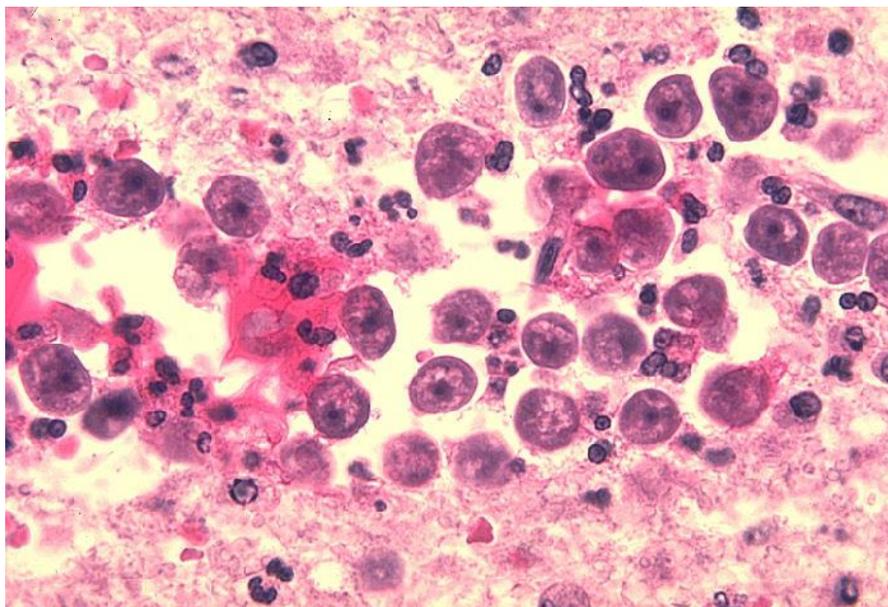
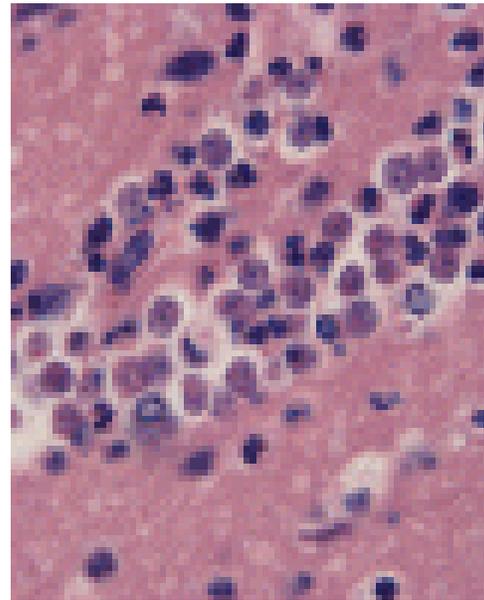
