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REVIEW

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Free-living Ameba Infections: Rare but Fatal

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Abstract

Free-living ameba infections, although rare, are recognized as causes of fatal infection in both immunocompetent and immunocompromised persons. *Naegleria fowleri* causes primary amebic meningoencephalitis (PAM), which is a rapid and fulminant central nervous system (CNS) infection in normal hosts. *Acanthamoeba* causes chronic or subacute granulomatous amebic encephalitis (GAE) in immunosuppressive persons. *Balamuthia* also causes GAE, but in competent hosts.

PAM should be considered in all patients with meningoencephalitis, especially if they have a recent history of swimming in fresh water. A clue that suggests the diagnosis is a negative CSF Gram stain finding in a patient with purulent meningitis.

Healthcare providers should be aware of *Acanthamoeba* as a potential pathogen in the HIV-infected patient population. Early diagnosis may allow for prompt intervention and increase a patient's chance of survival.

Clinicians, parasitologists, and pathologists must include free-living amebae in the differential diagnosis of possible pathogenic agents that cause sinusitis and cutaneous nodules or ulcers, with or without CNS involvement, especially when bacteria, fungi, or mycobacteria are not found by smear, biopsy, or culture. Typical motility of trophozoites on fresh examination is a key for diagnosis. Broad pseudopodia movement is typical for *Naegleria*, and it can turn into temporary flagellated form. Spiny cytoplasmic projection or acanthopodia is characteristic of *Acanthamoeba*.

Thoughtful history-taking, prompt simple laboratory examination, and awareness can be very rewarding.

Keywords: *Naegleria fowleri, Acanthamoeba* spp, *Balamuthia* spp, PAM, GAE, Thailand

Introduction

Free-living amebae belong to three groups: ameboflagellates (*Naegleria*), Acanthamoebidae (*Acanthamoeba)* and Leptomyxida (*Balamuthia*). They are distinct from other pathogenic protozoa due to their free-living existence. They lack an insect vector or human carrier state and have been isolated from air, soil, and water samples throughout the world [1-2]. Infections have become increasingly common with the appearance of AIDS and immunosuppressive agents.

While *fowleri* is only one species of *Naegleria* known to infect humans, several species of *Acanthamoeba* are implicated, including *A. culbertsoni, A. polyphaga, A. castellanii, A. astronyxis, A. hatchetti,* and *A. rhysodes*. An additional agent of human disease, *Balamuthia mandrillaris*, is a related leptomyxid ameba that is morphologically

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similar on light microscopy to *Acanthamoeba* [3-6].

N. fowleri is the etiologic agent of primary amebic meningoencephalitis (PAM), a rapidly fatal disease of the central nervous system in competent hosts. Carter and Fowler, for whom *N. fowleri* is named, reported the first cases of PAM in 1965, in Australia. Most patients with PAM have a history of water exposure that includes swimming or diving through a body of fresh water. Trophozoites penetrate the nasal mucosa and the cribriform plate, then ascend along the olfactory nerves to invade brain tissue, resulting in purulent meningitis and encephalitis. The organism produces multiple cytologic enzymes, which cause diffuse hemorrhage and necrosis of brain tissue [7-10].

Acanthamoeba spp and *B. mandrillaris* are opportunistic free-living amebae capable of causing granulomatous amebic encephalitis (GAE) in individuals with compromised immune systems. *Acanthamoeba* spp have been found in soil, brackish and sea water, sewage, swimming pools, contact lens equipment, medicinal pools, dental treatment units, dialysis machines, mammalian cell cultures, vegetables, human nostrils and throats, human and animal brains, skin, and lung tissues. *B. mandrillaris,* however, has not been isolated from the environment but has been isolated from autopsy specimens of infected humans and animals [11-16].

In AIDS patients, *Acanthamoeba* infection has been reported almost entirely in those with advanced HIV infection, as manifested by low $CD4^+$ cell counts [12,16-17]. Sinus, nasal, or palatal involvement has been described [18-20]. Most of those patients had insidious onset of symptoms, lasting from weeks to months. In most cases, the infection disseminated to other organs, most notably the skin, brain, and lungs.

