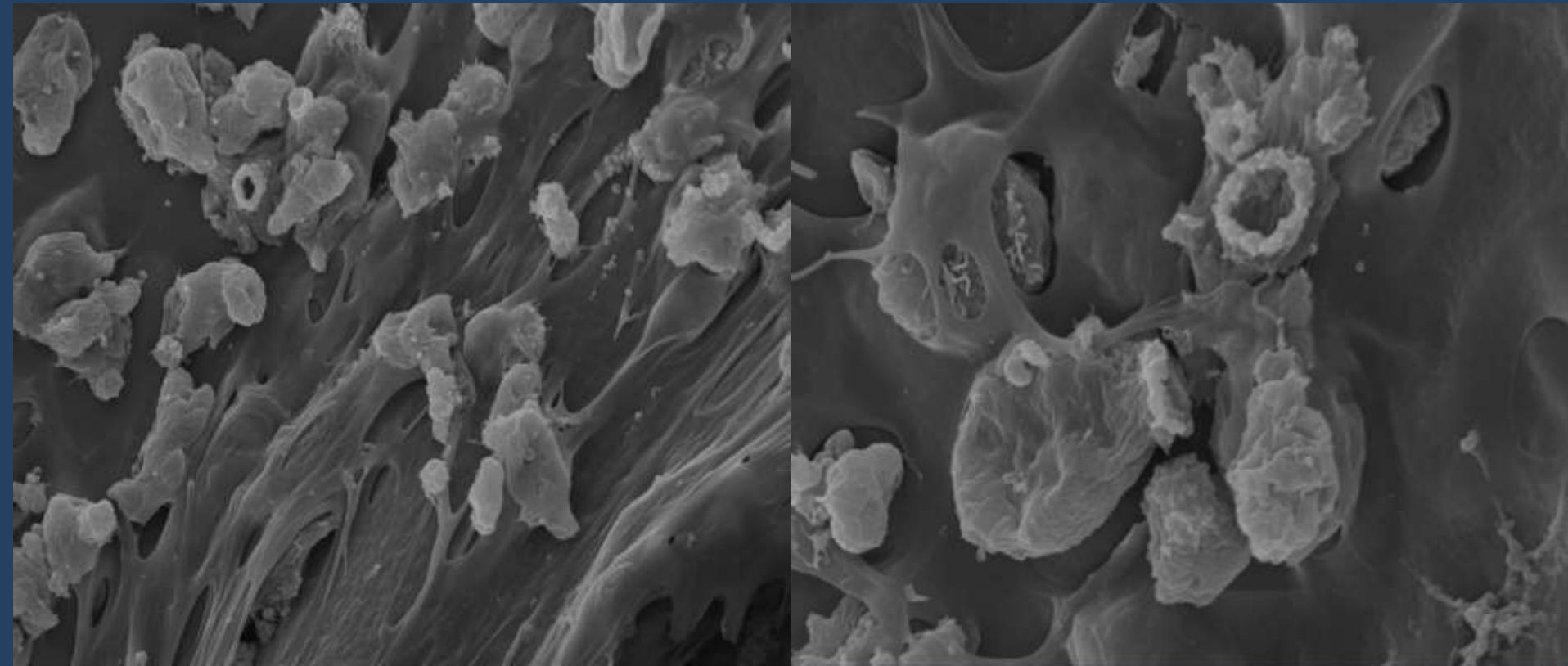


N. fowleri invading Collagen I



2 hours

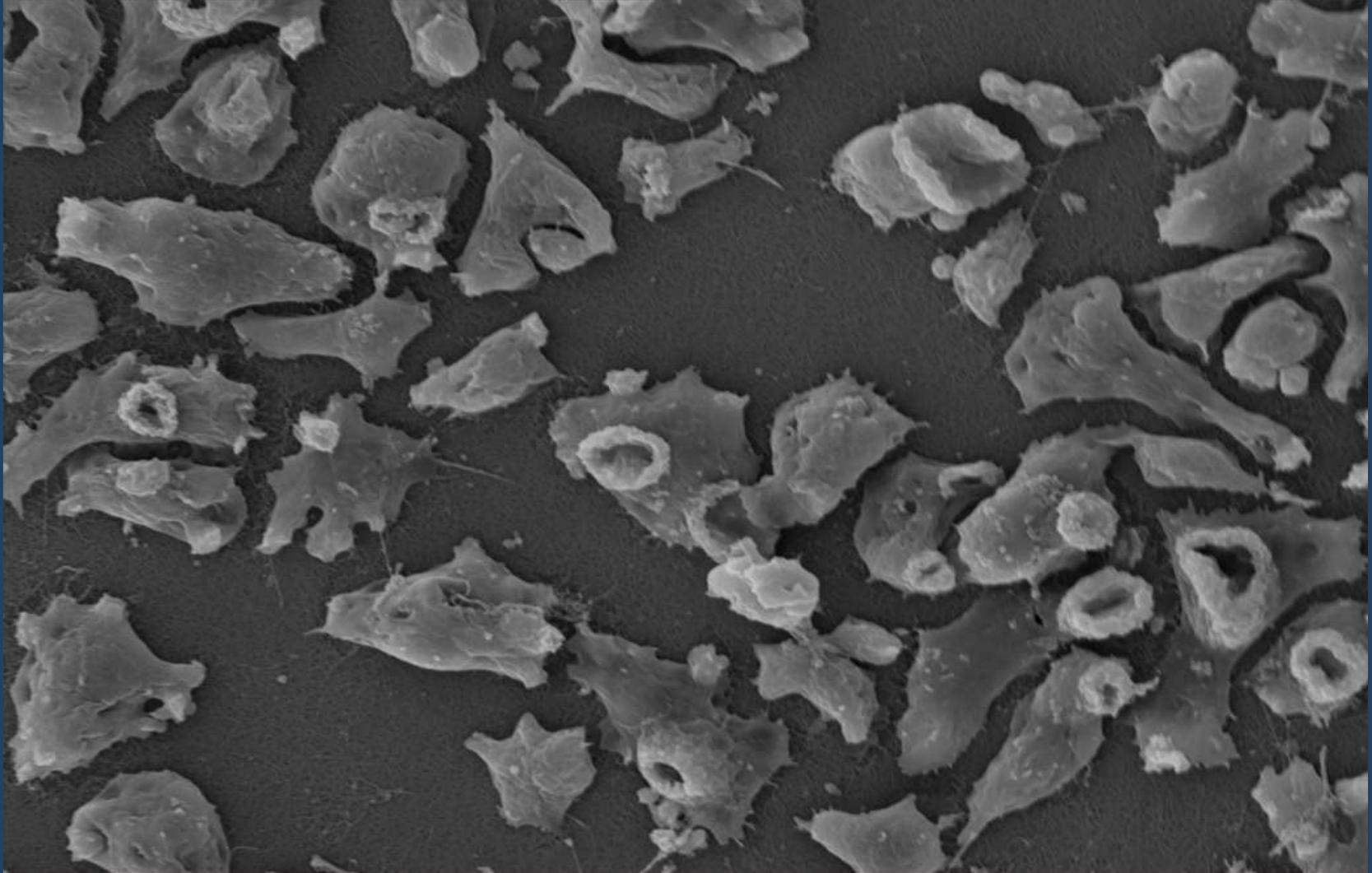
N. fowleri invading Matrigel



2 hours

Invasion Assay:

N. lovaniensis (nonpathogenic) on Collagen I



2 hours

Major difference: pathogenic *N. fowleri* is invasive, nonpathogenic *N. lovaniensis* is noninvasive.

Immune Response

Question:

Do humans respond immunologically to exposure to *Naegleria fowleri*?

Immune response

Innate Immunity – first line of defense after skin and mucus membranes is the **Complement system.** - A system of plasma proteins activated by pathogens leading to a cascade of reactions that occur on the surface of organisms.

Adaptive Immunity – occurs as an adaptation to infection.

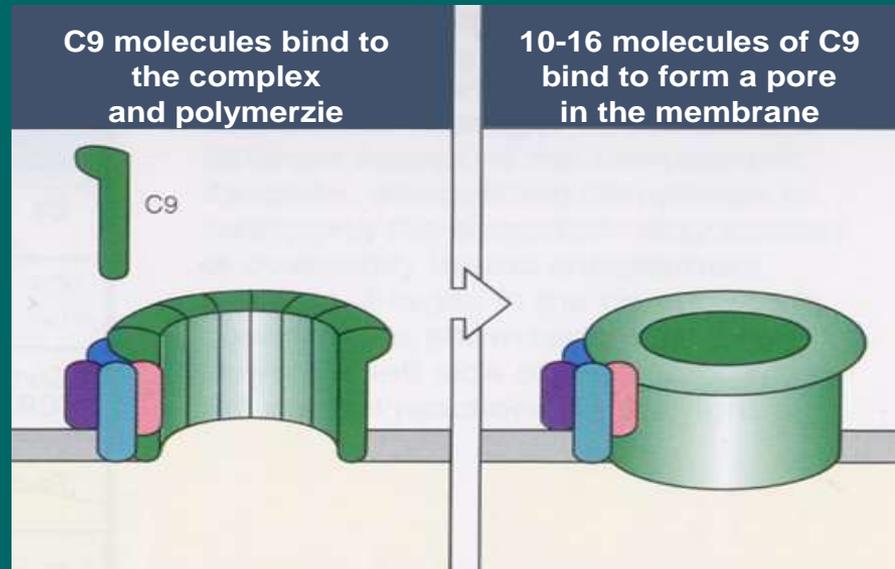
- a. Production of antibodies (B cells)**
- b. Cell-mediated (T cells)**

How does complement work?