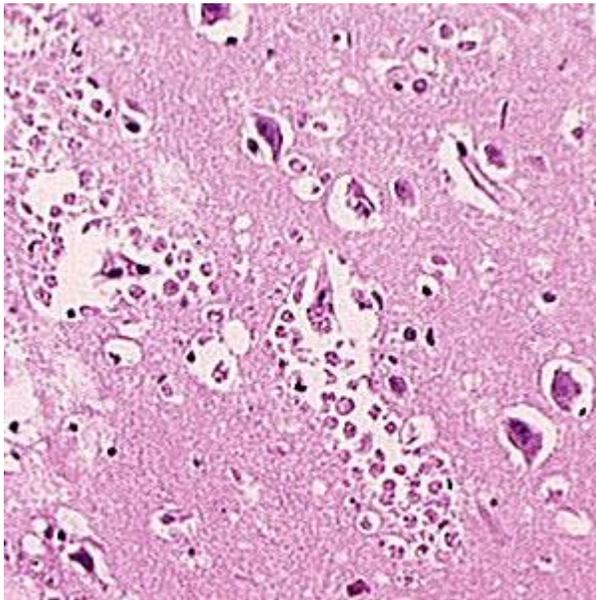
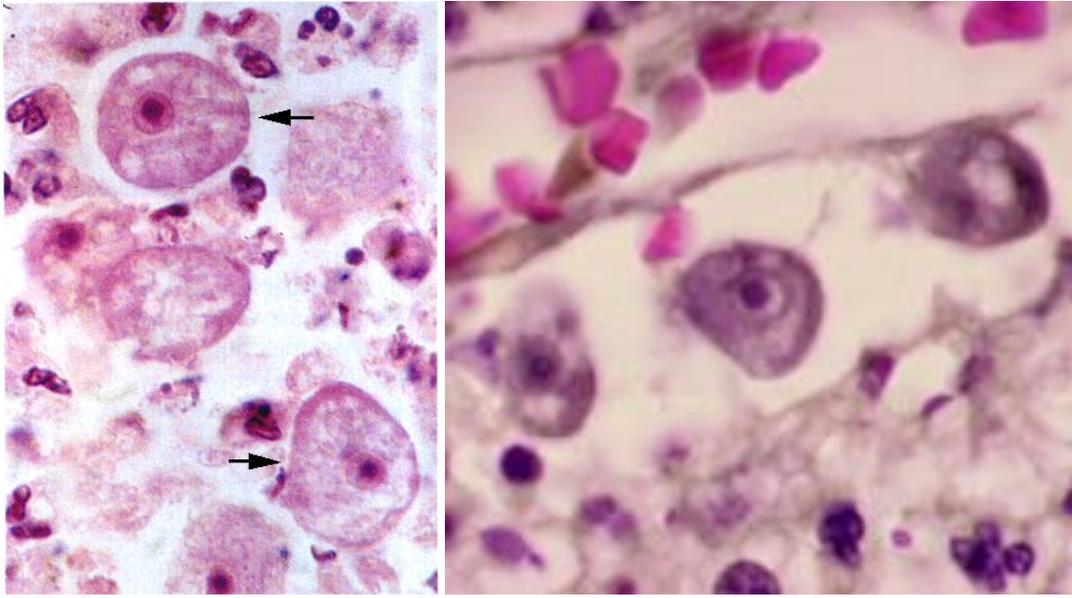