Life Cycle (Fig 1)

N. fowleri has three stages in its life cycle, *ie*, cysts, trophozoites and flagellated forms. The trophozoites replicate by promitosis (nuclear membrane remains intact. Trophozoites can turn into temporary flagellated forms which usually revert back to the trophozoite stage. Trophozoites infect humans or animals by entering the olfactory neuroepithelium and reaching the brain. *N. fowleri* trophozoites are found in the cerebrospinal fluid (CSF) and tissue, while flagellated forms are found only in the CSF.

Unlike *N. fowleri*, *Acanthamoeba* and *Balamuthia* have only two stages, cysts and trophozoites, in their life cycle. The trophozoites are the infective forms and are believed to gain entry into the body through the lower respiratory tract, ulcerated or broken skin, and invade the central nervous system by hematogenous dissemination. *Acanthamoeba* spp and *B. mandrillaris* cysts and trophozoites are found in the tissue.

Clinical manifestations

Acute primary amebic meningoencephalitis (PAM) presents with severe headache and other meningeal signs, fever, vomiting, and focal neurologic deficits, and progresses rapidly (< 10 days) and frequently to coma and death [7-10]. *Acanthamoeba* spp causes mostly subacute or chronic granulomatous amebic encephalitis (GAE), with a clinical picture of headache, altered mental status, and focal neurologic deficit, which progresses over several weeks to death [11,14-15]. In addition, *Acanthamoeba* spp can cause granulomatous skin lesions and, more seriously, keratitis and corneal ulcers following corneal trauma or in association with contact lenses [12-13]. Clinical features of the free-living amebae infections are compared in Table 1.

Alteration in taste or smell, sudden-onset headache (usually frontal or bitemporal), fever, nausea or vomiting, stiff neck and photophobia (later in the course of illness) will be recovered on history taking of PAM patients. GAE patients presented with mental status changes (86%), seizures (66%), hemiparesis (53%), meningismus (40%), visual disturbances (26%), ataxia (20%), fever, headache, skin lesions, including ulcers, nodules, or subcutaneous abscesses. Keratitis due to *Acanthamoeba* was mostly related to contact lens use, with a history of improper cleaning or use of homemade sodium chloride solution, swimming in fresh water and/or swimming pool. Foreign body sensation, severe pain, photophobia, tearing,

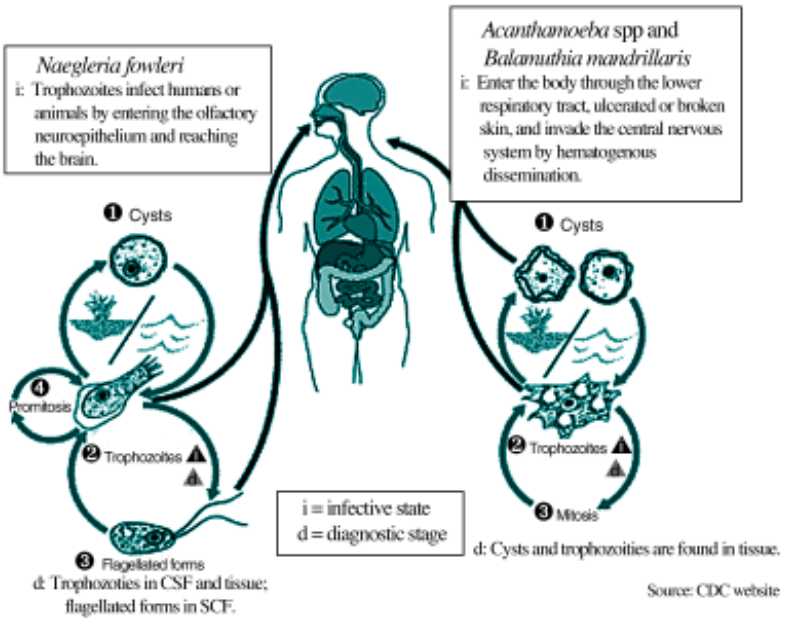

Fig 1 Life cycle of free-living amebae.

