Case Conference

West Coast Transplant ID Society

DATE: November 1,2023 PRESENTED BY: Poornima Ramanan MD

A 64-year-old male lung transplant recipient..

Chief complaints:

- presents with **headache** and **intermittent altered mentation** for **2-3 weeks**, 3 years after lung transplant (3/2022)
- On presentation to the hospital, **seizures** were observed

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A 64-year-old male lung transplant recipient..

Five weeks prior to presentation:

- admitted for ~3 weeks at a hospital in El Paso, Texas for hemoptysis and dyspnea
- CT chest showed new nodular opacities in left lung allograft
- Respiratory cultures grew *Aspergillus* sp not speciated or sent for susceptibilities
- Blood *Aspergillus* galactomannan was + at 1.35 and BDG was 295
- He was in ICU for few days but did not require mechanical ventilation or pressors
- He received ambisome for 3 weeks
- He did not have any CNS symptoms or brain imaging/LP done at OSH

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A 64-year-old male lung transplant recipient..

Transplant details:

- S/p single lung transplant in 2019 for interstitial lung disease
- CMV D-/R+, EBV D+/R+, Toxoplasma D-/R+
- Maintenance immunosuppression: Tacrolimus 1.5 mg twice daily, prednisone 5 mg daily
- No rejection episodes
- Post- transplant infectious complications:
  - Right native lung pulmonary aspergillosis/ fungal balls in May 2021, on voriconazole since May 2021. Had therapeutic levels at OSH indicating medication compliance
  - Intermittent reactivation CMV viremia
- Antimicrobial prophylaxis: Bactrim, valganciclovir (+ voriconazole)
- He moved to El Paso TX in 2020 and was intermittently lost to follow up

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A 64-year-old male lung transplant recipient..

Exposure history:

- Born and raised in Mexico
- Moved to the U.S. ~20 years ago
- Previously lived in Denver but moved to El Paso Texas in 2020
- Frequent travel (multiple times a year) to Ciudad Juarez, Mexico
- Lived with his wife
- Worked for a landscape company previously
- No relevant animal exposures
- No recent known sick contacts
- No known contact with someone with tuberculosis

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A 64-year-old male lung transplant recipient..

Physical exam:

Afebrile, stable vitals

General: ill-appearing, lying in bed, eyes closed, in restraints

HEENT: R sided conjunctival hemorrhage, no scleral icterus

Cardiovascular: regular rate and rhythm, no murmurs

Respiratory: symmetric expansion, no increased work of breathing, supplemental O2 via NC 2L

Abdomen: soft, nontender, normoactive bowel sounds

Extremities: moves all, no edema, no deformity

Skin: no open wounds, no rashes, normal temperature

Neuro: **oriented to person, place, year. No focal deficits noted. Awake but not completely alert**

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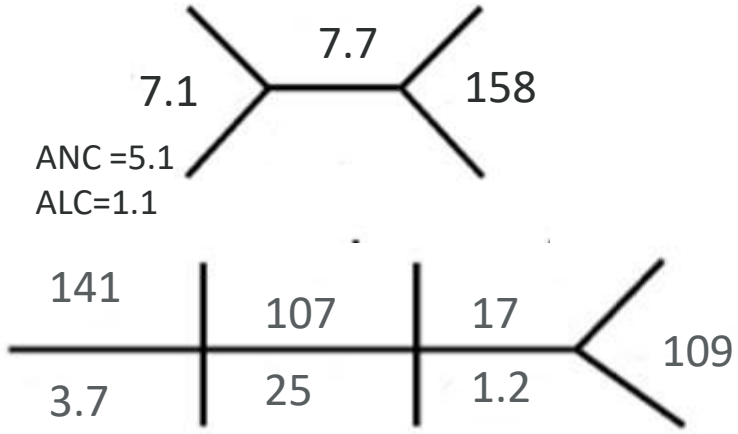
A 64-year-old male lung transplant recipient..

Laboratory data:



CMV Quantitative PCR on whole blood	EBV Quantitative PCR on whole blood
0 IU/mL	0 IU/mL
<1,000 ^	
0	
<1,000 ^	
<1,000 ^	
<1,000 ^	
	<1,000 ^
0	
12,950 ^	
212,000 ^	2,460 ^

Normal LFTs

Voriconazole level 3.9

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A 64-year-old male lung transplant recipient..

Imaging : MRI brain

Parenchyma:
Ring-enhancing lesions within the bifrontal lobes corresponding foci of mild diffusion restriction of the nonenhancing central component. The left inferior frontal lesion measures 14 mm diameter. The right frontal peripherally enhancing collection measures up to 15 mm diameter. There is adjacent more heterogeneous enhancement with some central nonenhancing areas more superficially measuring up to 2.5 cm diameter. Corresponding T2 FLAIR abnormality surrounding these lesions on one day prior MR brain. No acute infarct.

Extra-axial spaces:
No extra-axial hemorrhage, collections, or adverse intracranial mass effect. Pachymeningeal enhancement along the right frontal convexity.

Volume:
Parenchymal volume is normal. No hydrocephalus.

Calvarium:
Normal

Extra-cranial:
Orbits unremarkable. The visualized paranasal sinuses are clear. No large mastoid or middle ear effusions.

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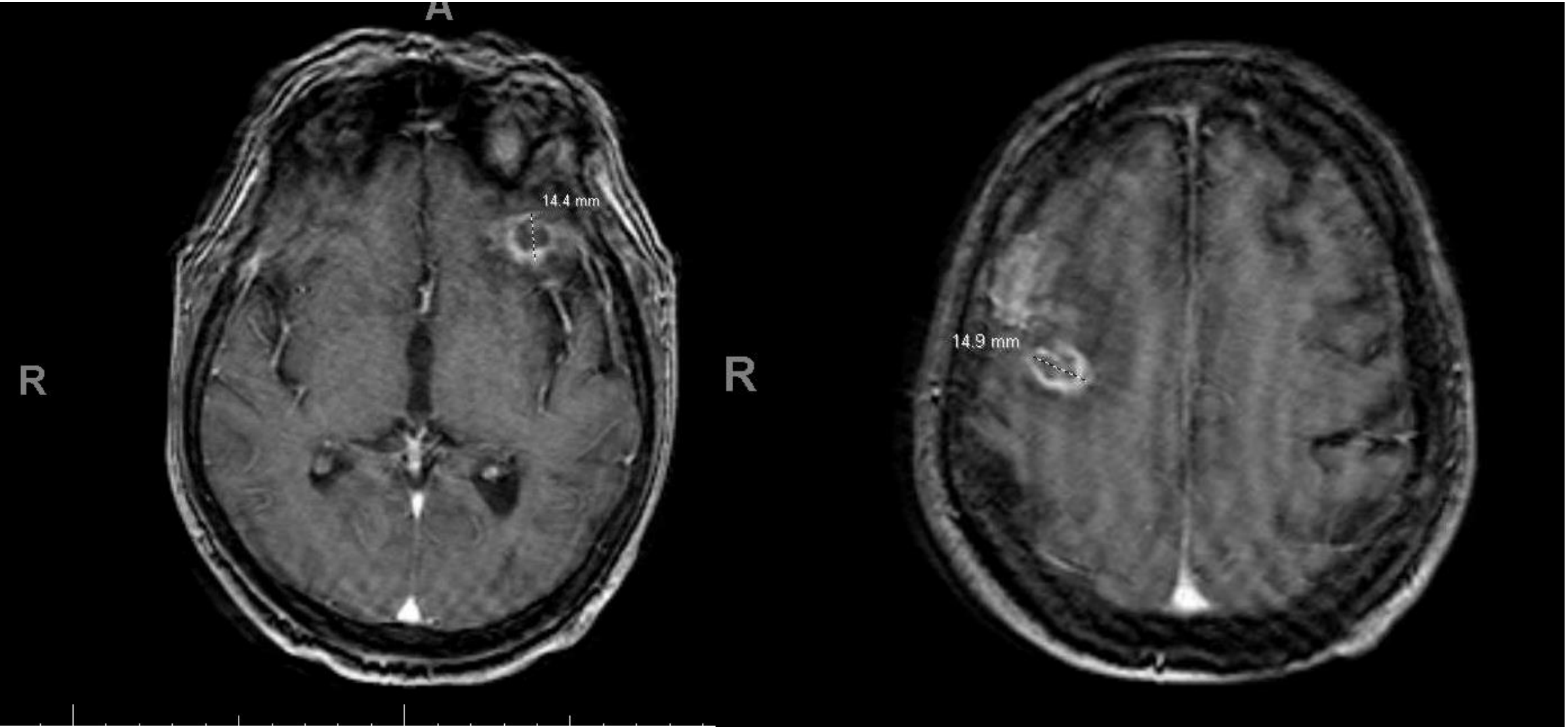
A 64-year-old male lung transplant recipient..

Imaging : MRI brain



A 64-year-old male lung transplant recipient..

Imaging - CT chest

Visualized portion of thyroid gland appears unremarkable. Tip of the left upper extremity PICC terminates in the lower SVC. Mild body wall anasarca. No intrathoracic adenopathy. Trace pericardial thickening without effusion. Minimal coronary artery calcifications.

The central airways are patent. Small volume left pleural effusion. No pneumothorax. Status post prior left unilateral transplantation. Limited evaluation of the transplanted lung given marked respiratory motion artifacts. However, within this limitation, there are focal areas of mostly groundglass predominant nodular opacities. The native left lung appears fibrotic containing multiple mycetomas.

Visualized upper abdomen appears unremarkable. Sequelae of remote right lower rib trauma. Mild multilevel degenerative changes in the thoracic spine.

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A 64-year-old male lung transplant recipient..

He undergoes bronchoscopy with BAL

OTHER BODY FLUIDS	
Fld color tube 2	Colorless
APPEARANCE BODY FLUID	Cloudy
SPECIMEN SOURCE	Bronchial...
NUCLEATED CELLS FLUID	3,875
RED BLOOD CELL CT BODY FLUID	227
Neutrophil, Fluid	78
Lymphocytes Body Fluid	1
MONOCYTES/MACROPHAGES BF	11
OTHER MONONUCLEAR CELLS BF	9
Eosinophils Body Fluid	1
Total Cells Counted on Differential	100
Bacteria, Body Fluid	Present

Final Cytologic Diagnosis
A. Lung, right, bronchoalveolar lavage:
- Marked acute inflammation, pulmonary macrophages, benign respiratory epithelial cells
- Fungal elements confirmed by GMS special stain (see comment)
- Negative for malignant cells

Adequacy: Satisfactory for evaluation.

