

Free-living amoebae in CSF & Amoebic Keratitis

Claire Rogers
Head of Teaching & Diagnostic Unit
Faculty of Infectious and Tropical Diseases

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FREE-LIVING AMOEBAE

- Unicellular protozoans
- Found in soil, water and air!
- Exist in 2 or 3 forms:
 - Trophozoite (feeding and proliferative)
 - Cyst (environmentally resistant)
 - Flagellate
- Certain species known to infect man

Some with fatal consequences

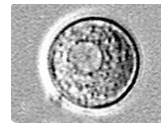


Naegleria fowleri

- Primary Amoebic Meningoencephalitis (PAM)
- Warm, stagnant bodies of water contaminated with sewage or other decaying matter eg. lakes, hot springs, wastewater from power plants, unchlorinated swimming pools
- Exists as trophozoite, flagellate and cyst



Naegleria fowleri



www.cdc.gov/parasite/images



PRIMARY AMOEBIC MENINGOENCEPHALITIS

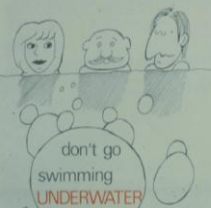
- Infects healthy, active, young people
- Infection by swimming, diving or washing in infected water.
- Onset of symptoms is usually 2-5 days after last exposure to water.
- Severe headache, vomiting, fever, stiff neck rapidly progressing to coma

Fatal within a week after onset of symptoms



AMOEBIC MENINGITIS

WARNING
keep your head above water



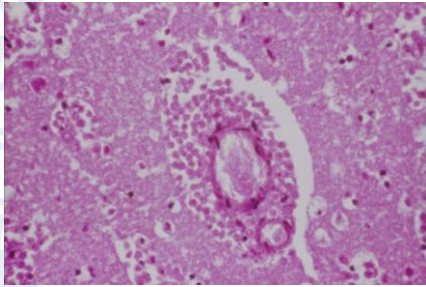
In all thermal pools keep your head above water to avoid the possibility of developing the serious illness - AMOEBIC MENINGITIS

This disease can be caught in thermal pools if water enters the nose while swimming or diving

Issued by The Department of Health
NEW ZEALAND



Brain section (H&E) showing amoebae in perivascular space in *N. fowleri* infection



BATHUK1078



Treatment of PAM

- Few reported cases of successful treatment
- amphotericin B



Granulomatous Amoebic Encephalitis (GAE)

- *Balamuthia mandrillaris*
- *Acanthamoeba* spp.

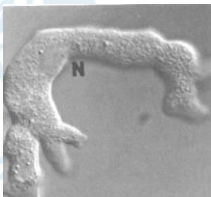


Balamuthia mandrillaris

- First described in an isolate from brain of a pregnant mandrill
- Exists as a trophozoite and cyst
- Found in soil



Balamuthia mandrillaris



Atlas of human protozoa: Rondanelli & Scaglia



www.dpd.cdc.gov/dpdx



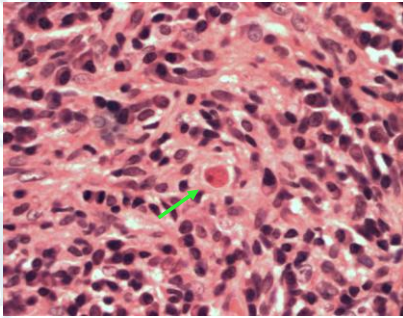
Balamuthia mandrillaris

- infects immunocompromised and immunocompetent (esp. children)
- often manifests as a skin lesion
- infection moves to brain in 3-6 months
- Can disseminate to other sites eg. kidneys, lungs
- Early signs include fever, personality change, stiff neck, seizures, localized neurological symptoms

Usually fatal



Balamuthia mandrillaris trophozoite in skin

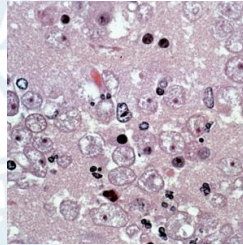


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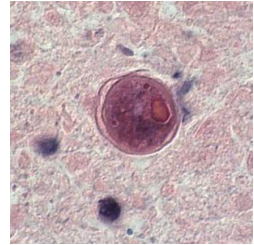
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Balamuthia mandrillaris in brain

Trophozoites



Cyst



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Treatment of B.mandrillaris

- Optimal therapy yet to be determined
- 7 reported cases of success with combination of flucytosine, pentamidine, fluconazole, sulphadiazine, azithromycin/clarithromycin.

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Acanthamoeba spp

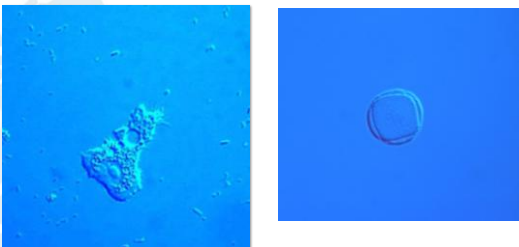
Widely distributed throughout the environment

Isolated from saltwater, freshwater, tapwater, sewage, chlorinated water, soil and air!