Table 1 Comparison of *N. fowleri* and *Acanthamoeba* spp infections.

Clinical features	*N. fowleri*	*Acanthamoeba* spp
Disease	Primary amebic meningoencephalitis (PAM)	Granulomatous amebic encephalitis (GAE) with dissemination to other tissues and keratitis in contact lens wearer
Primary site of entry	The olfactory mucosa and neuroepithelium	Respiratory tract with hematogenous dissemination, and skin
Patient's age	Young and healthy adult	5–60 years (mean 32 years) with immunocompromised status
Male:female ratio	1:1	6:1.
Frequency:	About 300 cases of PAM have been reported internationally, mostly from USA, Australia, and Europe	In the US, keratitis occurs in 1.65-2.01 people per million contact lens wearers GAE is very rare
Incubation period and clinical course	3-8 days with acute and rapidly fatal course dies within 7-10 days of onset of symptoms	Subacute to chronic but is terminally fatal. Duration of illness until death ranges from 7-120 days (average 39 days)
Prognosis	Mortality rate approximately 95%	GAE has a poor prognosis and high mortality rate Keratitis usually responds to medical therapy

blepharospasm, conjunctivitis, and blurred vision were also reported [16].

Neurological involvement, including nuchal rigidity, positive Kernig and Brudzinski signs, mental status changes, seizures and coma were found in PAM and GAE. The latter also presented skin lesions, including ulcers, nodules, and subcutaneous abscesses. Conjunctivitis and hypopyon were common in *Acanthamoeba* keratitis.

Clinical diagnosis

Diagnosis of PAM is difficult and always delays. If PAM is suspected, serologic testing has no role in diagnosis; a lumbar puncture must be performed. With a lumbar puncture, opening pressure and protein levels are elevated (usually > 100 mg/dl); glucose levels may be within reference ranges or low. The number of white blood cell in CSF is elevated by several hundred to thousands, with polymorphonuclear predominance. Although CSF parameters are consistent with purulent meningitis, Gram stain findings are negative for bacteria. Examine a fresh-centrifuged specimen of CSF for the presence of trophozoites; trichrome and Giemsa stainings may also be employed. Occasionally, amebae can be observed on Gram-stained smears. Confirmation of *N. fowleri* infection requires a culture or an indirect fluorescent antibody test, which is performed at a reference laboratory. The use of molecular methods, including polymerase chain reaction, monoclonal antibody probes, and DNA probes, in the diagnosis of PAM is increasing [21-23].

GAE may be diagnosed by lumbar puncture, skin biopsy, and brain biopsy. Lumbar puncture may be contraindicated if signs of increased intracranial pressure are present. Cerebrospinal fluid examination reveals an increased number of white blood cells (as many as 800 cells/l, primarily lymphocytes), elevated protein, and decreased glucose. In keratitis cases, stain eye scrapings and cyst walls with calcofluor white and examine specimen by fluorescent microscopy [24]. Conducting polymerase chain reaction (PCR) of biopsy specimens is also useful [25].

CT scan of the brain is not diagnostic in PAM but may show diffuse gray matter enhancement and loss of the midbrain cisterns and the subarachnoid space. In GAE, CT scan should be obtained before lumbar puncture. Findings include multiple non-enhancing lesions in the cerebral cortex and ventriculomegaly.

Diagnostic tests, laboratory studies and procedures of both PAM and GAE are summarized in Table 2.

Laboratory diagnosis

Laboratory diagnosis can be made by finding motile trophozoites of *N. fowleri* (Fig 2). The organism moves in a directional manner by extension of large, broad pseudopodia. The trophozoite measures 7-20 m and has a fine granular cytoplasm that may contain vacuoles. The single nucleus contains a prominent central karyosome which may appear to be surrounded by a halo (Fig 3). Peripheral chromatin is absent. Flagellate forms of this parasite can be induced within 2-20 hours by transferring the ameboid form from tissue or CSF to water and incubating at 37˚C. This characteristic helps to differentiate trophozoites of *N. fowleri* from those of *Acanthamoeba* spp, which have no such flagellate form [1-2]. Table 3 concludes key laboratory points for diagnosis of PAM [26].