Comment
The fungal hyphae show septations and branching at 45 degree angles, which is compatible with Aspergillus spp. The AFB stain is negative for acid fast bacilli. Correlate with microbiological, serologic and other clinical and radiographic findings.

BAL fungal cultures grew ***Aspergillus flavus***

BAL *Aspergillus* galactomannan **6.99**

BAL RPP + for **Influenza A**

BAL M.tb PCR negative

BAL PJP PCR negative

Rest of ID work up negative

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A 64-year-old male lung transplant recipient..

Laboratory data:

	Day 1		Day 4
COLOR CSF	Colorless	Colorless	Colorless
APPEARANCE CSF	Clear	Clear	Clear
CSF TUBE NUMBER	#1	#4	#4
NUCLEATED CELL COUNT CSF	36 ▲	37 ▲	23 ▲
CSF Red Blood Cell Count	8 ▲	2 ▲	62 ▲
LYMPHOCYTES CSF		98 ▲	99 ▲
MONOCYTES/MACROPHAGES CSF		2 ▼	1 ▼
Total Cells Counted on Differential...		100	100
Glucose CSF	44		60
PROTEIN TOTAL, CSF	85 ▲		103 ▲

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Final Cytologic Diagnosis
A. Cerebrospinal fluid:
- Pleocytosis with lymphocytes, rare plasmacytoid cells, monocytes and
red blood cells (see comment)
- Negative for malignant cells or intracytoplasmic fungal forms
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A 64-year-old male lung transplant recipient..

So, to recap..

We have a single lung recipient from Mexico with known native lung *Aspergillus* mycetomas on therapeutic level of voriconazole who presented with intermittent fevers, encephalopathy with CSF lymphocytic pleocytosis, new multiple ring enhancing brain lesions , new GGO and nodular opacities in lung allograft (BAL + for Influenza A and *Aspergillus flavus*) and CMV viremia

At this point, patient is on the following antimicrobial therapy:

1. **Ambisome** – for presumed disseminated aspergillosis (remember, he received ambisome at OSH)
2. IV **ganciclovir** + **cytogam** – reactivation breakthrough CMV viremia (CMV drug resistance testing showed pan-sensitive virus)
3. **Oseltamivir** (10-day course for Influenza A LRTI)
4. **Meropenem** (empiric)

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Differential diagnosis

What further work up would you want?

A 64-year-old male lung transplant recipient..

More infectious diseases work up:

ID work up from blood/serum

Quantiferon TB Gold negative

Beta- D glucan **230**

Serum Aspergillus galactomannan **2.6**

Serum cryptococcus antigen negative

Serum and urine *Histoplasma* antigen negative

Coccidiodes serology negative

Cysticercosis IgG negative

Echinococcus IgG negative

Serum *Toxoplasma* PCR negative

Blood cultures negative

ID work up from CSF

JC PCR negative

CMV PCR negative

HSV PCR negative

VZV PCR negative

EBV PCR + at 1030

HHV-6 PCR negative

Adenovirus PCR negative

CrAg negative

M.Tb PCR negative

Toxoplasma PCR negative

West Nile IgM and IgG negative

CSF Fungitell negative

Bacterial, fungal and AFB culture NGTD

CSF free living amoebae PCR negative

CSF broad range PCR negative

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Repeat MRI brain after 10 days shows worsening brain lesions

Any additional thoughts?

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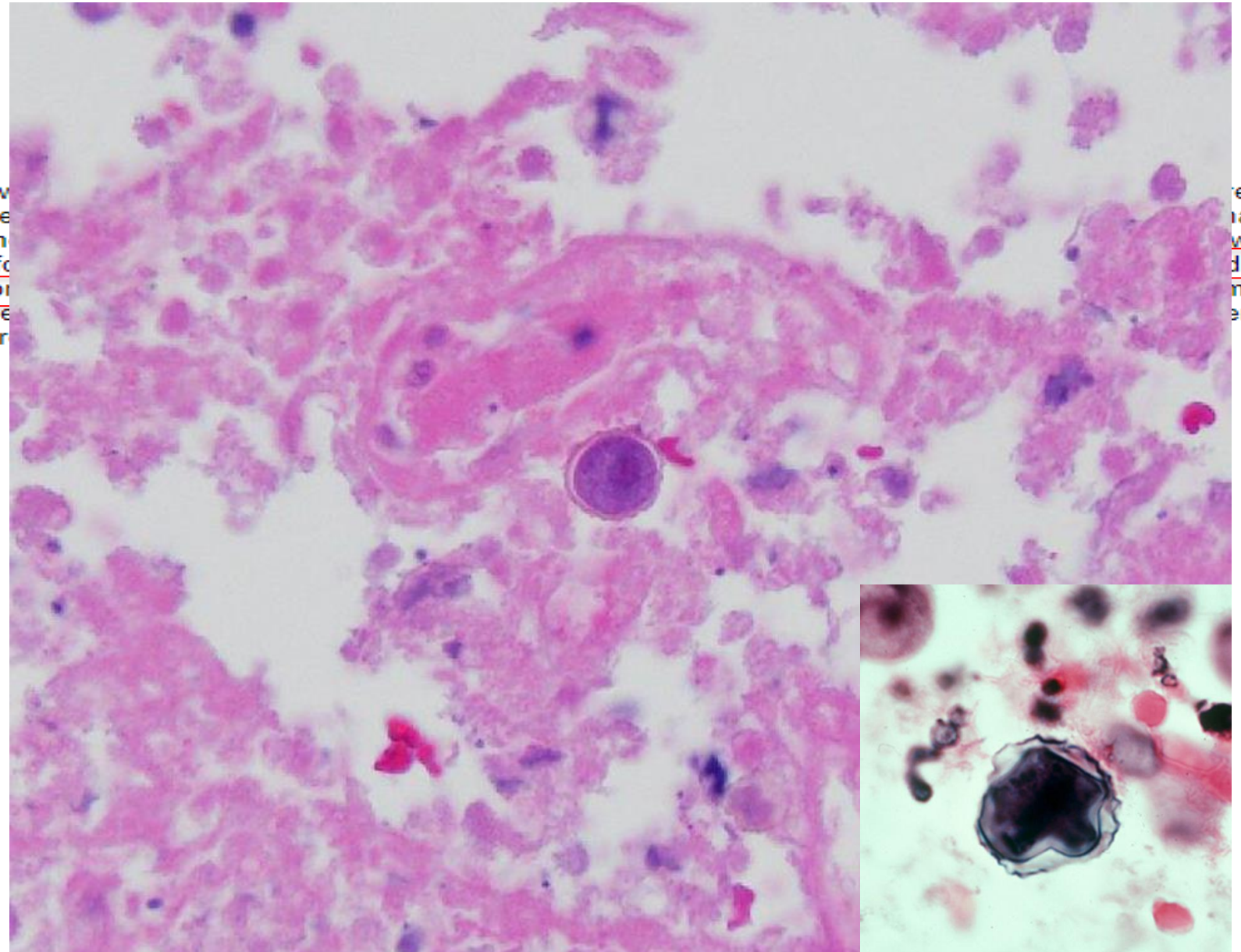
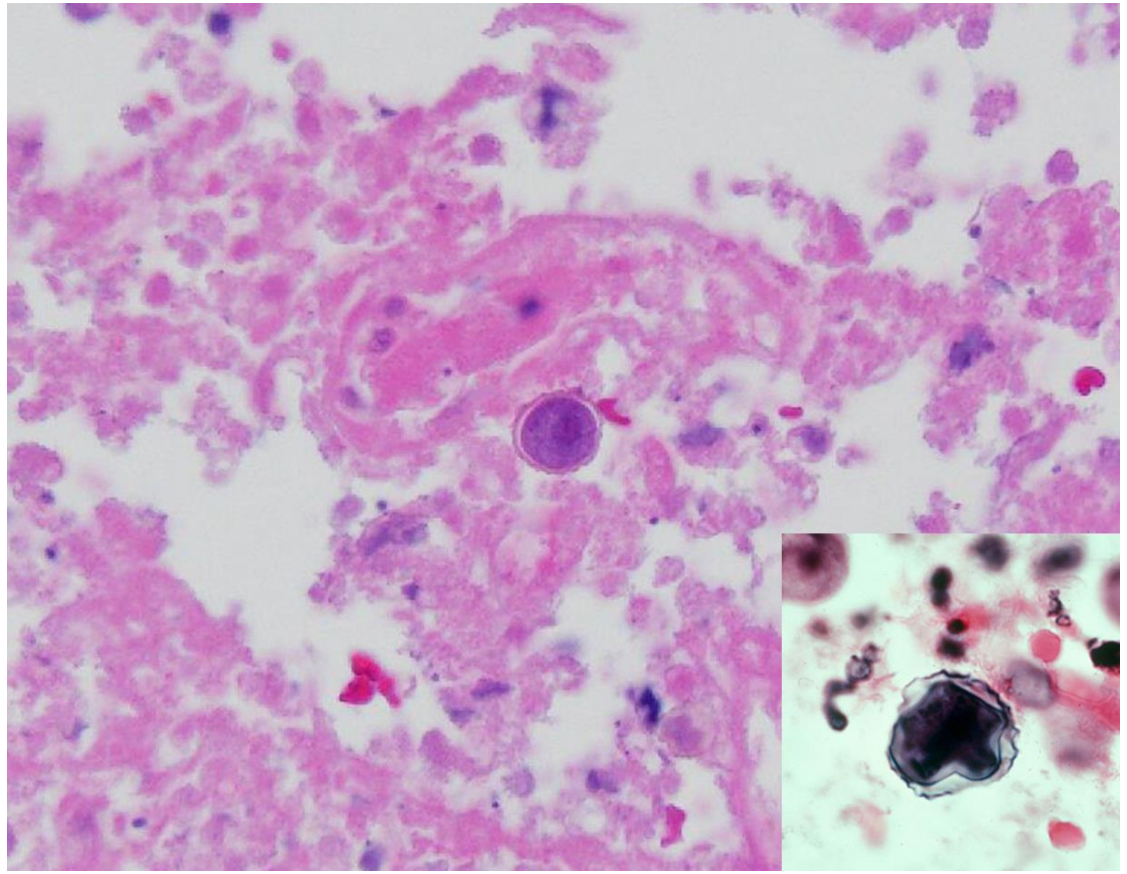


What IS that thing?