Membrane attack complex of complement (C5b-C9) lyses organisms.

The Complement System

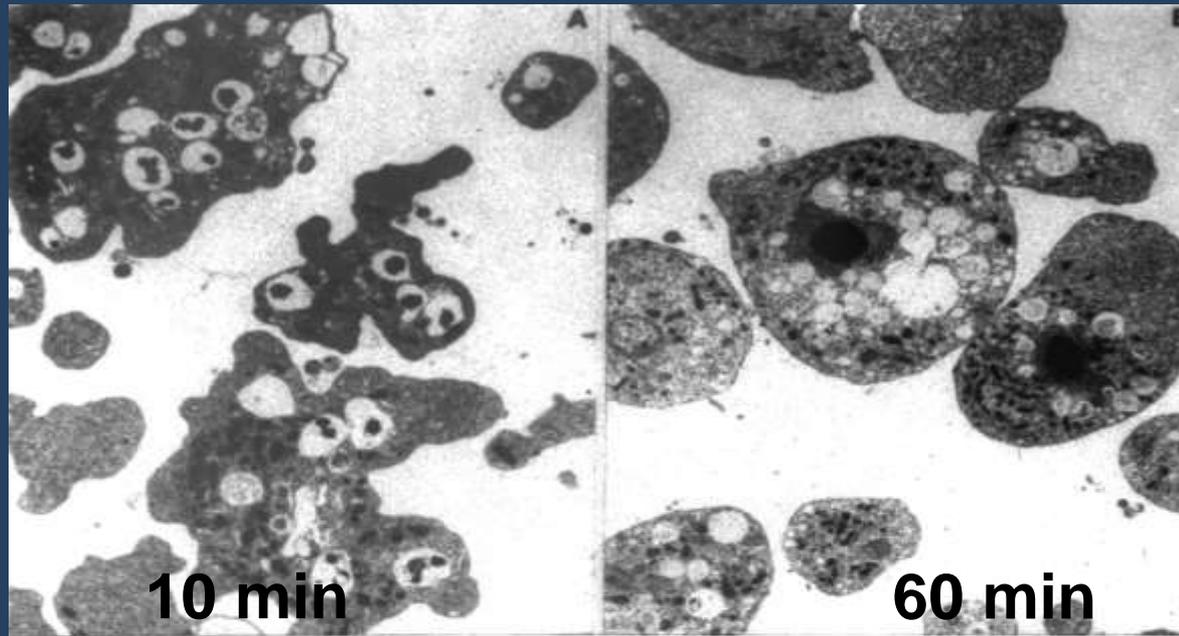
- Body's first line of defense against foreign invaders
- C5b-C9 = membrane attack complex (MAC)
- MAC responsible for lysis of pathogen



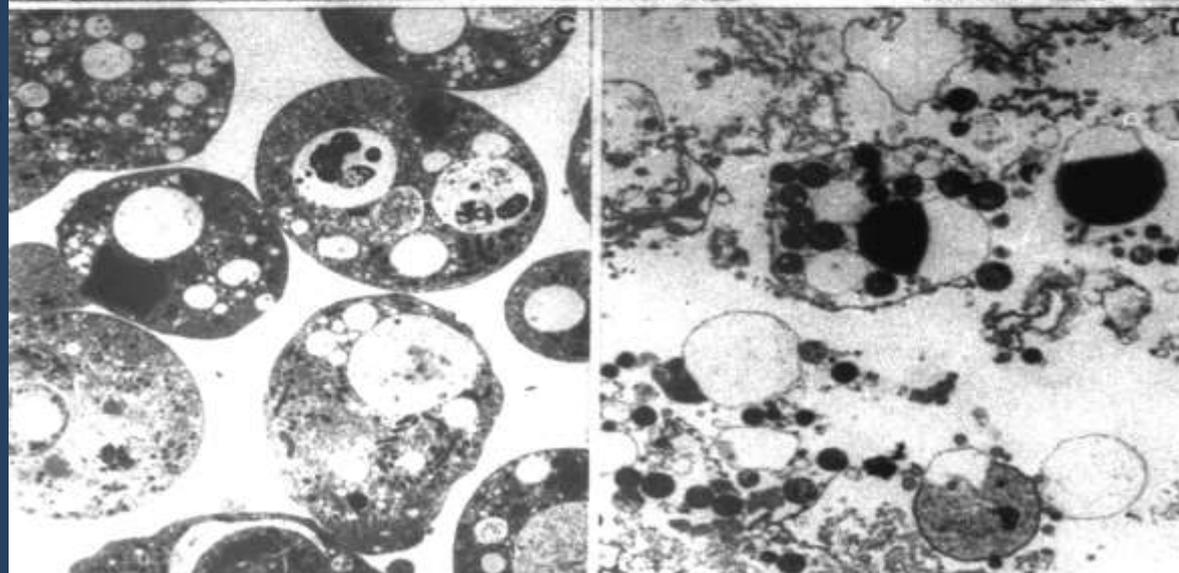
Janeway, C. A., et al. (1999). *Immunobiology: The immune system in health and disease*.

Effect of Human Serum Complement on *Naegleria Amebae*

**Pathogenic
N. fowleri
Complement
resistant
(Not lysed)**

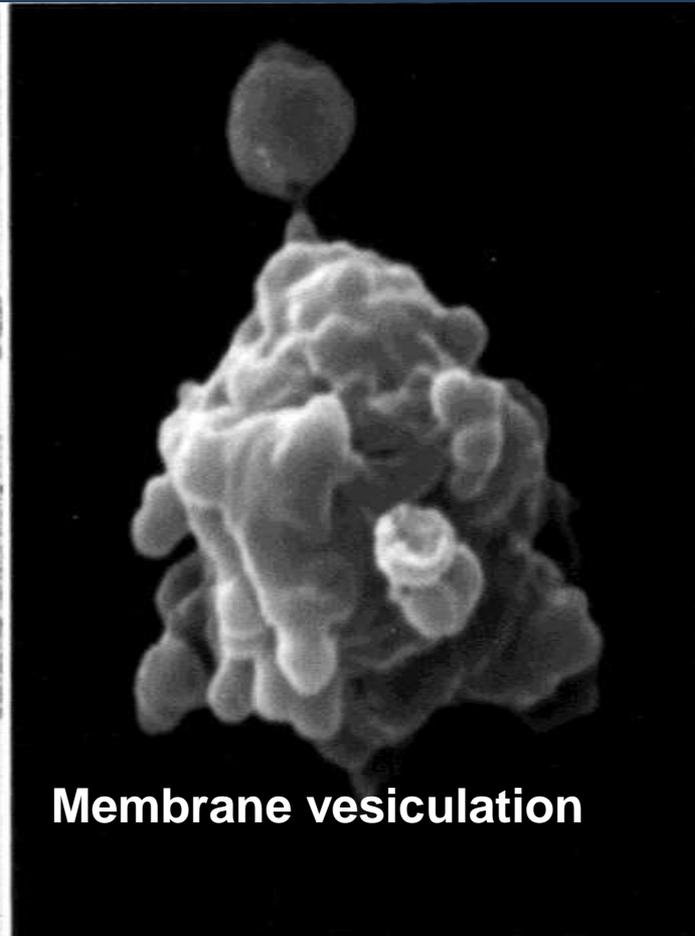
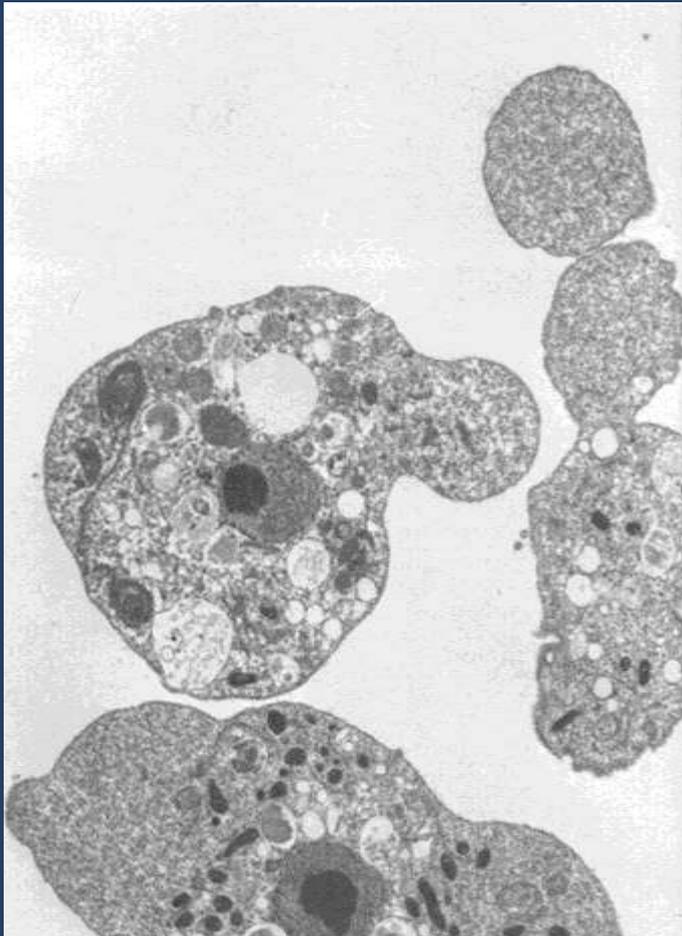


**Nonpathogenic
N. gruberi
Complement
sensitive
LYSED**



Mechanisms of Complement Resistance

Extrude MAC of complement on vesicles, leaving behind an intact amoeba. A CD59-like protein found on the amoeba.