Patients with GAE have moderate-to-severe cerebral edema. Necrotizing granulomas containing perivascular trophozoites and cysts most frequently are located in the cerebellum, midbrain, and brain stem. Multinucleated giant cells may be present within the granulomas. The leptomeninges are spared, except when directly overlying areas of cortical involvement.

Unlike *Naegleria, Acanthamoeba* never produce flagella. The trophozoite form is characterized by spiny cytoplasmic projections termed acanthopodia (Fig 2). Trophozoite size may vary from 10-45 m. The karyosome is large and centrally placed within the nucleus (Fig 3). Peripheral chromatin is absent. *Acanthamoeba* spp cysts are round and smaller, ranging in size from 10-20 µm. Cysts have a single nucleus and are double-walled, with the outer wall having a wrinkled appearance. The cysts of both *Naegleria* and *Acanthamoeba* are resistant to desiccation and

Table 2 Comparison of diagnostic tests, laboratory studies and procedures between PAM and GAE.

	PAM	GAE
Diagnostic test	CSF study with direct visualization of *Naegleria* under light microscope can be stained with Heidenhain's iron hematoxylin and Wheatley's trichrome stain CSF findings mimic those of bacterial meningitis, with a predominantly polymorphonuclear leukocytosis and increased protein and decreased glucose concentration Occasionally, amebae can be observed on Gram-stained smears Confirmation of *N. fowleri* infection requires a culture or an indirect fluorescent antibody test, which is performed at a reference laboratory	Usually the trophozoites are not seen in CSF smear and cytology usually reveals a lymphocytic pleocytosis
Lab studies	Peripheral neutropenia Serologic testing has no role in the diagnosis of acute PAM	Cerebrospinal fluid examination reveals an increased number of white blood cells (as many as 800 cells/l, primarily lymphocytes), elevated protein, and decreased glucose
Procedures	Lumbar puncture, opening pressure is elevated. Protein levels are also elevated (usually >100 mg/dl) Glucose levels may be within reference ranges or low The CSF WBC is elevated by several hundred to thousands with polymorphonuclear predominance Examine a wet mount of CSF for the presence of trophozoites; trichrome and Giemsa staining of CSF may also be employed Use of molecular methods, including polymerase chain reaction, monoclonal antibody probes, and DNA probes, in the diagnosis of PAM is increasing	Obtain lumbar puncture, skin biopsy, and brain biopsy Lumbar puncture may be contraindicated if signs of increased intracranial pressure are present

mild chlorination and can be carried by water and through the air [1-2].

With keratitis, amebic cysts and trophozoites are found within the cornea. An acute or mixed inflammatory infiltrate may contain giant cells. Corneal revascularization may occur.

Treatment

Few people have survived PAM, and no standard treatment regimen has been developed. Early diagnosis, medication, and aggressive supportive care are necessary to have any chance of a successful outcome. Successful outcomes have been reported with high-dose systemic and intrathecal amphotericin B, as well as rifampin and sulfisoxazole [27-28]. No routine surgical care is indicated. Poungvarin and Jariya [29] reported a nonlethal case of PAM in Thailand and recommended the regimen of low dose amphotericin B for a prolonged period instead of a high dose over a short period.