1. *Prototheca*
2. *Coccidioides*
3. *Acanthamoeba*
4. *Scedosporium*
5. *Toxoplasma*
6. *Naegleria*



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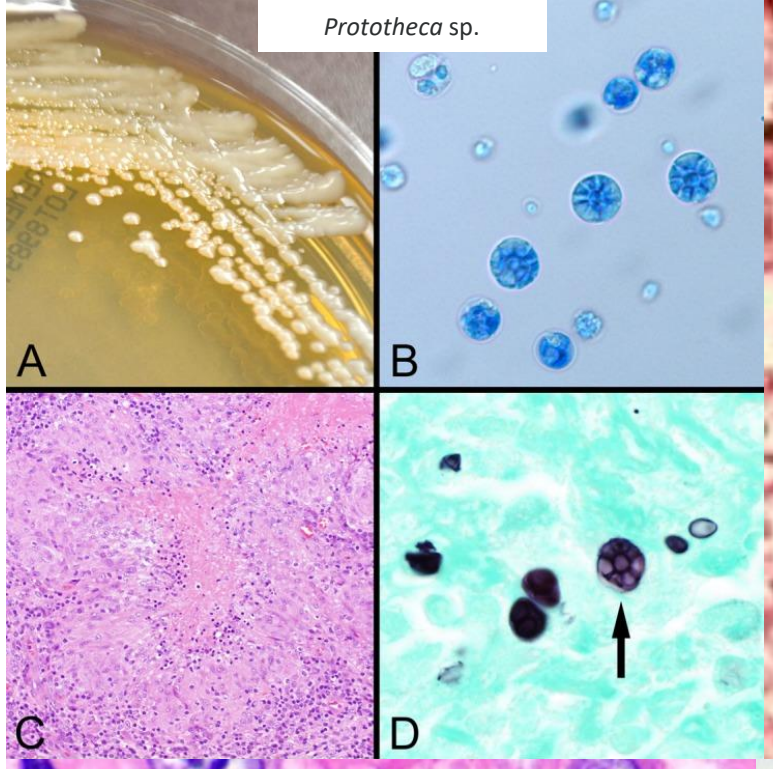
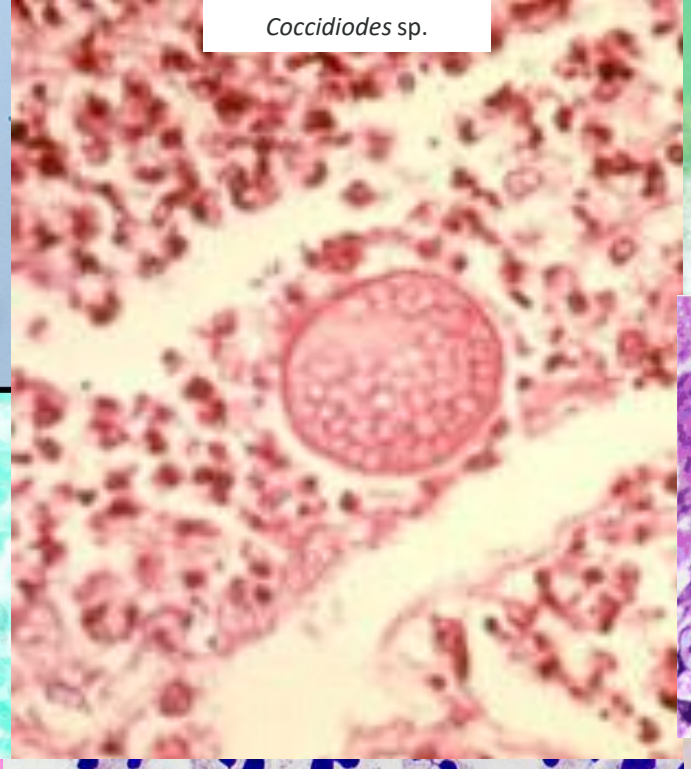
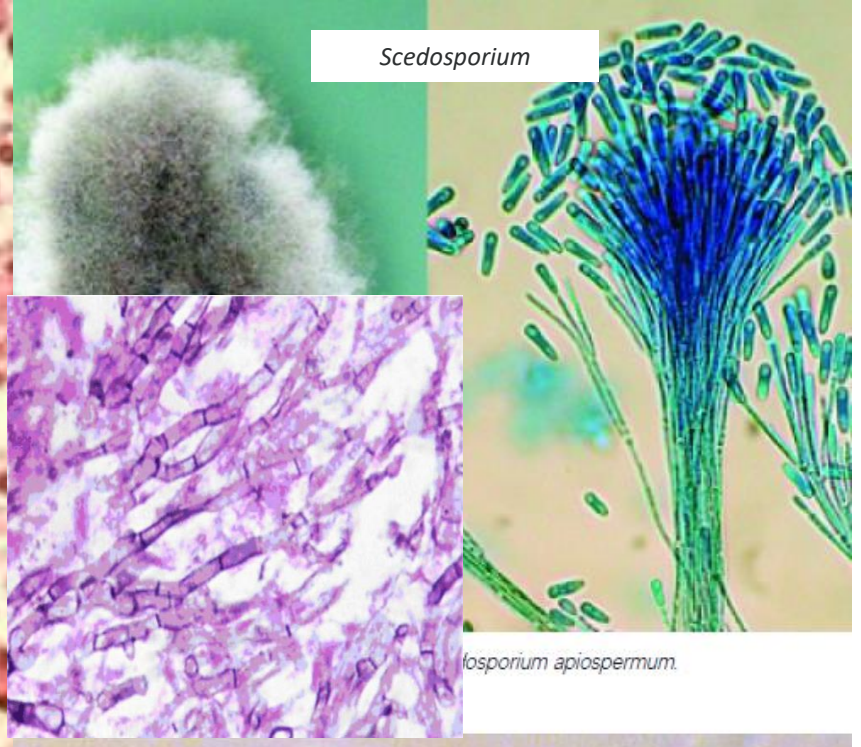
Prototheca sp.



Coccidiodes sp.

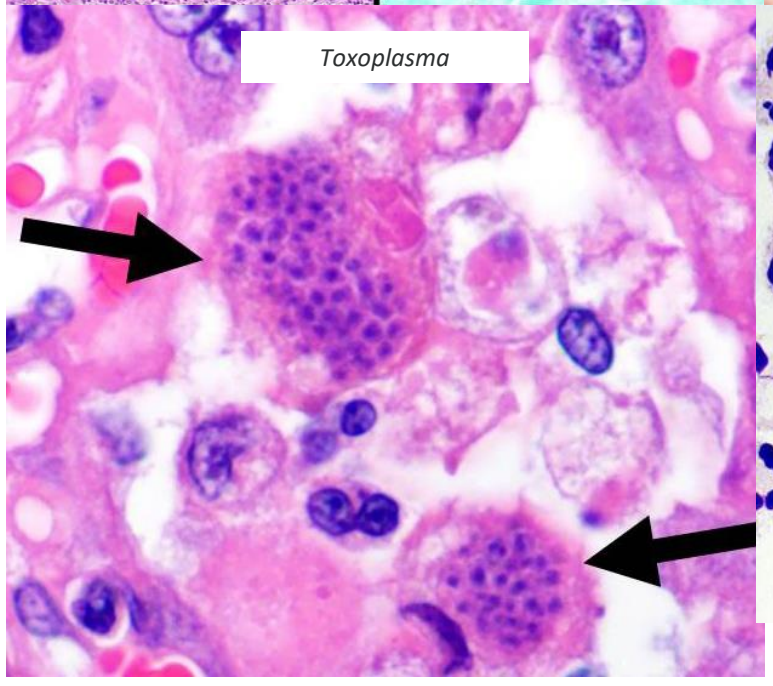


Scedosporium

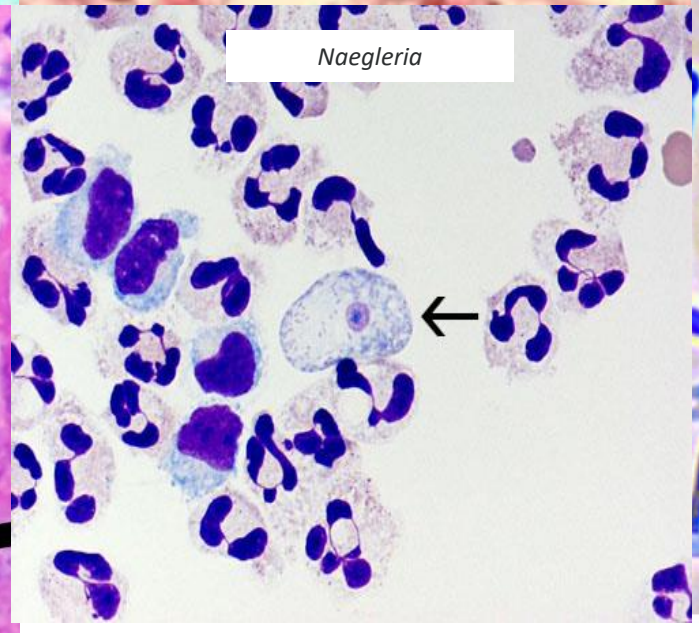


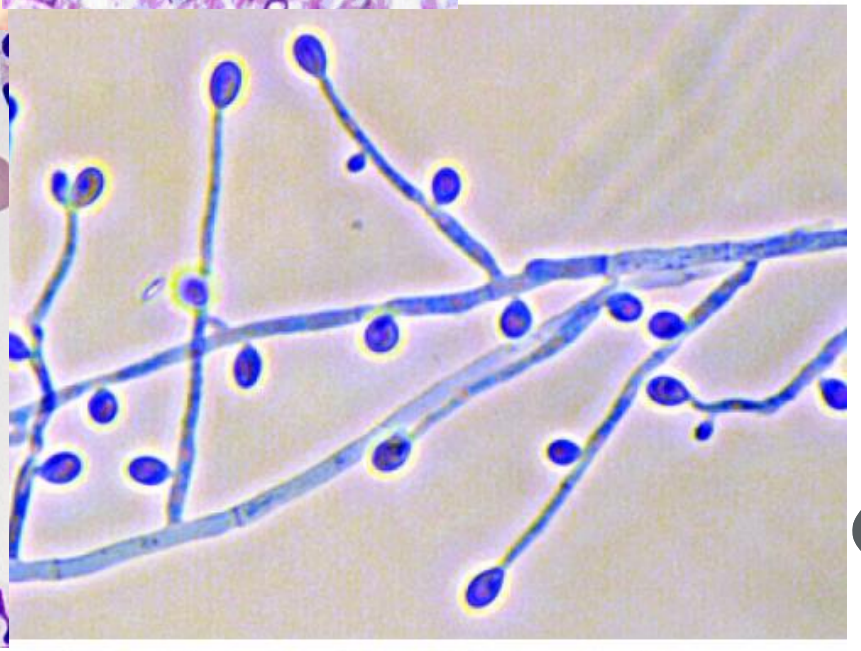
fosporium apiospermum.