Are antibodies protective?

Most human serum contains IgM & IgG antibodies to *Naegleria* in endemic areas.

A study by Ferrante & Thong (1979) indicated that an antibody response does not appear to be protective since the amebae internalize and degrade antibody.

Another study by Lallinger et al 1987 suggested that antibodies slow the progression to death but do not result in a cure.

Could IgA antibodies to *N. fowleri* in mucus membranes inhibit adherence of amebae to the nasal mucosa ?

Antibody Response

Rivera et al (2001) detected higher levels of IgA in serum and saliva in individuals with respiratory infections than in healthy individuals in endemic areas.

Cursons et al (1979) reported that serum obtained before death from a patient with PAM showed very low levels of antibody.

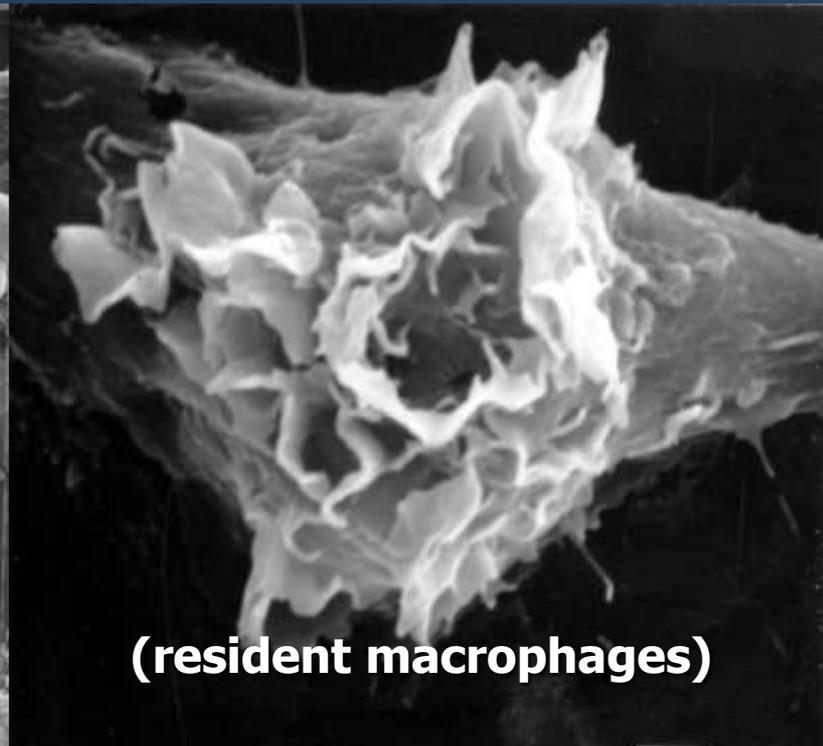
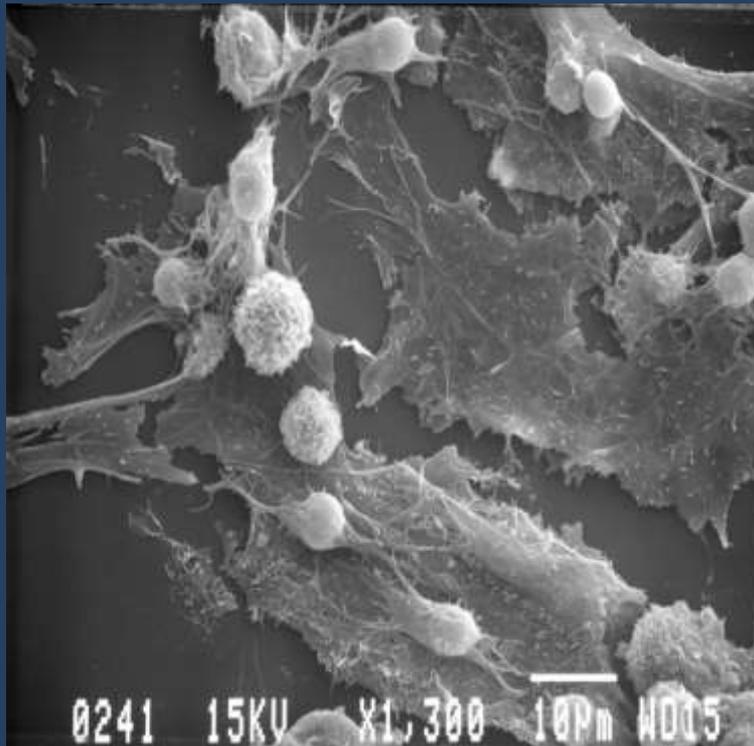
Cain et al (1979) obtained serum before death of a PAM patient reported levels were within normal limits.

Seidel et al (1982) reported an antibody titer of 1:4096 after 42 days of infection in a patient who survived following treatment with IV and intraventricular Amphotericin B.

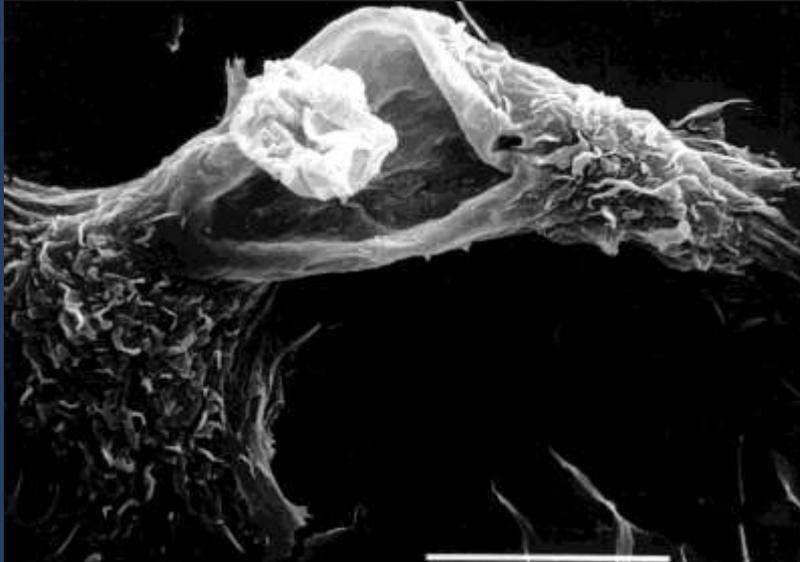
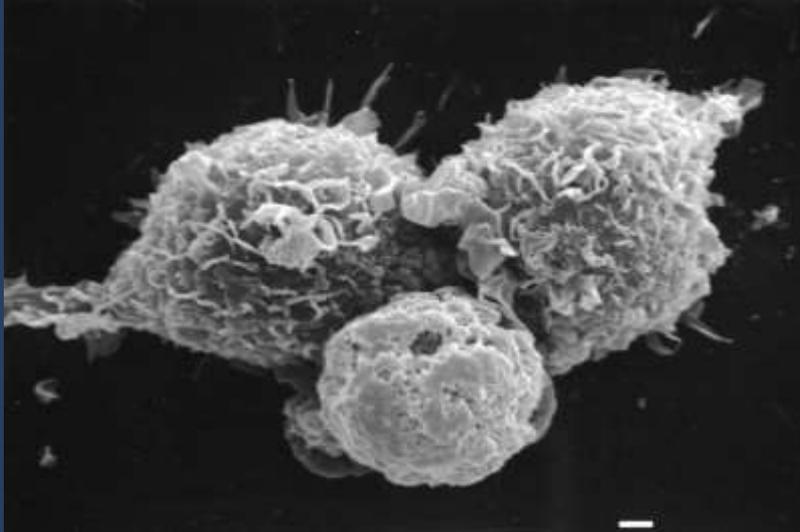
Immune System in the brain