Granulomatous amebic encephalitis usually

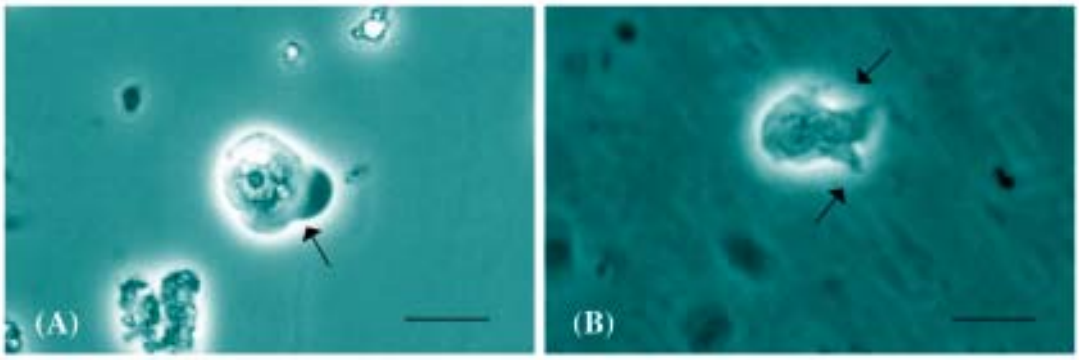

Fig 2 Typical characteristic movement of (A) *Naegleria* and (B) *Acanthamoeba*. A: *Naegleria* trophozoite in saline fresh smear. Observe broad pseudopods (arrow). The trophozoite measures 7-20 µm and has a granular cytoplasm, 40x, Bar = 10 µm. B: *Acanthamoeba* trophozoite in saline fresh smear. Observe spiny cytoplasmic projections (arrows) termed acanthopodia. The trophozoite size may vary from 10-45 µm 40x, Bar = 10 µm.

Table 3 Key points for diagnosis of PAM [26].

1.	Never refrigerate the specimen(s) prior to examination.
2.	A clue to diagnosis is a negative CSF Gram stain finding in a patient with purulent meningitis, since PAM will usually mimic pyogenic meningitis (CSF will contain increased numbers of leukocytes).
3.	The *Naegleria* trophozoites will mimic leukocytes if put in a counting chamber. Motility is more likely to be seen if a drop of CSF is placed directly on a slide and a cover-glass is added.
4.	Organisms can be cultured on non-nutrient agar plated with *E. coli*.

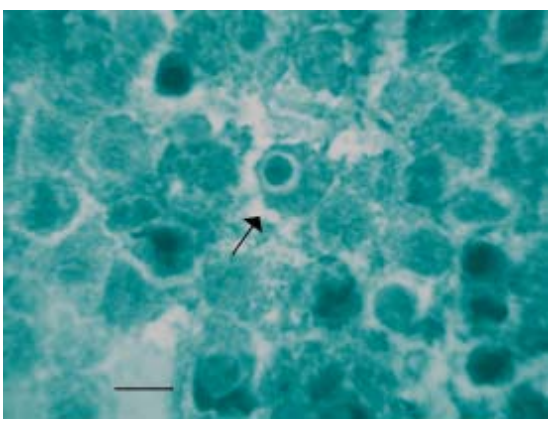

Fig 3 Single nucleus containing a prominent central karyosome surrounded by a halo, Trichrome stain, 110x, Bar = 10 µm.

has a prolonged clinical course. Early diagnosis by brain biopsy holds hope for successful treatment because there are multiple drugs, such as ketoconazole, miconazole, 5-flucytosine and pentamidine, effective against the causative organisms *in vitro* [30-31]. Treatment for keratitis is topical. Two types of preparations are used [32]: diamides (propamide isethionate and hexamide) and cationic antiseptics (polyhexamethyl biguanide and chlorhexidine). These agents are not commercially formulated for ophthalmic use. Many authorities recommend using a diamide and a cationic antiseptic immediately after corneal debridement every hour for 48 hours [33-36]. These agents are then continued hourly during waking hours for another 3 days. The frequency is then reduced to every 3 hours. Two weeks may

be required before a response is observed, and the total duration of therapy frequently is 3-4 weeks. No clear consensus exists for the use of steroids. Those who use steroids should continue antiamebic therapy for several weeks after the steroids are stopped. McClellan and co-workers [37] showed that steroid use was significantly greater among patients for whom medical therapy failed than for those whose medical therapy was successful. A study by Park *et al* [38], revealed no difference in response to medical therapy for those who used topical steroids compared with those who did not. However, in this study, patients treated with topical steroids required a longer duration of medical therapy (38.5 weeks versus 20 weeks). Penetrating keratoplasty may be necessary in patients who do not respond to medical therapy.