Toxoplasma



Naegleria





Scedosporium apiospermum conidiophores and conidia.

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Final diagnosis

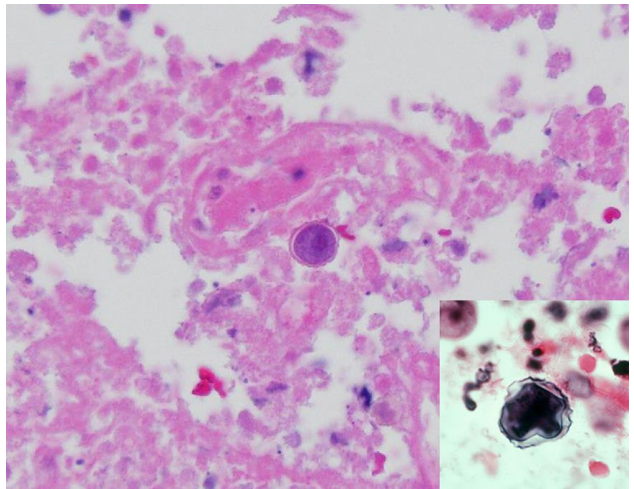
(confirmed by PCR, culture and histopath of brain tissue)

I. **Granulomatous Amebic Encephalitis due to *Acanthamoeba* sp.**

II. CMV viremia

III. Probable pulmonary aspergillosis in allograft + native lung mycetomas

IV. Influenza A LRTI



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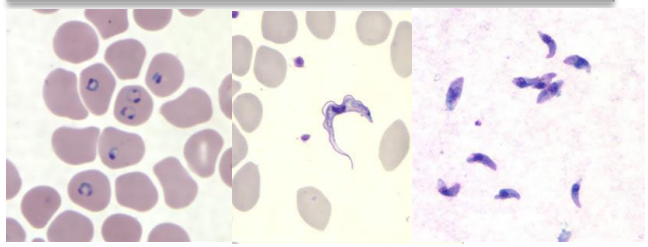
Parasites

Helminths

Protozoa



Ectoparasites

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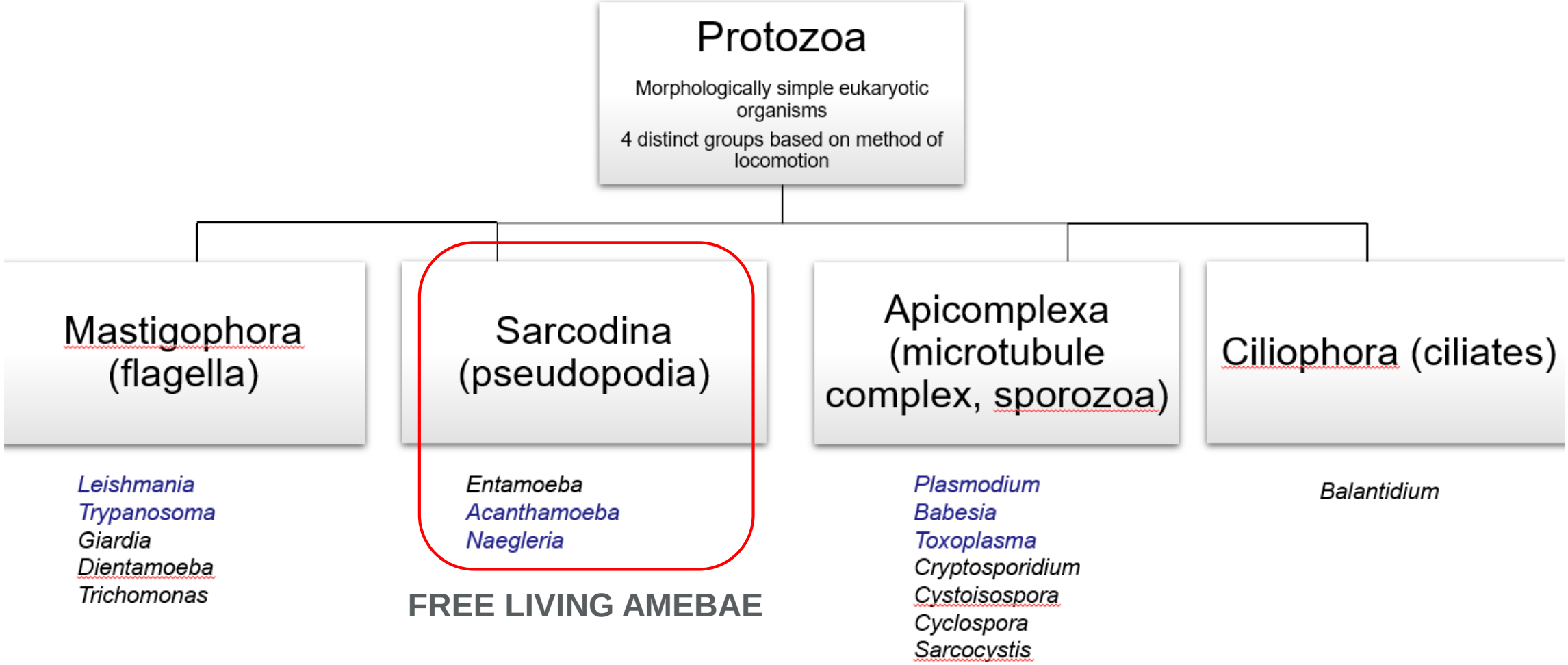
Protozoa
Morphologically simple eukaryotic organisms
4 distinct groups based on method of locomotion
Mastigophora (flagella)
Sarcodina (pseudopodia)
Apicomplexa (microtubule complex, sporozoa)
Ciliophora (ciliates)
Leishmania
Trypanosoma
Giardia
Dientamoeba
Trichomonas
Entamoeba
Acanthamoeba
Naegleria
FREE LIVING AMEBAE
Plasmodium
Babesia
Toxoplasma
Cryptosporidium
Cystoisospora
Cyclospora
Sarcocystis
Balantidium

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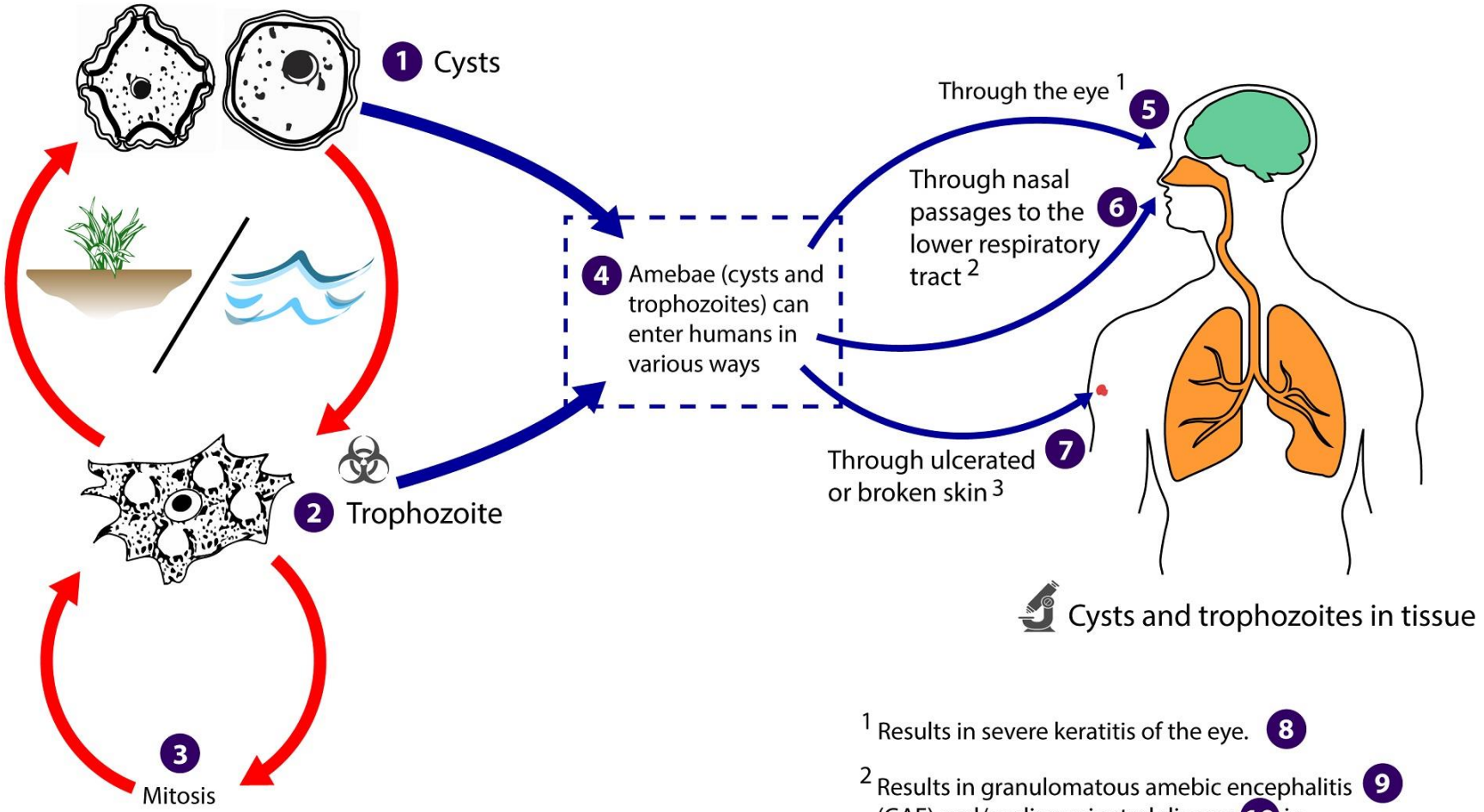
Acanthamoeba spp.