Microglia and astrocytes :
respond to infection or injury

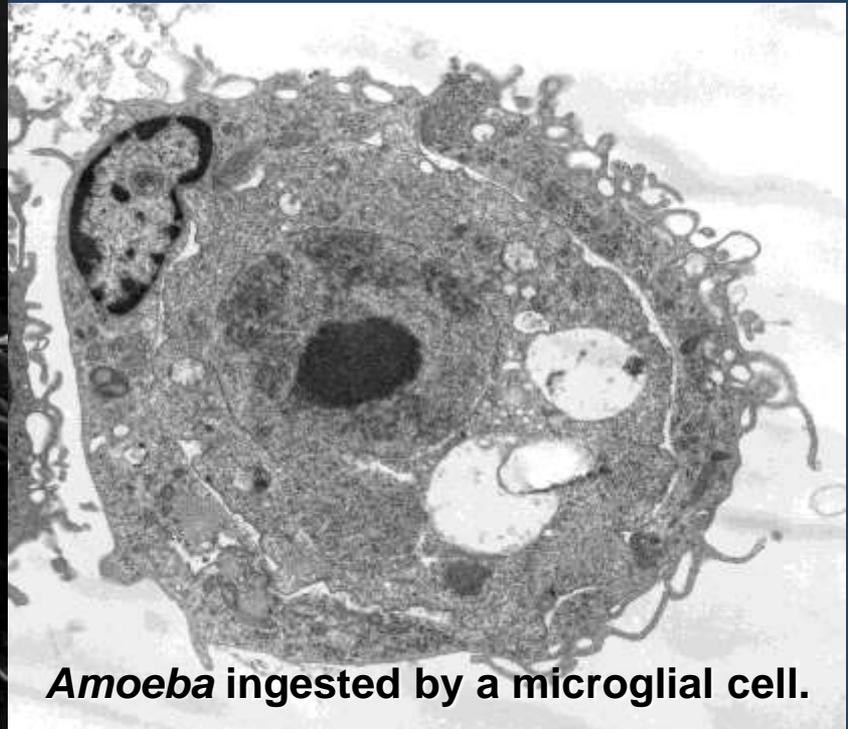
Activation of these cells can be helpful or harmful



Microglia Response to amoebae

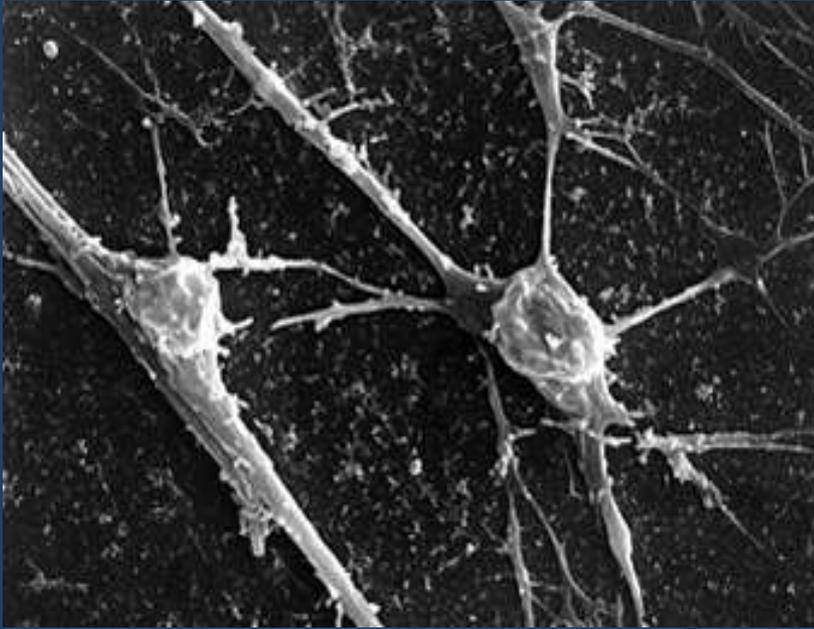


Microglia can lyse and ingest trophozoite and cyst forms.



***Amoeba* ingested by a microglial cell.**

Microglia



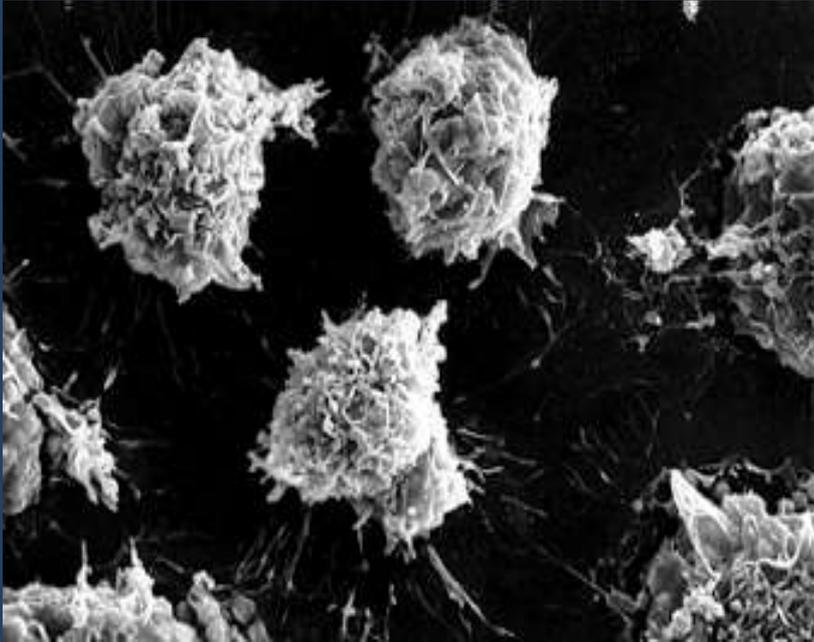
**Resident population of
macrophages in the
brain**

**Activated after brain injury
or infection**

**Release proinflammatory
cytokines: IL-1 β , IL-6,
TNF- α**

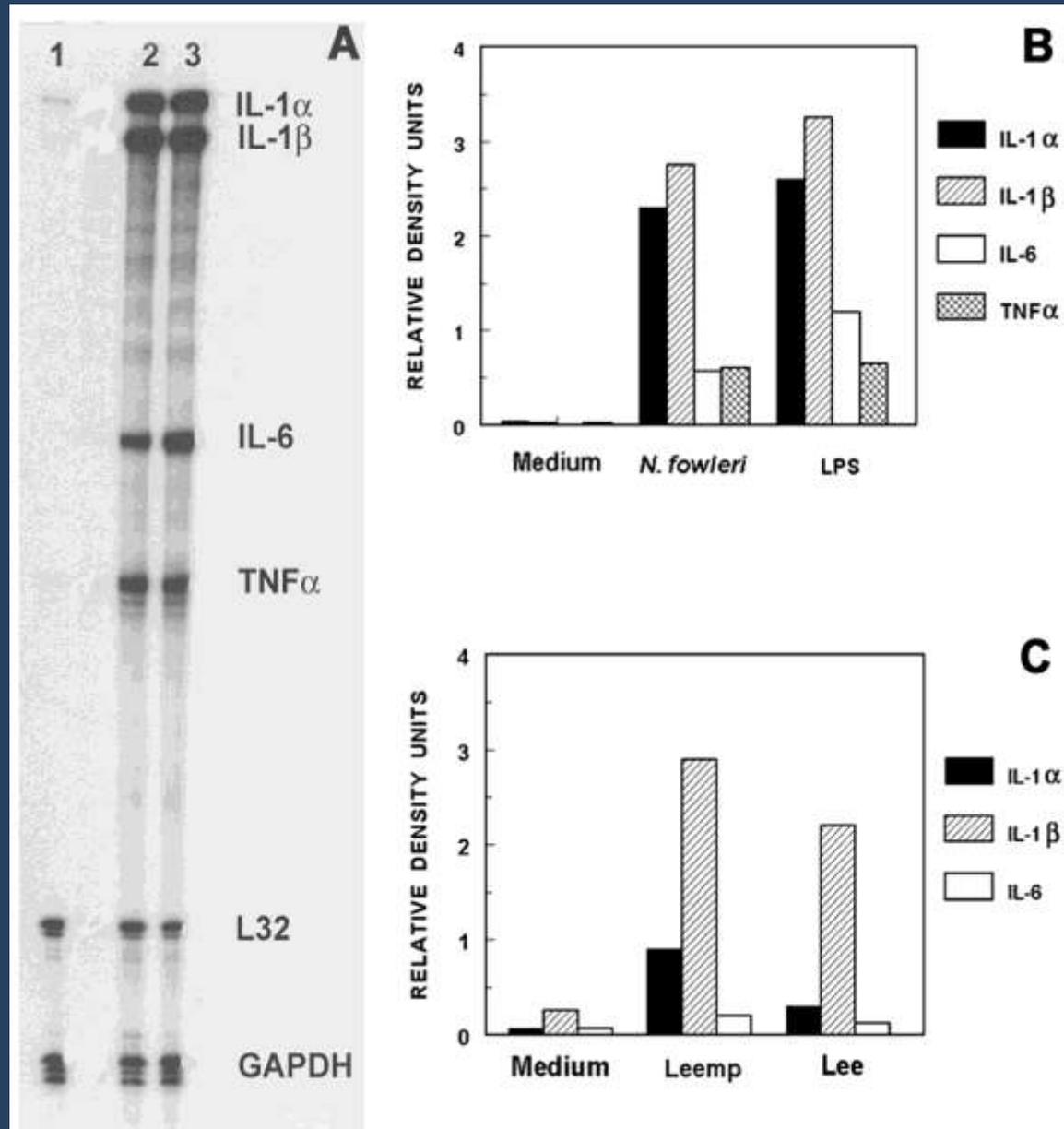
Produce nitric oxide

Causing inflammation



The Interaction of *N.fowleri* with Brain Microglial Cells.

Highly pathogenic strains induce higher levels of proinflammatory cytokines.



Why do some people become infected, others do not?