Free-living ameba infections in Thailand

The first reported case of PAM in Thailand was a 5-year old boy from the northeast [39]. Since then, about 12 cases of PAM have been reported from all over the country [29,40-43]. It was found that more than two-thirds of cases were children, disease occurred during summer months and the majority had a history of swimming and water exposure. The mortality rate was very high, with all but one case dying. These findings correspond with infections reported worldwide.

Twelve cases of GAE have been reported in Thailand, and four patients with GAE were reported by Sangruchi *et al* [44]. One case was diagnosed antemortem by brain biopsy. The remaining cases were diagnosed as GAE postmortem. One patient had a chronic skin ulcer with free-living ameba trophozoites. No other organ involvement was observed. Pathological findings included acute and chronic necrosis and fibrin thrombi in the brain. The immune status of the patients was not clearly identified.

Acanthamoeba cysts and trophozoites were isolated from the first two keratitis patients from King Chulalongkorn Memorial Hospital [32]. The patients developed decreased vision, severe photophobia, severe eye pain, and foreign body sensation after minor corneal trauma. The lesion included generalized superficial punctate keratitis, stromal corneal ulcer with keratic precipitate and uveitis in one case, and corneal ulcer with abscess in another. Both cases were diagnosed by isolation and culture, and confirmed by restriction fragment length polymorphism analysis of mitochondrial DNA.

Another series from Siriraj Hospital, found 9 out of 102 cases (8.8%) who presented with chronic corneal ulcers were diagnosed as *Acanthamoeba* keratitis [45]. Of six patients, four cases were successfully treated with chlorhexidine solution and two with antibiotic medical therapies. Keratoplasty was performed in a case with perforated corneal ulcer and enucleation in one blind patient with advanced and extensive keratitis who failed to respond to aggressive medical treatment.

Recently, there was an exotic sinusitis case infected by both *Acanthamoeba* and *Naegleria* [46]. Detailed history taking revealed that he had spent half a day diving and fishing in a stagnant pond one day earlier. He had congested left nostril with minimal blood-tinged secretion from the middle meatus. Diagnosis was made by fresh smears of nasal discharge and confirmed by culture of sinus aspiration fluid in cell-free nutrient. An extensive Caldwell-Luc operation was performed and intravenous amphotericin B with oral ketoconazole and amoxycillin/clavulanic acid were prescribed. His symptoms were relieved. At the sixth week, when the antral irrigation fluid culture was negative for *Acanthamoeba,* only a few, non-active *Naegleria* remained. Due to rising serum creatinine (2.5 mg/dl) and slight anemia, the treatment was stopped. When seen 3 months later, the patient was well.

This patient was an unusual case, infected by both amebae via sinus opening and no CNS invasion, which had never been previously documented. However, six cases of *Acanthamoeba* sinusitis had been reported [18-20], but infection by *Naegleria* alone and mixed infection had not yet been documented. Half a liter of water from that pond was collected for examination and cysts were identified, each with one nucleus containing a large central karyosome surrounded by a halo.

Conclusion

Three free-living amebae, *Naegleria fowleri*, *Acanthamoeba* spp and *Balamuthia* spp are of medical important. PAM caused by *N. fowleri*, is a rapid and fulminant central nervous system (CNS) infection in normal hosts. *Acanthamoeba* causes chronic or subacute granulomatous amebic encephalitis (GAE) in immunosuppressive persons. *Balamuthia* also causes GAE, but in competent hosts.

Free-living amebae infections are rare, but always fatal. *N. fowleri* infection should be considered in all patients with meningo-encephalitis, especially if they have a recent history of swimming in fresh water. Unlike *Naegleria* infections, most cases of acanthamebiasis and balamuthiais lack a clear source of acquisition. *Acanthamoeba* is a potential pathogen in the HIV-infected patient population.

Clinicians, parasitologists, and pathologists must include free-living amebae in the differential diagnosis of possible pathogenic agents that cause CNS infection, sinusitis and cutaneous nodules or ulcers, especially when bacteria, fungi, or mycobacteria are not found on smear, biopsy, or culture. Early diagnosis may allow for prompt intervention and increase a patient's chance of survival.

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