Infective stage

Diagnostic stage

[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

Clinical syndromes

1. **Severe keratitis**, especially in contact lens users
2. **Cutaneous** acanthamebiasis
3. **Disseminated** infection (skin, lungs, brain) in immunocompromised patients
4. **Granulomatous amoebic encephalitis** (GAE)
    - Gradual onset with progressive worsening over weeks to months
    - Signs of meningoencephalitis
    - High fatality rate, very few known survivors

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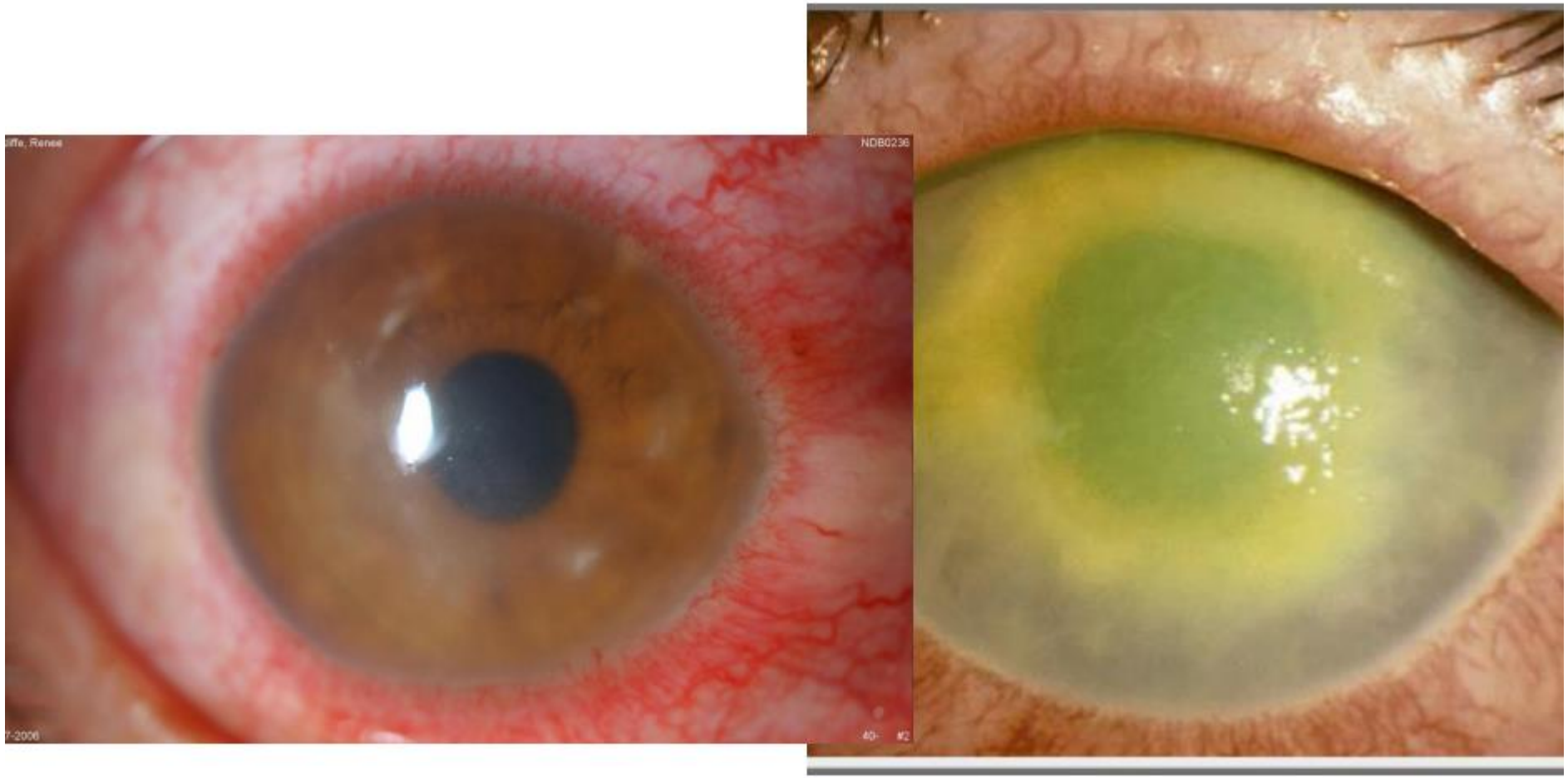
Acanthamoeba keratitis



CDC

Cutaneous Acanthamebiasis (isolated cutaneous or disseminated disease)

- Lesions may be single or multiple
- Chronic lesions may appear crusted, ulcerated, indurated, nodular and may have eschar
- May be mistaken for fungal or mycobacterial infection or cutaneous leishmaniasis

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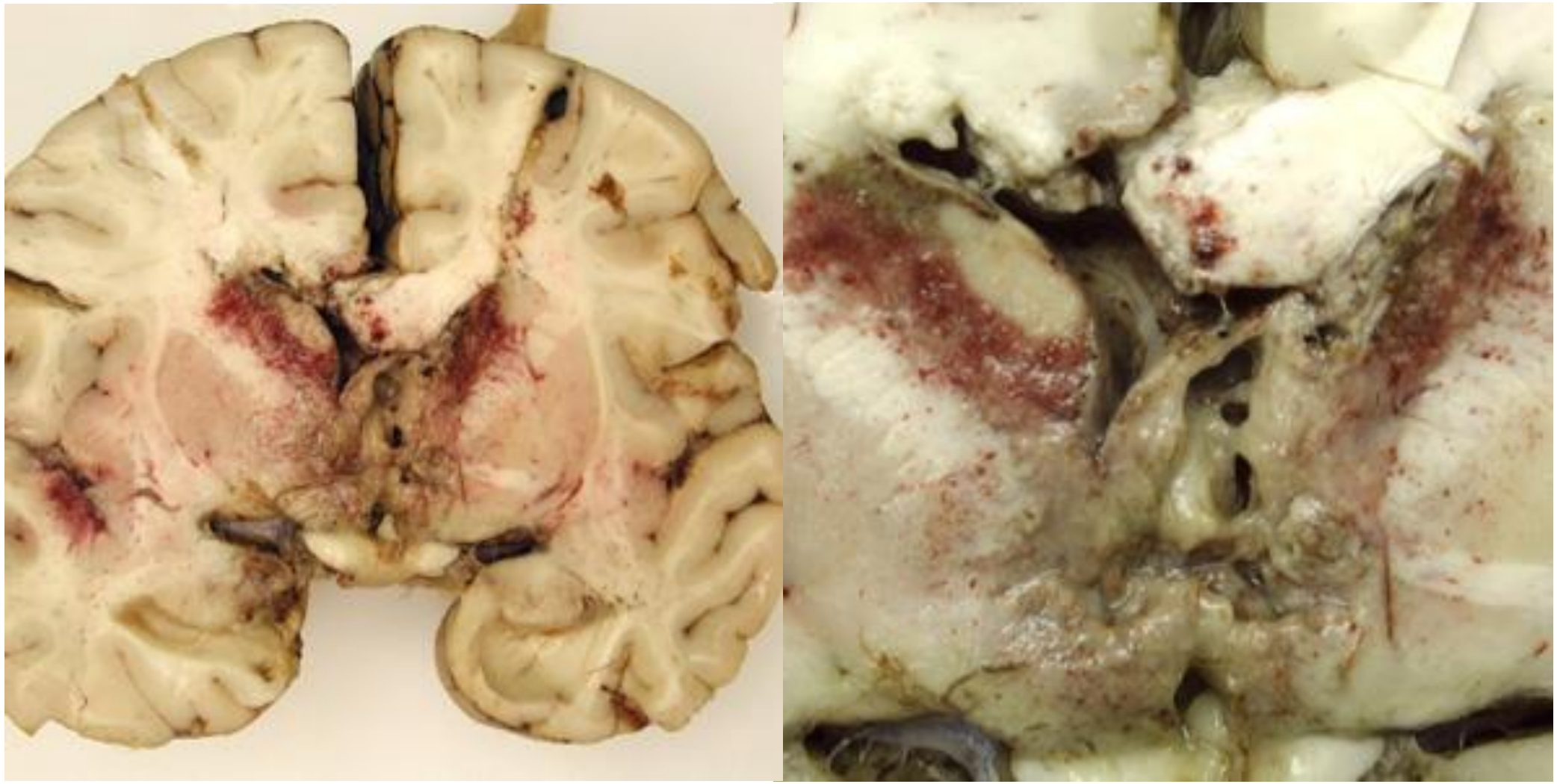
Granulomatous Amebic Encephalitis (GAE)



Gross specimen of brain tissue from a patient who died of GAE. The autopsy specimen revealed **extensive necrotizing granulomatous amebic encephalitis (GAE)** with a subependymal necroinflammatory process. Image courtesy of Cook Children's Hospital, Fort Worth, Texas.