**Lack of IgA antibodies at the mucosal
surface.**

Complement deficiency?

**A structural defect in nasal passage or in
the cribriform plate?**

Exposure to a large number of amebae?

Exposure to a virulent strain?

Follow these rules to Prevent Amoebic Meningitis.



If you must jump in fresh water hold your nose.



If you have been away- run the tap for a few minutes before use.



Keep your head above water in the bathtub.



Keep sprinklers and hoses away from the nose.



Disinfect swimming pool correctly. Empty and clean small collapsible pools daily.

The Health Survivor of your local



Acanthamoeba

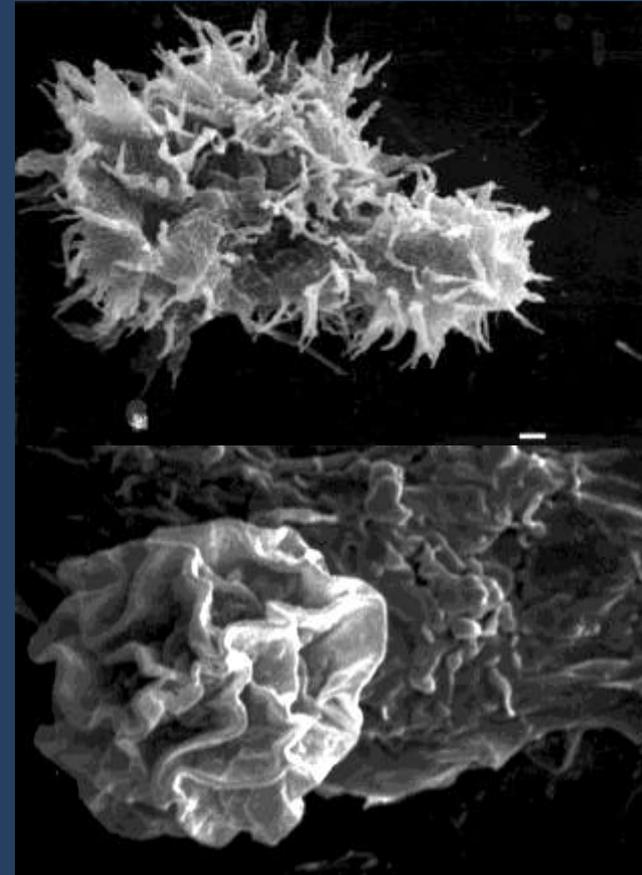
Opportunistic pathogen –

Granulomatous amoebic encephalitis

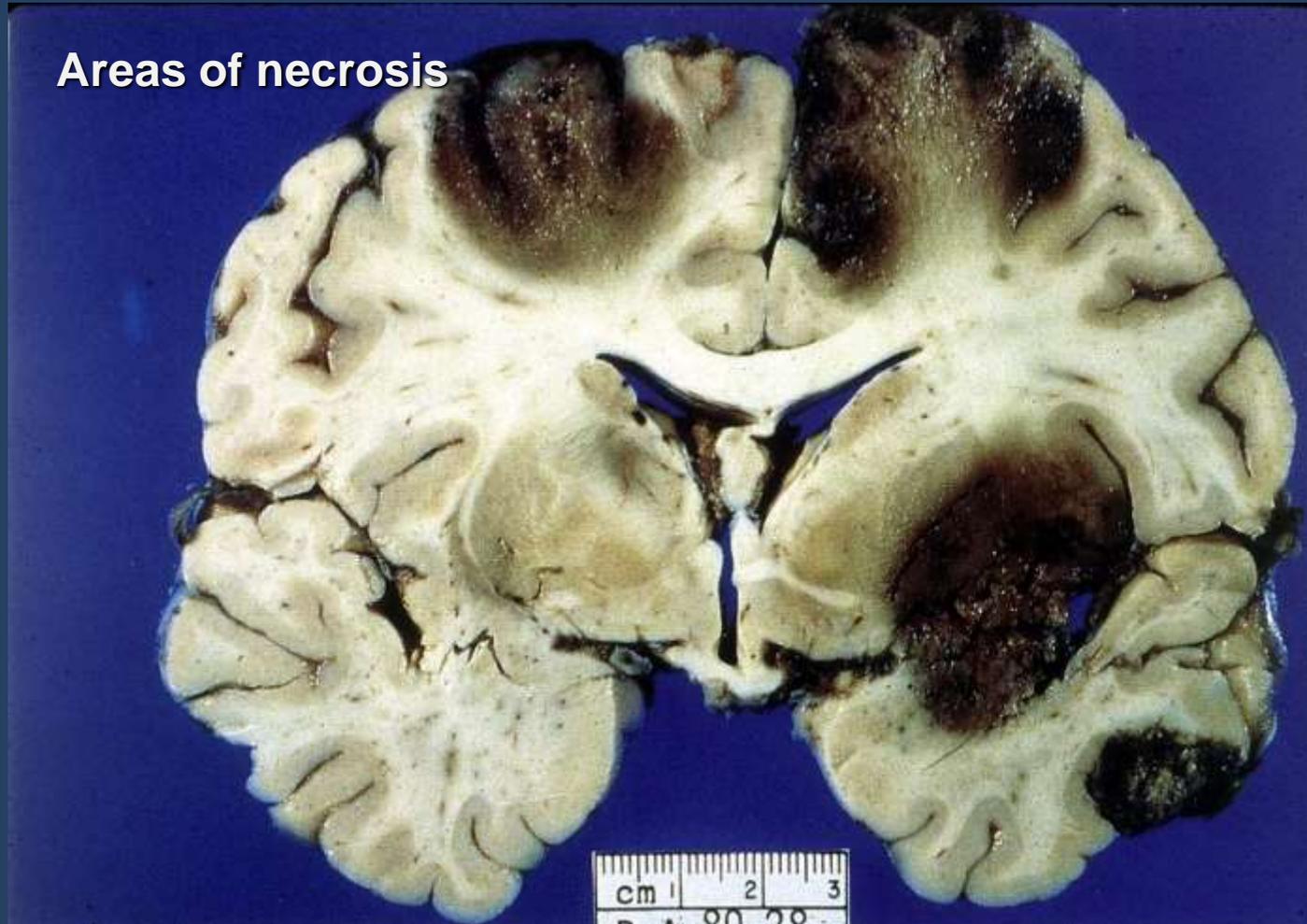
Chronic infection – one month or longer to manifest disease.

Cutaneous amebiasis

Amebic keratitis - immunocompetent individuals, associated with contact lens wear and poor hygiene.



Granulomatous Amebic Encephalitis caused by *Acanthamoeba*



Symptoms: Headache, seizures, patient disoriented

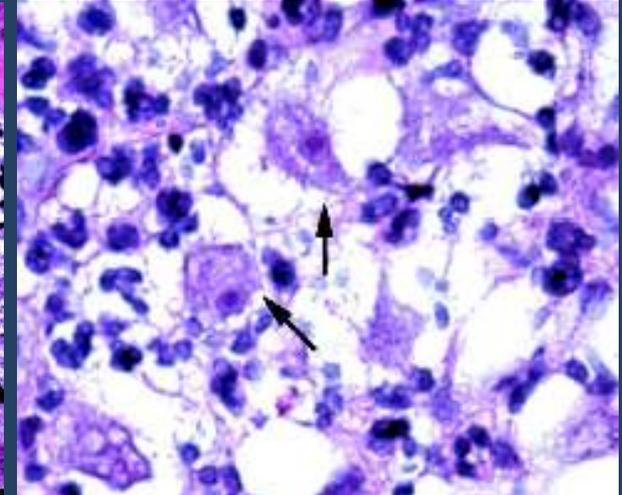
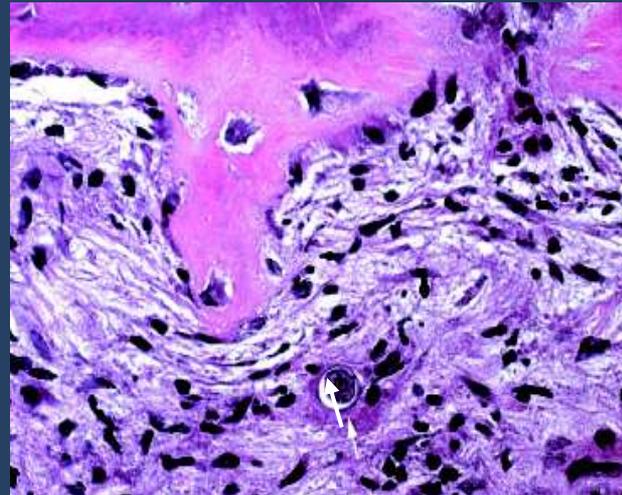
(Martinez AJ 1985 Free-Living Amebas: CRC Press Inc. Boca Raton, FL)

Clinical Case of Acanthamoebiasis

- 61 year-old female on immune suppressive treatment due to kidney transplant
- Referred for evaluation of osteomyelitis of the finger, subcutaneous nodules on both legs, and ulcerated lesions on arms.