## PARASITOLOGY CASE HISTORY 8 (HISTOLOGY)

(Lynne S. Garcia)

A 12 year-old male presented with a one-day history of fever and headache on the right side. Two days after admission, he developed a stiff neck and became lethargic and unresponsive to pain stimuli. A CT scan revealed a lesion in the right frontal lobe and diffuse cerebral edema. The patient expired on the 6<sup>th</sup> day after admission. The history revealed he had been swimming in a freshwater pond several days prior to admission. Biopsies of brain tissue were taken; the stained sections are seen below:





- Based on these images, what is your diagnosis?

***Scroll Down for Answer and Discussion***

### **Answer and Discussion of Histology Quiz #8**

This is a case of primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri*. Culbertson and his colleagues in 1958 were the first to develop the concept that free-living soil and water amoebae could cause disease in humans. A number of fatal cases of acute meningoencephalitis have been reported since that time. Almost all patients whose cases were documented in the years immediately following this awareness of amebic infections had a history of swimming in freshwater lakes or ponds a few days prior to the onset of symptoms.

Infections of the central nervous system (CNS) caused by free-living amoebae have been recognized only since the mid-1960s, and our understanding of

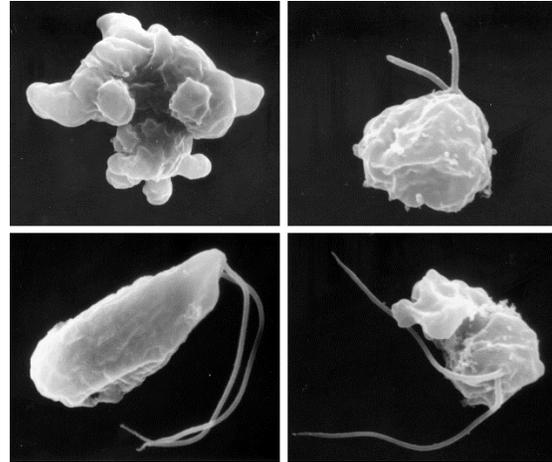
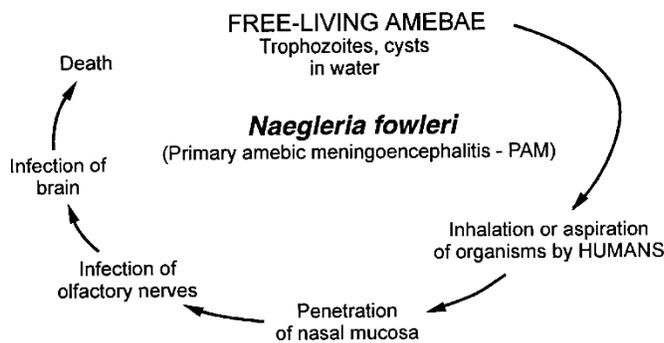
this disease process is still incomplete. One type of meningoencephalitis (PAM) is a fulminant and rapidly fatal disease that affects mainly children and young adults. The disease closely resembles bacterial meningitis but is caused by *N. fowleri*, an organism found in moist soil and freshwater habitats. Close to 200 cases of PAM have occurred worldwide, and approximately 90 of those cases have been reported from the United States. Until recently, it was thought that this infection was limited to humans; however, infections have also been reported in other animals.

There are both trophozoite and cyst stages in the life cycle, with the stage primarily depending on environmental conditions. Trophozoites can be found in water or moist soil and can be maintained in tissue culture or other artificial media.

**Morphology of Trophozoites.** The trophozoites can occur in two forms, ameboid and flagellate. Motility can be observed in hanging-drop preparations from cultures of cerebrospinal fluid (CSF); the ameboid form (the only form recognized in humans) is elongate with a broad anterior end and tapered posterior end. The size ranges from 7 to 35  $\mu\text{m}$ . The diameter of the rounded forms is usually 15  $\mu\text{m}$ . There is a large, central karyosome and no peripheral nuclear chromatin (see original biopsy images above). The cytoplasm is somewhat granular and contains vacuoles. The ameboid-form organisms change to the transient, pear-shaped flagellate form when they are transferred from culture or teased from tissue into water and maintained at a temperature of 27 to 37°C. The change may occur very quickly (within a few hours) or may take as long as 20 hours. The flagellate form has two flagella at the broad end. Motility is typical, with either spinning or jerky movements. These flagellate forms do not divide, but when the flagella are lost, the ameboid forms resume reproduction.

**Morphology of Cysts.** Cysts from nature and from agar cultures look the same and have a single nucleus almost identical to that seen in the trophozoite. They are generally round, measuring from 7 to 15  $\mu\text{m}$ , and there is a thick double wall.

**The life cycle** can be seen below on the left. Note the association between the infection and exposure to freshwater containing the infective organisms. The image on the right shows the ameboid form (upper left) and the flagellated forms (upper right and bottom row).



**Clinical Disease.** Amebic meningoencephalitis caused by *N. fowleri* is an acute, suppurative infection of the brain and meninges. With extremely rare exceptions, the disease is rapidly fatal in humans. The period between contact with the organism and onset of clinical symptoms such as fever, headache, and rhinitis may vary from 2 to 3 days to as long as 7 to 15 days.

The amebae may enter the nasal cavity by inhalation or aspiration of water, dust, or aerosols containing the trophozoites or cysts. The organisms then penetrate the nasal mucosa, probably through phagocytosis of the olfactory epithelium cells, and migrate via the olfactory nerves to the brain. Cysts of *N. fowleri* are generally not seen in brain tissue.