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Diagnosis of free-living amebae infection is often challenging

A If you don't think about it, you will likely miss the diagnosis

1. Histopathology
2. PCR of brain tissue, CSF or skin or lung or sinus
3. Amoebic culture on non-nutrient agar plates overlaid with E.coli
4. Wet mount or Giemsa stain of CSF may show trophozoites

Test
Free-living Ameb
Specimen Sourc
Acanthamoeba sp
Critical resu
Naegleria fowl

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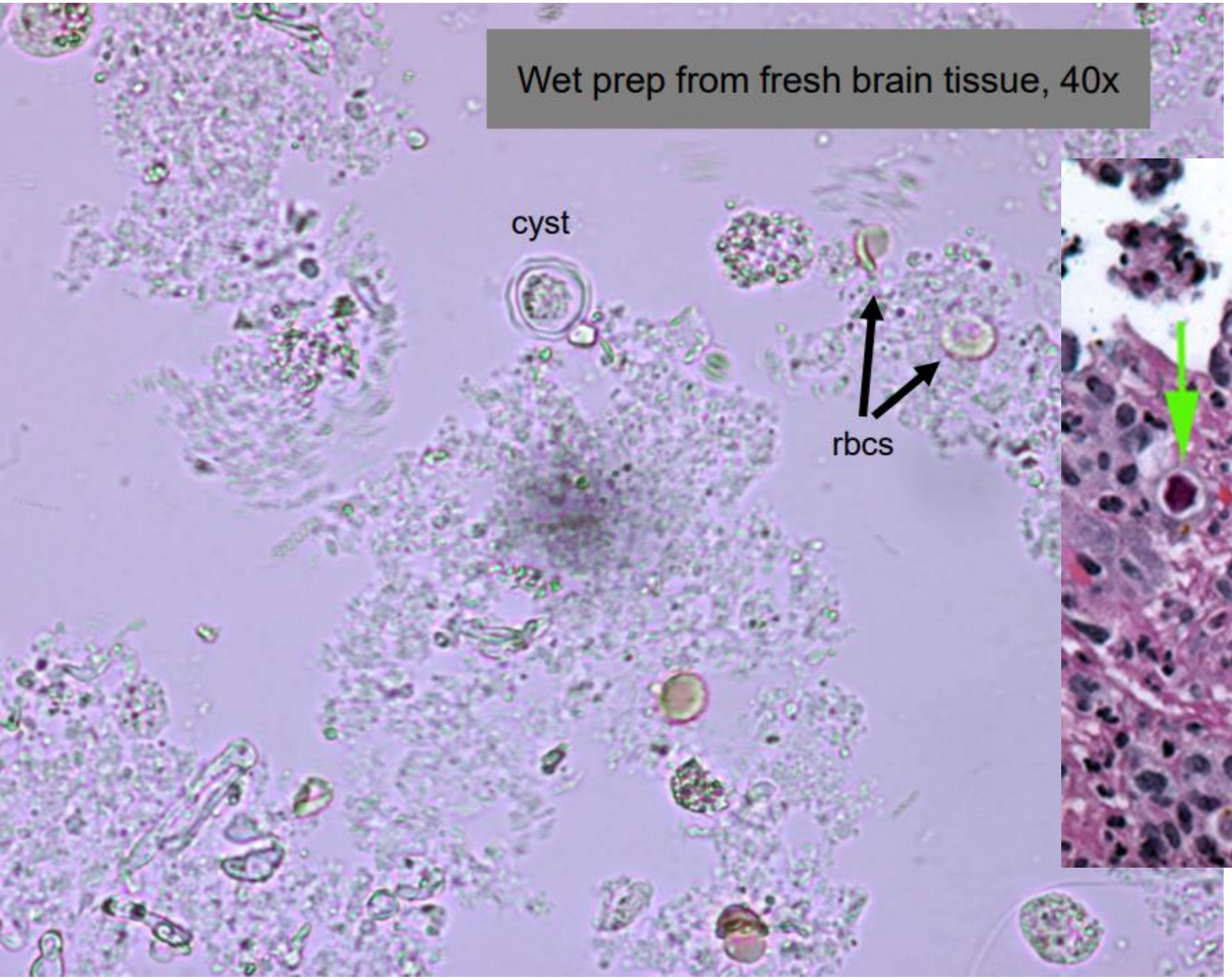

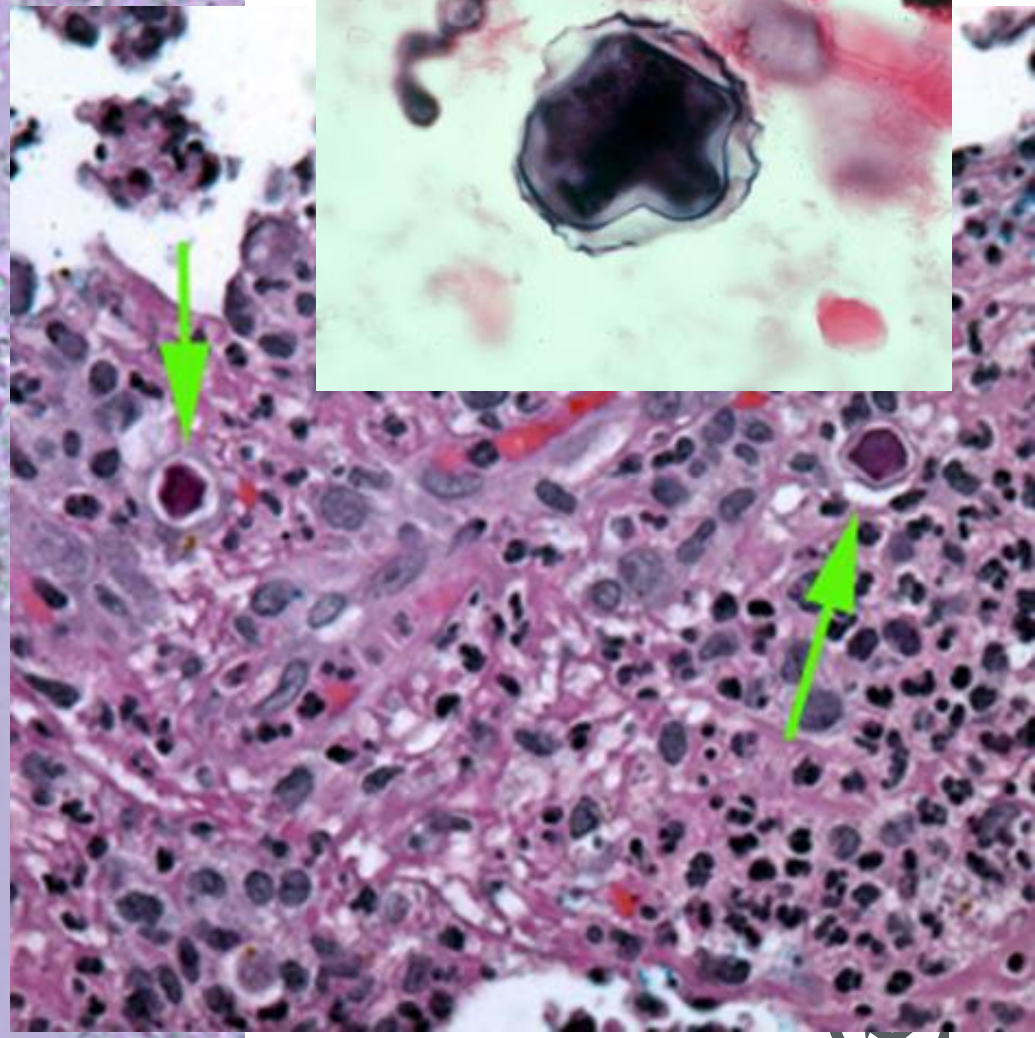

Cyst of *Acanthamoeba* sp. from brain tissue, stained with hematoxylin and eosin (H&E)
CDC, parasitewonders.com

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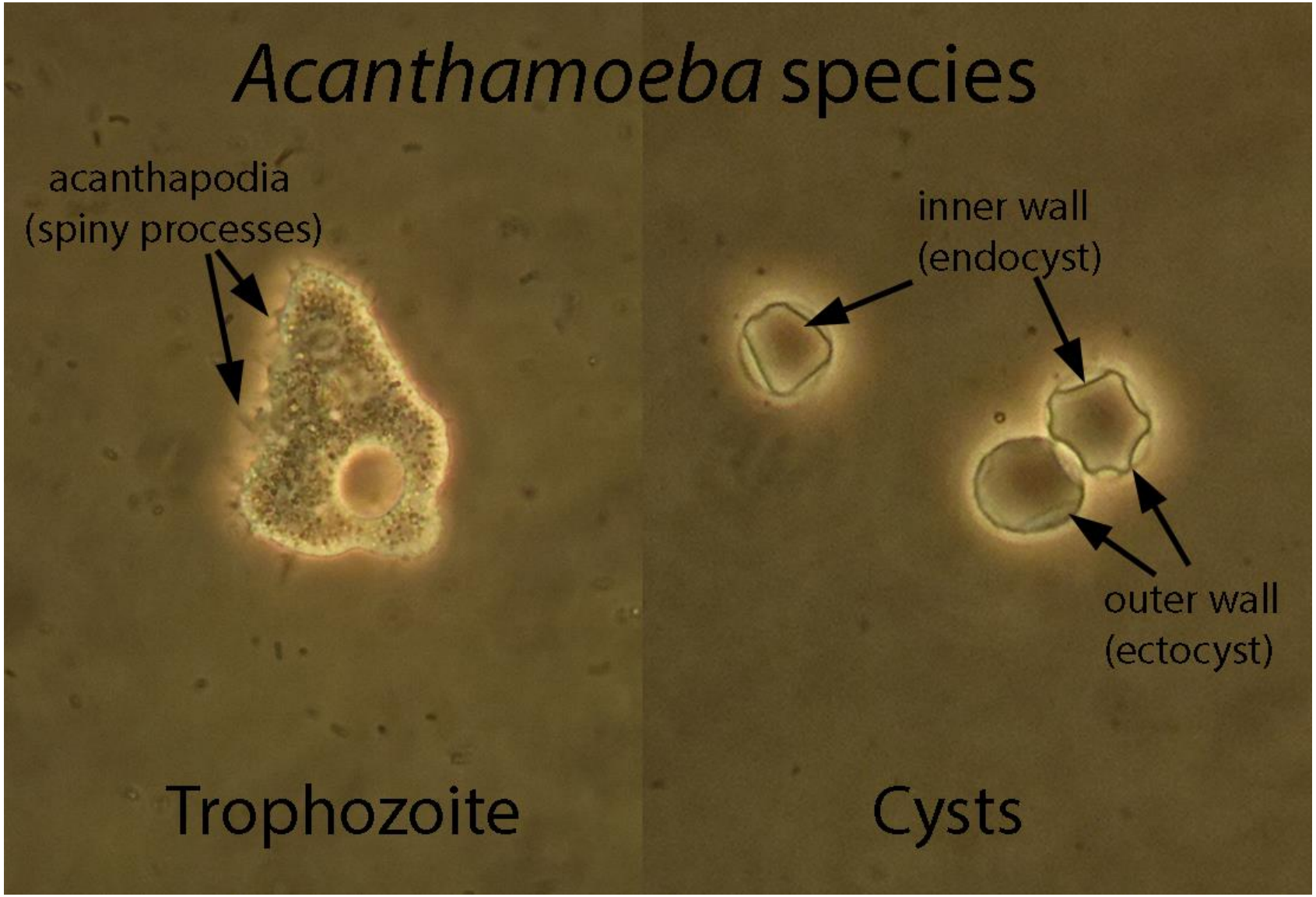
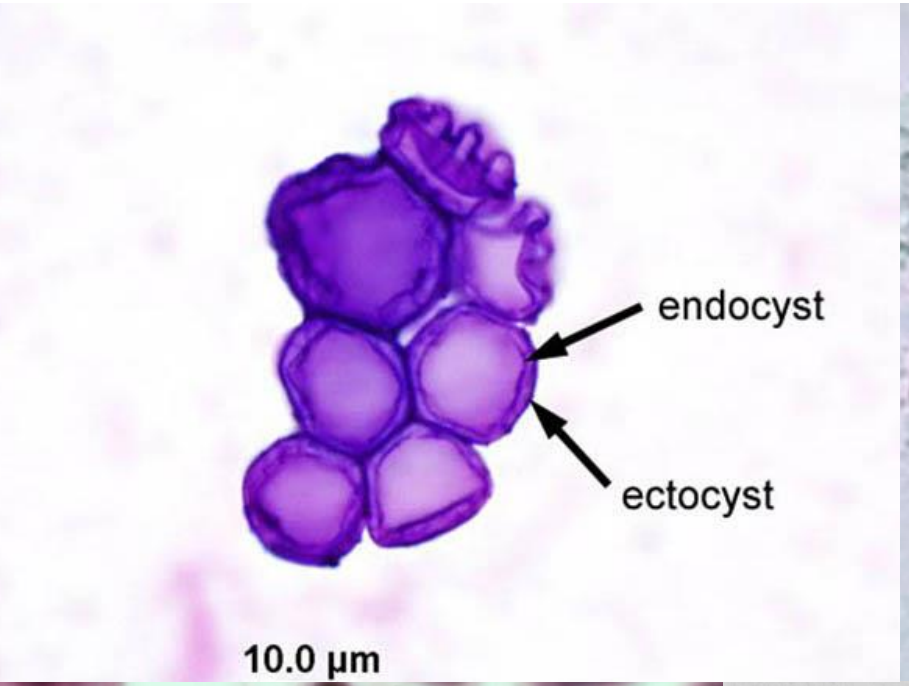
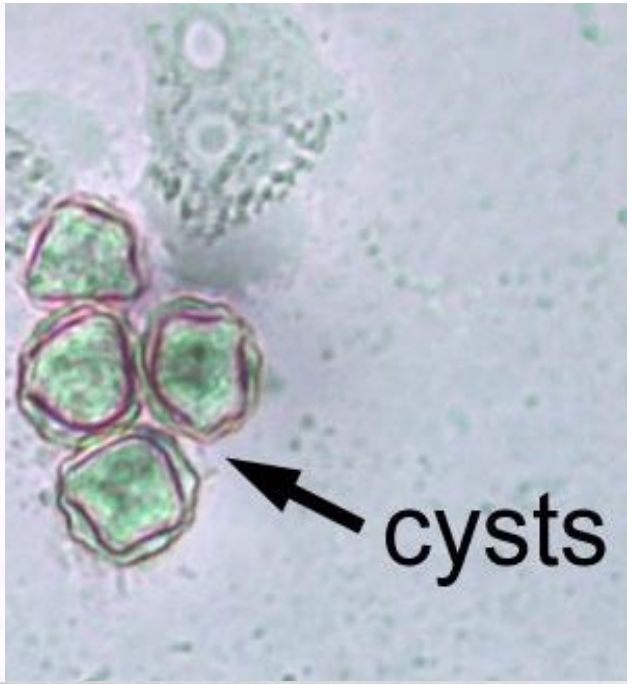