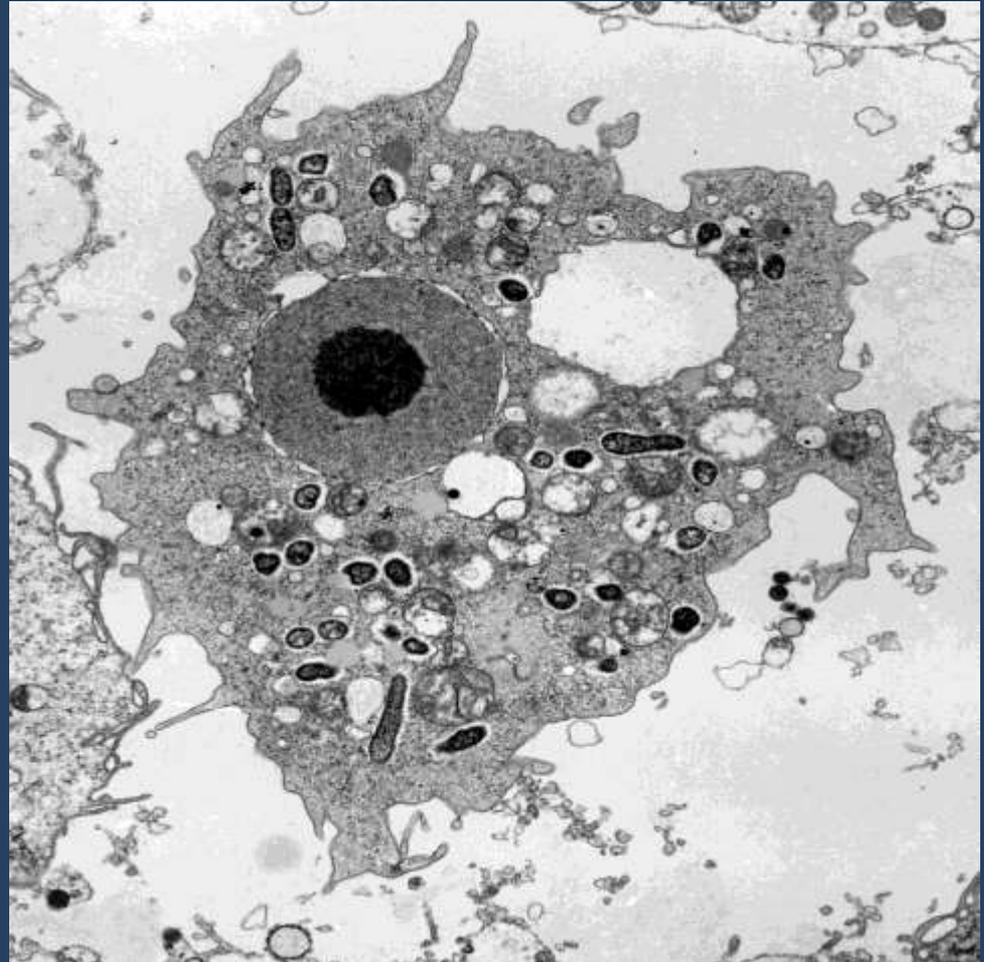


- Patient died from disseminated Acanthamebiasis



Clinical Isolate (JH1)

- Immunoblot analysis indicated isolate was reactive with polyclonal antiserum raised against *Acanthamoeba* spp.
- Electron microscopy of cultures revealed bacteria within amebae.
- Bacteria were non-cultureable in standard bacteriological media.



Cutaneous Acanthamoebiasis in an immunosuppressed patient



Patient first presented with lymphoma

A Case Study of a Patient at VCU Medical Center

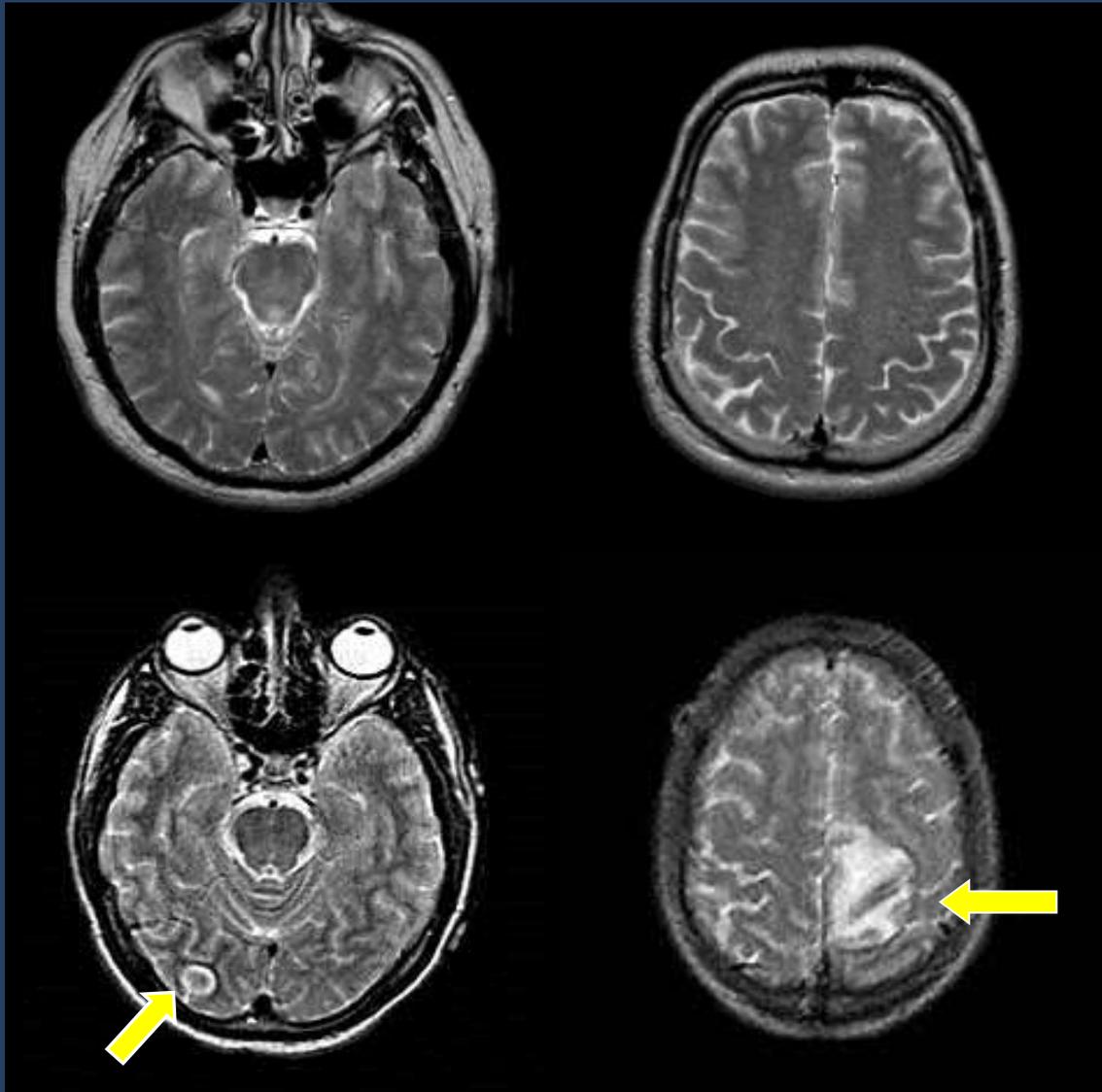
▶ Patient information

- 41 year old man presented to an outside hospital in January 2005**
- right sided weakness for 3 days**
- seizure episodes**

▶ Patient was transferred to VCU Medical Center

- Laboratory tests were normal and urine and CSF cultures were negative for bacteria**
- MRI scan showed four lesions in his brain**