Exists as trophozoite and a cyst

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Acanthamoeba spp



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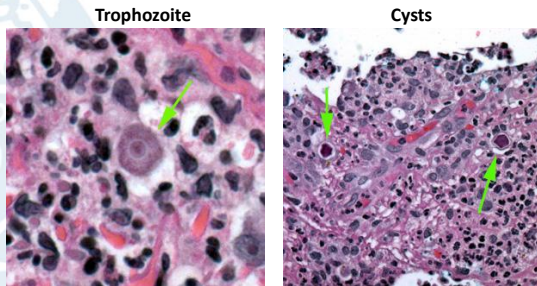
Granulomatous amoebic encephalitis

- infects immunocompromised
- route of entry- intranasal
 - via break in the skin
- chronic disease, slow deterioration
- Fever, headache, behavioural abnormalities

Usually fatal

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Acanthamoeba spp in tissue



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Treatment of Acanthamoeba GAE

- No single drug effective
- Some success with combinations of amphotericin B, azithromycin, fluconazole, flucytosine.
- Outcome is influenced by how early treatment is initiated, strain differences in antimicrobial sensitivity and virulence and immune status of the host

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Diagnosis of FLA

- Clinical suspicion
- History of recent exposure to water / history of graze/skin lesion
- CSF – direct examination / culture
- Serology
- Molecular methods

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Diagnosis of PAM

- Microscopic examination of CSF
- Culture of CSF or biopsies (or environmental samples)
- Molecular diagnosis

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Examination of CSF

- Microscopic examination of CSF for presence of trophozoites
- Do not refrigerate
- Gentle centrifugation
- Examine within 2-3hrs
- CSF ↑ cell count
- ↑ CSF protein, ↓ glucose

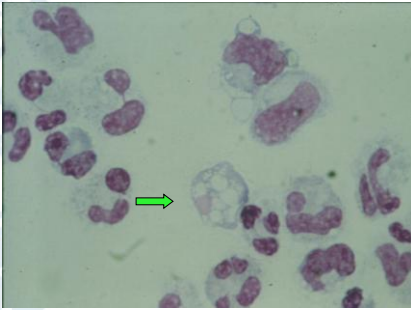
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Examination of CSF for *N.fowleri*

- Small nucleus with large karyosome
- Very active with bulging pseudopodia
- Transformation into flagellate is rapid
- Flagellate – 2 flagellae
- Very fast directional swimming

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Diagnostic CSF concentrate stained with Giemsa (W.Australia)



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Diagnosis by culture

- From CSF or biopsies
- 1.5% Page's non-nutrient agar spread with *Escherichia coli*
- Incubate at 40°C, check daily up to 5 days
- Cat 3 organism

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DIAGNOSIS OF GAE

- Direct examination of CSF
- Culture from CSF
 - Acanthamoeba: NNA + *E.coli*, 35°C
 - *B.mandrillaris*: cell culture (eg human brain microvascular endothelial cells)
- CSF ↑ cell count
- ↑ CSF protein, normal glucose

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DIAGNOSIS OF GAE

- Serology
- Pre- or post-mortem diagnosis from brain or other tissue
- Molecular methods

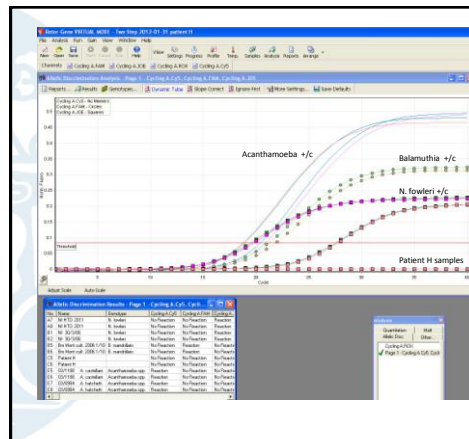
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Multiplex real-time PCR

Qvarnstrom et al 2006

- Simultaneous detection of *Acanthamoeba* spp, *N.fowleri* and *B.mandrillaris*
- 18SrRNA gene
- Detection limit 1 amoeba
- Results within 2-5hrs

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The future

- Recognition of other pathogenic FLA
- The effect of global warming



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Amoebic Keratitis

- Painful, vision threatening infection of the cornea
- Caused by Acanthamoeba spp
- Associated with trauma to the cornea and contamination with amoebae
- Inflammation of cornea, photophobia, intense pain, ring infiltrate, ulceration, loss of vision
- Often misdiagnosed as viral keratitis
- **Up to 93% of cases occur in contact lens wearers**



Risk factors contributing to AK

- Poor hygiene in the use of contact lens
- Swimming whilst wearing lens
- Use of home-made saline or inadequate disinfecting systems
- Working with soil and rubbing eyes



Treatment of Acanthamoeba keratitis

- Successful treatment can use topical diamidines (propamidine), polyhexamethylene biguanide or chlorhexidine
- Cases caught early can be additionally helped by debridement of the corneal surface.
- When a graft is needed it is important to continue treatment since the graft can be reinvaded from the periphery



Diagnosis of AK

- Successful treatment relies on early diagnosis
- Culture from corneal scrapes or biopsies, swabs, lens or lens fluid
- Stained smears/biopsies
- Confocal microscopy



Real-time PCR on ocular specimens

- Use of Qvarnstrom assay as singleplex for detecting Acanthamoeba cysts and trophozoites
- Commercial extraction kit
- 100% sensitivity and specificity compared to culture
- 4 PCR positives, culture negative samples were true positives
- PHMB found to have inhibitory effect on real-time PCR

Thompson et al 2008 J Clin Micro