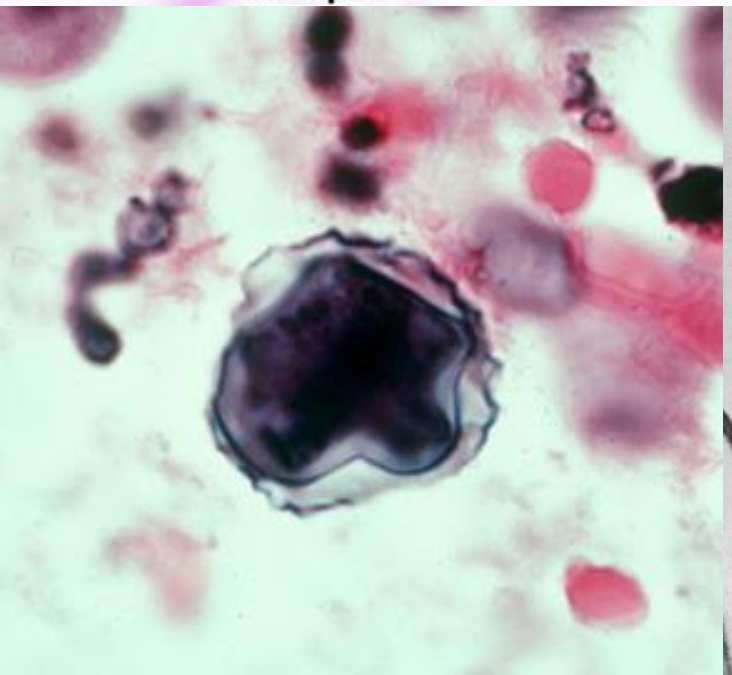
Acanthamoeba spp. and *Balamuthia mandrillaris* can show both cysts and trophozoites in human infection

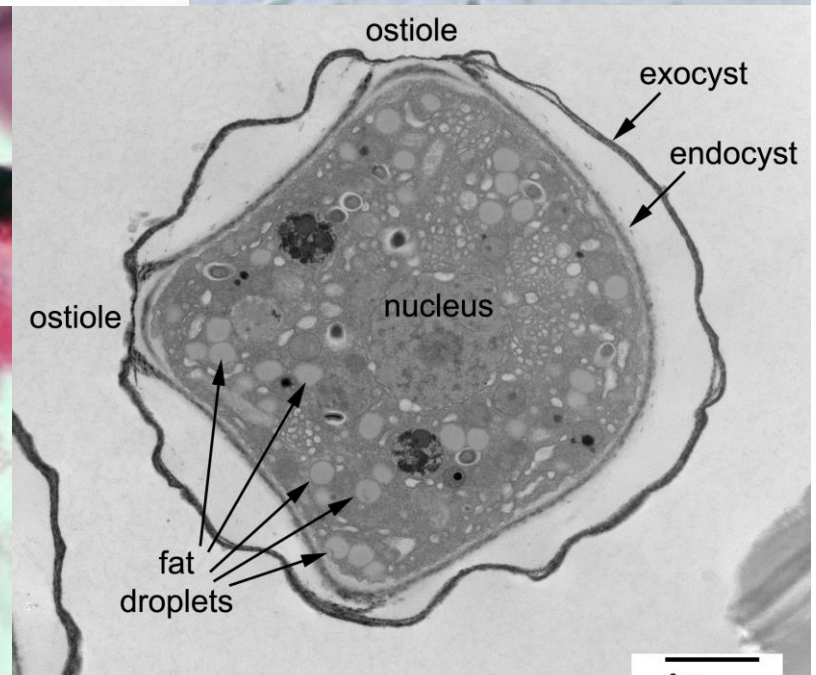
In contrast, only trophozoites are seen with *Naegleria* infection.The cysts are not seen in human infection

Acanthamoeba cysts









Acanthamoeba cysts with 2 walls - a wrinkled outer wall (exocyst) and inner star-shaped or polygonal wall (endocyst)

CDC, parasitewonders.com

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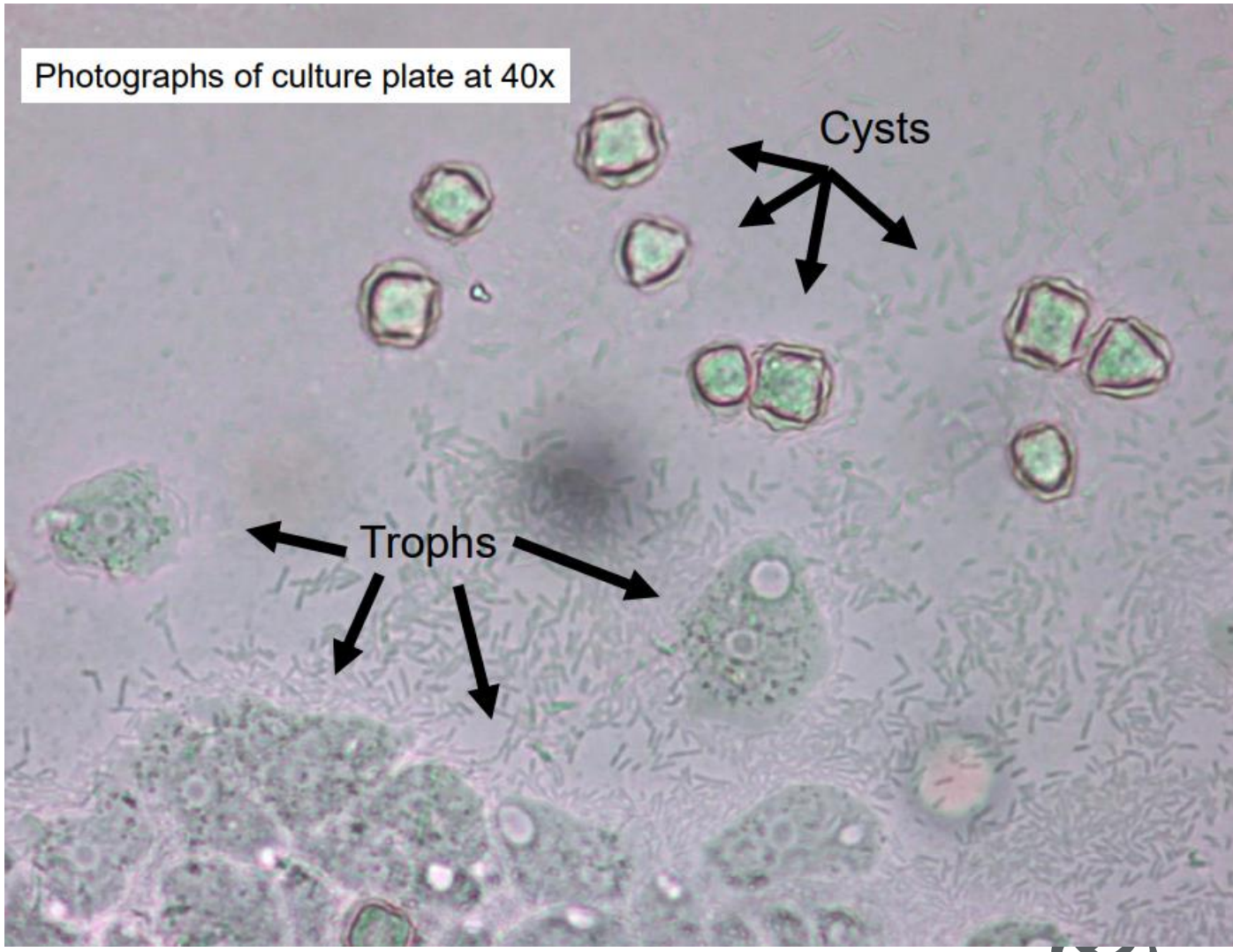
Culture of *Acanthamoeba* and *Naegleria*