MRI Brain Scans – MCV



Normal brain scan

VCU patient

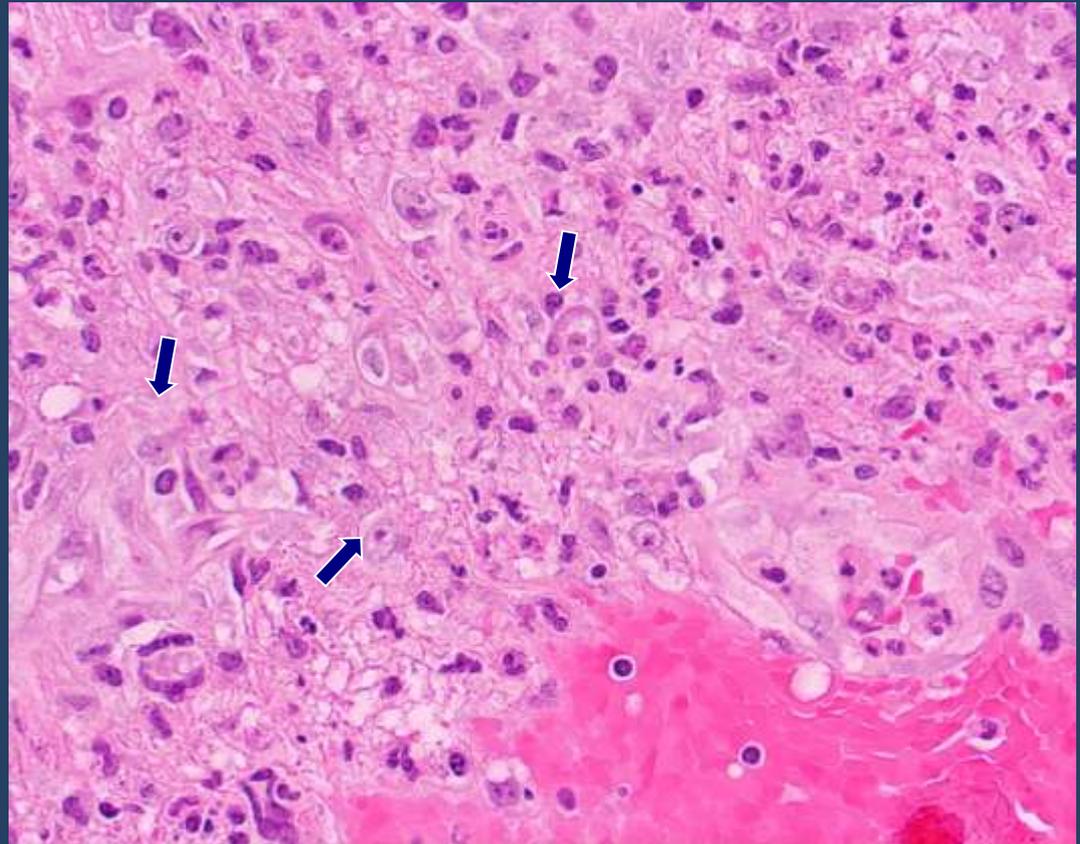
Occipital lobe

Frontoparietal lobe

A Case Study of a Patient at VCU Medical Center

Arrows indicate multiple trophozoites in the brain tissue

- Free within necrotic brain parenchyma
- Within the perivascular space
- Engulfed in giant cells

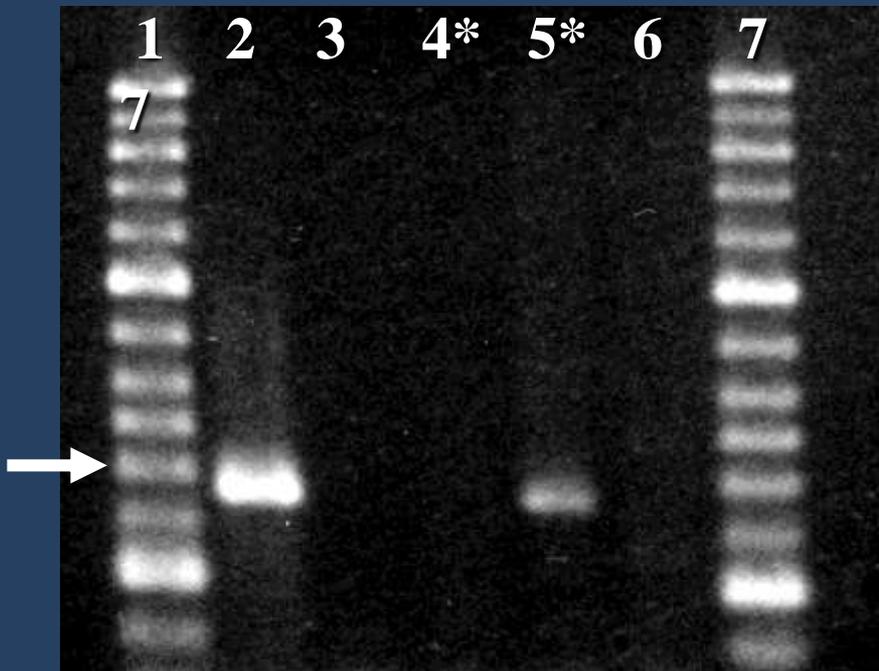


H&E staining of brain tissue

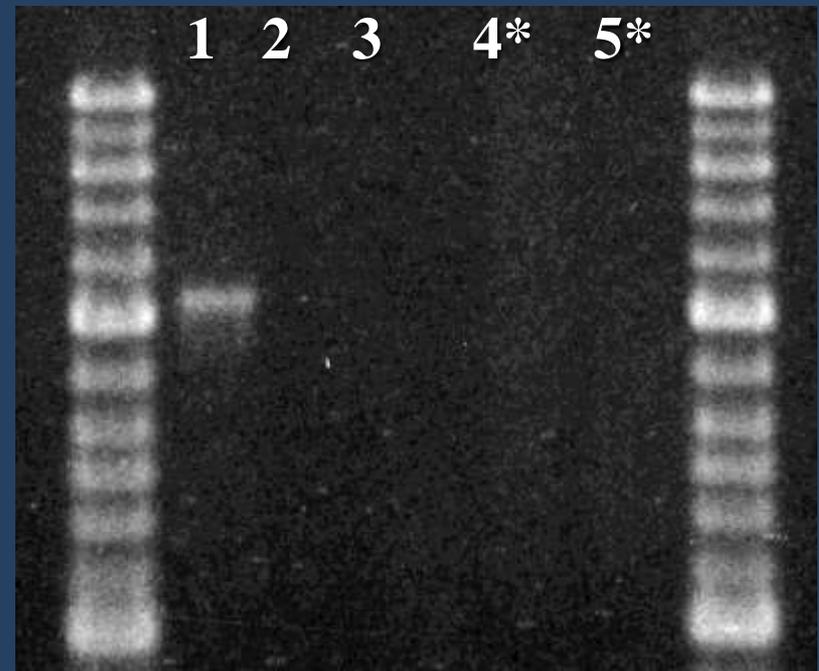
PCR analysis of human brain.

DNA extracted from paraffin-embedded brain tissue was subjected to PCR analysis.