Early symptoms include vague upper respiratory distress, headache, lethargy, and occasionally olfactory problems. The acute phase includes sore throat, stuffy blocked or discharging nose, and severe headache. Progressive symptoms include pyrexia, vomiting, and stiffness of the neck. Mental confusion and coma usually occur approximately 3 to 5 days prior to death. The cause of death is usually cardiorespiratory arrest and pulmonary edema.

PAM can resemble acute purulent bacterial meningitis, and these conditions may be difficult to differentiate, particularly in the early stages. The CSF may have a predominantly polymorphonuclear leukocytosis, increased protein concentrations, and decreased glucose concentration like those seen with bacterial meningitis. Unfortunately, if the CSF Gram stain is interpreted incorrectly (identification of bacteria as a false positive), the resulting antibacterial therapy has no impact on the amebae and the patient will usually die within several days.

Extensive tissue damage occurs along the path of amebic invasion; the nasopharyngeal mucosa shows ulceration, and the olfactory nerves are inflamed and necrotic. Hemorrhagic necrosis is concentrated in the region of the olfactory bulbs and the base of the brain. Organisms can be found in the meninges, perivascular spaces, and sanguinopurulent exudates.

**Diagnosis.** Clinical and laboratory data usually cannot be used to differentiate pyogenic meningitis from PAM, and so the diagnosis may have to be reached by a process of elimination. A high index of suspicion is often mandatory for early diagnosis. Although most cases are associated with exposure to contaminated water through swimming or bathing, this is not always the case. The rapidly fatal course of 3 to 6 days after the beginning of symptoms (with an incubation period of 1 day to 2 weeks) requires early diagnosis and immediate chemotherapy if the patient is to survive.

Analysis of the CSF shows decreased glucose and increased protein concentrations. Leukocytes may range from several hundred to 20,000 cells/mm<sup>3</sup>. Gram stains and bacterial cultures of CSF are negative; however, the Gram stain background can incorrectly be identified as bacteria, thus leading to incorrect therapy for the patient.

A definite diagnosis could be made by demonstration of the amebae in the CSF or in biopsy specimens. Either CSF or sedimented CSF should be placed on a slide, under a coverslip, and observed for motile trophozoites; smears can also be stained with Wright's or Giemsa stain. CSF, exudate, or tissue fragments can be examined by light microscopy or phase-contrast microscopy. Care must be taken not to mistake leukocytes for actual organisms or vice versa. It is very easy to confuse leukocytes and amebae, particularly when one is examining CSF by using a counting chamber, hence the recommendation to use just a regular slide and coverslip. Motility may vary, and so the main differential characteristic is the spherical nucleus with a large karyosome.

**Epidemiology.** Although rare worldwide, most cases of PAM have been reported in the United States, Australia, and Europe (France). A large number of cases in developing countries go unnoticed. In particular, religious, recreational, and cultural practices such as ritual ablution and/or purifications, Ayurveda, and the use of neti pots for nasal irrigation can contribute to this devastating infection. With increasing water scarcity and public reliance on water storage, here we debate the need for increased awareness of primary amoebic meningoencephalitis and the associated risk factors, particularly in developing countries.

Nasal cleansing/irrigation using neti pots can provide relief to patients with sinusitis including symptoms of facial pain, headache, cough, rhinorrhea (allergic rhinitis) and nasal congestion. Routine nasal cleansing can reduce medication used by patients with sinusitis and provide relief for hay fever, common cold, and other chronic sinus and nasal symptoms. The basis of such an adjunct therapy is that the nasal cavity is washed to flush out excess mucus and debris from the nose and sinuses and moisten the mucous membranes. It is recommended that nasal irrigation be performed using saline solution (0.9% non-iodized sodium chloride in purified or filtered warm water, with or without inclusion of a buffering agent such as sodium bicarbonate). Sterile water is also an option.

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