Place specimen on non-nutrient “tap water” agar plated with a lawn of *Escherichia coli* for nutrients

incubate at 35ºC, check daily up to 5-7 days

Balamuthia does not grow in routine culture; requires cell culture which is not done in most clinical labs



Disseminated Acanthamoeba infection in a heart transplant recipient treated successfully with a miltefosine-containing regimen: Case report and review of the literature. Transplant Infectious Diseases 2017

Year	Patient Age in yrs/Gender	Location	Organ	Time to infection (months)	Immunosuppresive regimen	Type of infection	Treatment regimen	Diagnosis (dx) confirmation	Outcome	First author Reference
1982	38/M	Pennsylvania	Kidney	30	Azathioprine, MPS	Skin, lung, brain	Broad-spectrum antibiotics	Histopathologic staining of biopsy specimen	Died	Martinez[17]
1994	31/M	Texas	Kidney	10	Azathioprine, cyclosporine, Pred	Skin	Pentamidine, topical chlorhexidine/ketoconazole	IF staining of biopsy tissue section	Cured	Slater[9]
1999	39/F	South Carolina	Lung	72	Azathioprine, Pred, Tac	Skin	5-fluorocytosine, itraconazole, pentamidine, topical chlorhexidine/ketoconazole	Histopathologic staining of abscess fluid[a]	Cured	Oliva[10]
2001	38/M	France	Bilateral lung	36	MPS, Tac	Skin	Itraconazole pentamidine, topical chlorhexidine/ ketoconazole	IF staining of tissue at autopsy	Died	Van Hamme[18]
2002	61/F	Maryland	Kidney	12	MMF, Pred, Tac	Skin, bone	Amikacin, AmB, azithromycin, imipenem	Culture of tissue at autopsy	Died (postmortem dx)	Steinberg[7]
2005	49/F	Florida	Bilateral lung	7	MMF, Pred, Tac	Sinus	AmB, caspofungin, voriconazole	IF staining of biopsy tissue section	Cured	Vernon[11]
2006	60/M	Texas	Bilateral lung	9	MMF, Pred, Tac	Skin, lung, brain	AmB, ciprofloxacin, imipenem, itraconazole, vancomycin	Histopathologic staining at autopsy[a]	Died (postmortem dx)	Duarte[19]
2006	60/M	Texas	Lung	Not reported	Azathioprine, Pred, Tac	Skin, lung, brain	Broad-spectrum antibiotics	IF staining and PCR analysis of brain biopsy specimen	Died	Readinger[20]
2006	51/M	Utah	Kidney	3	MMF, prednisolone, Tac	Skin, brain	AmB, azithromycin, flucytosine, metronidazole, pentamidine, ritampin, sulfadiazine	IF staining of tissue at autopsy	Died (postmortem dx)	McKeller[21]
2006	40/M	Pennsylvania	Multiple organs	9	ATG, Tac	Brain	Not reported	IF staining of tissue at autopsy	Died (postmortem dx)	Mendez[22]
2007	40/M	Spain	Multiple organs	9	Tac	Brain	Not reported	Histopathologic staining at autopsy	Died (postmortem dx)	Gene[23]
2007	52/F	Florida	Lung	36	MMF, Pred, Tac	Skin	AmB, voriconazole	IF staining of biopsy tissue section	Cured	Walia[12]
2007	39/M	France	Heart	22	Cyclosporine, MMF, Pred	Skin, lungs, kidneys	5-fluorocytosine, itraconazole, pentamidine	IF staining of biopsy tissue section, confirmed by culture and PCR	Died	Barete[5]
2007	36/F	India	Kidney	48	Not reported	Brain, lungs, pancreas	Broad-spectrum antibiotics	IF staining of biopsy tissue section	Died (postmortem dx)	Mutreja[24]
2008	41/M	United Kingdom	Liver	14	Azathioprine, cyclosporine, Pred	Brain	Co-trimoxazole, rifampicin, surgical resection	IF staining of biopsy tissue section	Cured	Fung[25]
2010	63/M	New York	Liver	12	Alemtuzumab, cyclophosphamide, daclizumab, doxorubicin, etoposide, MMF, Pred, rituximab, Tac, vincristine	Skin, lung, brain	AmB, caspofungin, flucytosine, miltefosine, pentamidine, voriconazole, topical ketoconazole	IF staining of biopsy tissue section	Died	Young[13]
2013	62/M	California	Bilateral lung	6	MMF, prednisolone, Tac	Skin, brain	Amikacin, flucytosine, pentamidine, intrathecal AmB	light microscopy and PCR of CSF fluid	Died	Afshar[26]
2013	58/M	New York	Kidney	24	MPS, MMF, Pred, rituximab, Tac, ATG	Brain	Broad-spectrum antibiotics, ganciclovir, pyrimethamine, sulfadiazine, voriconazole	IF staining of tissue at autopsy	Died (postmortem dx)	Satlin[6]
2014	63/M	Mississippi	Kidney	6	MMF, Tac	Brain	Azithromycin, fluconazole, flucytosine, miltefosine, sulfadiazine	Histopathologic staining—brain biopsy specimen[a]	Died	Zamora[14]
2015	64/F	Arizona	Kidney	7	MMF, Pred, Tac	Brain	Azithromycin, fluconazole, flucytosine, miltefosine, pentamidine, sulfadiazine	IF staining of brain biopsy section	Died	Salameh[15]
2015	59/F	California	Lung	10	Not reported	Skin, sinus	Inpatient: 5-flucytosine, azithromycin, bactrim, intranasal pentamidine; Discharge: azithromycin, bactrim, miltefosine, voriconazole	IF staining of biopsy tissue section	Cured	Kandakuri[16]
2015	60/F	California	Heart	5	MMF, Pred, Tac, ATG	Skin, sinus, bone	Fluconazole, flucytosine, miltefosine	IF staining of biopsy tissue section	Cured	Brondfield (present

3 clusters of transplant associated, donor derived *Balamuthia mandrillaris* infection reported

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Transmission of *Balamuthia mandrillaris* through Solid Organ Transplantation: Utility of Organ Recipient Serology to Guide Clinical Management

Morbidity and Mortality Weekly Report (*MMWR*)

Balamuthia mandrillaris Transmitted Through Organ Transplantation --- Mississippi, 2009

Weekly
September 17, 2010 / 59(36);1165-1170

Notes from the Field: Transplant-Transmitted Balamuthia mandrillaris --- Arizona, 2010

Weekly
September 17, 2010 / 59(36);1182

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How would you treat this infection?

AMEBIC MENINGOENCEPHALITIS, primary and granulomatous

	Drug	Adult dosage	Pediatric dosage
Naegleria			
Drug of choice:	Amphotericin B[1,2]	1.5 mg/kg/d IV in 2 doses x 3d, then 1 mg/kg/d x 6d plus 1.5 mg/d intrathecally x 2d, then 1 mg/d every other day x 8d	1.5 mg/kg/d IV in 2 doses x 3d, then 1 mg/kg/d x 6d plus 1.5 mg/d intrathecally x 2d, then 1 mg/d every other day x 8d

Acanthamoeba

Drug of choice: Several patients with granulomatous amebic encephalitis (GAE) have been successfully treated with combinations of **pentamidine, sulfadiazine, flucytosine,** and either **fluconazole** or **itraconazole.**[3] GAE in an AIDS patient was treated successfully with **sulfadiazine, pyrimethamine** and **fluconazole** combined with surgical resection of the CNS lesion.[4] Chronic *Acanthamoeba* meningitis was successfully treated in 2 children with a combination of oral **trimethoprim/sulfamethoxazole, rifampin** and **ketoconazole**.[5] Disseminated cutaneous infection in an immunocompromised patient was treated successfully with IV **pentamidine, topical chlorhexidine** and 2% **ketoconazole** cream, followed by oral **itraconazole**[6] and with **voriconazole** and **amphotericin B lipid complex**.[7] Other reports of successful therapy have been described.[8] Susceptibility testing of *Acanthamoeba* isolates has shown differences in drug sensitivity between species and even among strains of a single species; antimicrobial susceptibility testing is advisable.[9]

Balamuthia mandrillaris

Drug of choice: *B. mandrillaris* is a free-living ameba that causes subacute to fatal granulomatous amebic encephalitis (GAE) and cutaneous disease. Two cases of *Balamuthia* encephalitis have been successfully treated with **flucytosine, pentamidine, fluconazole** and **sulfadiazine** plus either **azithromycin** or **clarithromycin** (**phenothiazines** were also used) combined with surgical resection of the CNS lesion.[10] Another case was successfully treated following open biopsy with **pentamidine, fluconazole, sulfadiazine** and **clarithromycin**.[11]

Sappinia diploidea

Drug of choice: A free-living ameba once thought not to be pathogenic to humans. *S. diploidea* has been successfully treated with **azithromycin, pentamidine, itraconazole** and **flucytosine** combined with surgical resection of the CNS lesion.[12]

Back to our patient…

- After consulting with CDC, patient was started on a cocktail of IV pentamidine + miltefosine + flucytosine + sulfadiazine + posaconazole
- Patient and [redacted] declined brain surgery for debridement of lesions
- [redacted] continued to clinically deteriorate and eventually opted for home hospice
- [redacted] was discharged on oral regimen of miltefosine + Bactrim + posaconazole
- [redacted] passed away ~ 3 weeks later

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Take home points…

- Keep free living amebae infections in your differentials for immunocompromised patients presenting with CNS +/- skin, sinus, lung, eye disease
- Diagnosis is often challenging and requires tissue biopsy +/- PCR and culture
- A negative CSF PCR does not rule out CNS disease
- Treatment is challenging. Consult with CDC
- Prognosis for GAE is poor, > 90 % mortality

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