1.kb 2. + Control 3. - Control 4. Control brain 5.*Patient X 6.Human DNA

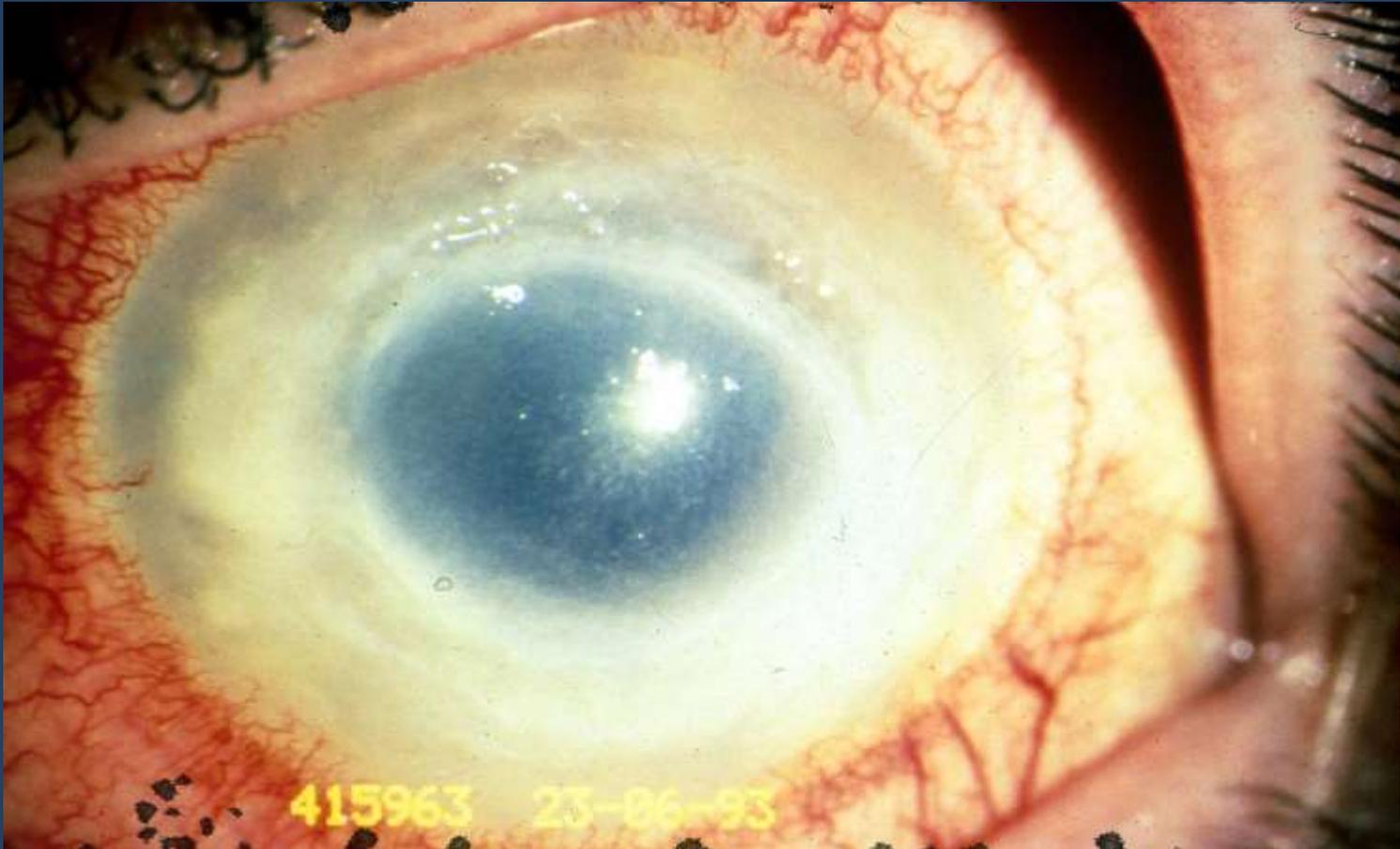


Acanthamoeba-specific primers



Balamuthia-specific primers

Amoebic keratitis — immunocompetent individual

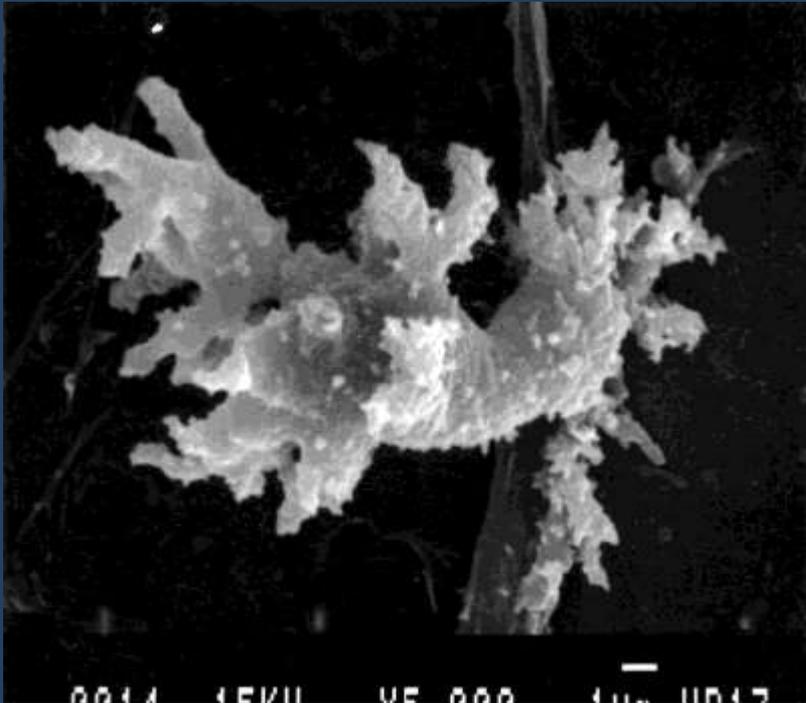


What is a major cause (predisposing factor) of *Acanthamoeba* keratitis? Contact lens wear

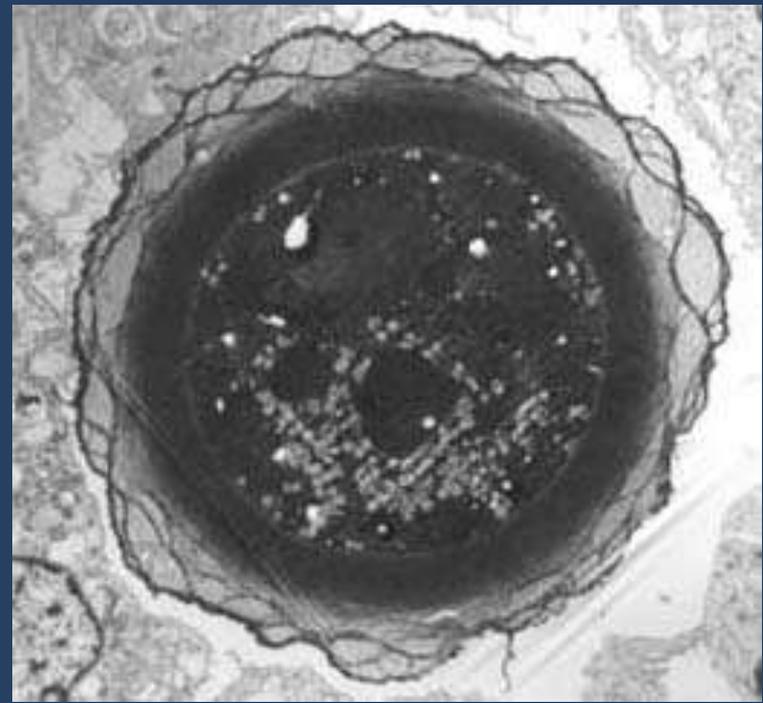
Balamuthia mandrillaris

- **Recognized as a pathogen in the 1990s**
- **First isolated from the environment in 2003 from a soil sample from a plant at the home of a child who died of GAE.**
- **No history of swimming, most likely acquired through soil**
- **Causes sub-acute to chronic granulomatous amebic encephalitis (GAE), as well as granulomatous skin and lung lesions**

Balamuthia mandrillaris



Trophozoite – infective stage

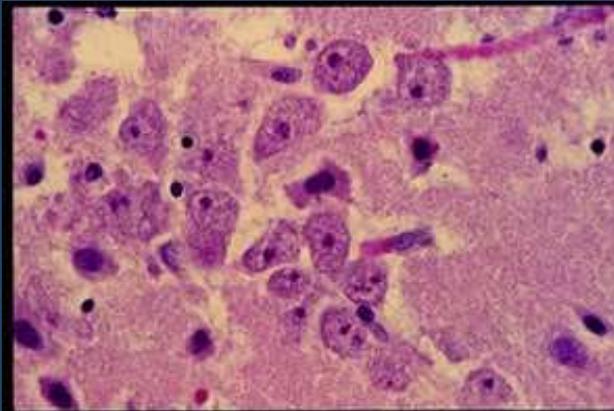


Cyst

Balamuthia Infections



www.itg.be



www.dpd.cdc.gov



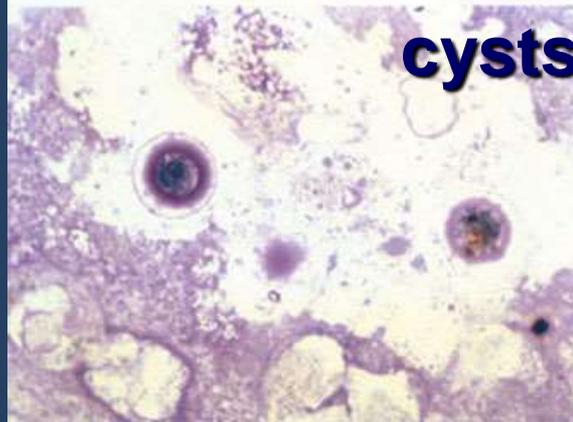
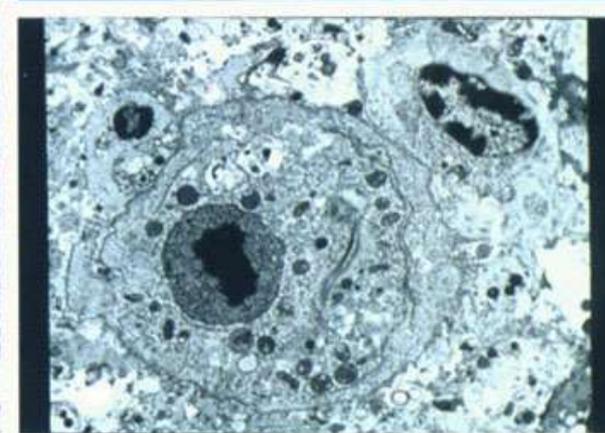
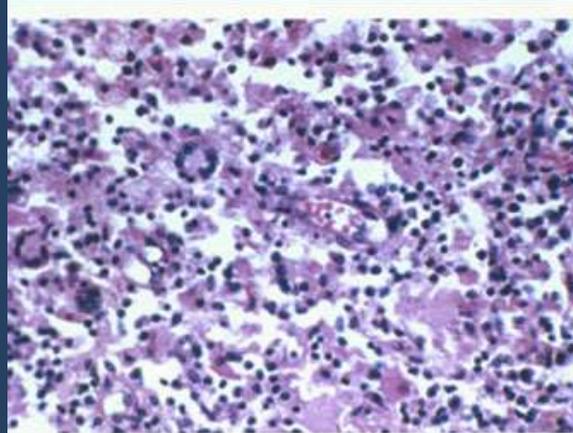
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Balamuthia mandrillaris
BAE

Brain with
Necrotizing
Encephalitis

Brain Xs with
Granulomatous Rx

Trophozoite (TEM)
Cysts



GAE – *Balamuthia* sp.

Symptoms & Clinical Signs

Confusion, Somnolence, Irritability, Seizures, Hemiparesis, Aphasia, Cranial nerve palsies, Diplopia, Nausea, Headache, Dizziness.

Laboratory Diagnosis

**CSF - Moderate Mononuclear pleocytosis,
No Bacteria or Fungi
Elevated Protein, Low Glucose
Amebic Trophs & Cysts – Not Usually Seen,
Brain Bx – Necrosis, Perivascular Amebic Trophs & Cysts
Immunofluorescence, PCR & Real Time PCR**

Differential Diagnosis

Acute Pyogenic (Bacterial) Meningitis

In Summary

- ▶ Although infections with FLA are rare, these infections are almost always fatal.
- ▶ *Acanthamoeba* & *Balamuthia* are opportunistic pathogens.
- ▶ *Naegleria* is not an opportunistic pathogen.
- ▶ Pathology is due to the direct lytic action of amebae on brain tissue as well as the over production of proinflammatory cytokines and chemokines. (Inflammation)

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help make others aware of the
disease to prevent further
infection.**

