

Amebic Encephalitis and Keratitis

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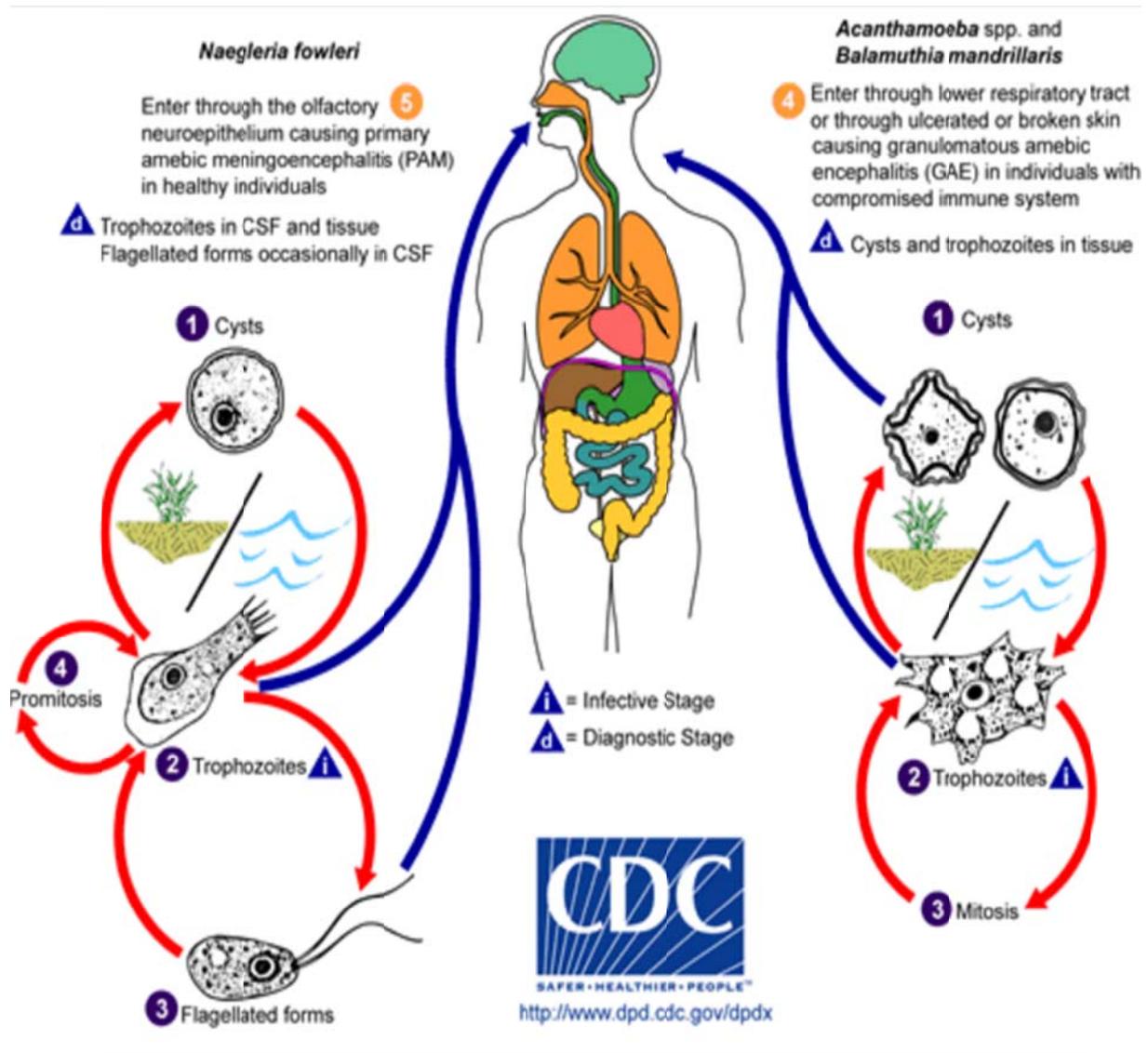
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Free-living amoebae are unicellular protozoa common to most soil and aquatic environments. Of the many hundreds of species of free-living amoebae, only the following are known to cause diseases in humans.

1. members of the genus *Acanthamoeba*
2. *Naegleria fowleri*
3. *Balamuthia mandrillaris*

Acanthamoeba and *B. mandrillaris* trophozoites may be recognized by the presence of slender, spine-like processes. When rounded, the cells measure 25 to 40 μm in diameter. The finely granular cytoplasm, as a rule, contains a single nucleus with a large, dense central nucleolus surrounded by a nuclear clear zone. Water and digestive vacuoles are usually visible in the cytoplasm. The double-walled cysts are generally polygonal, spherical, or star-shaped, 15 to 20 μm in diameter, with a nucleus containing a large dense central nucleolus surrounded by a clear nuclear halo. The smooth inner wall of the cyst contacts the wrinkled outer wall at a number of points, forming pores, opercula, or ostioles. Reproduction is by binary fission of the trophozoites.



1-Acanthamoeba

Acanthamoeba is a genus of amoebae, one of the most common protozoa in soil, and also frequently found in fresh water and other habitats. The cells are small, usually 15 to 35 µm in length and oval to triangular in shape when moving. The pseudopods form a clear hemispherical lobe at the anterior, and there are various short filose extensions from the margins of the body. These give it a spiny appearance, which is what the name *Acanthamoeba* refers to. Cysts are common. Most species are free-living bacterivores, but some are opportunists that can cause infections in humans and other animals.

History

The first suggestion that *Acanthamoeba* could cause disease in humans came in 1958 during polio vaccine safety trials. Plaques appeared in cell cultures used to prepare vaccine and were thought to be virus induced because mice and monkeys died from encephalitis following inoculation of tissue culture fluid. However, these plaques were found later to be caused by amoebae. The first cases which clearly established *Acanthamoeba* as causative agents of disease in humans were reported in the early 1970s. These included reports of amoebic encephalitis, amoebic keratitis and skin infections. Consequently, since different free-living amoebae can infect the central nervous system (CNS), the term “granulomatous amoebic encephalitis” (GAE) has been used for CNS infections caused by *Acanthamoeba* spp. while the term “primary amoebic meningoencephalitis” has been reserved for CNS infections caused by *Naegleria fowleri*.

Ecology- Source of Infection

Acanthamoeba is a genus of environmental free-living amoebae found in most soil and water habitats. The genus contains numerous species, of which *A. polyphaga*, *A. castellanii* and *A. culbertsoni* have been identified most frequently as causing human disease.

Acanthamoeba have been isolated from soil, dust, natural waters (lakes, rivers, hot springs), artificial waters (swimming pools, hot tubs, drinking water systems, slime layers in pipes, taps, a heating, ventilating, and air conditioning, HVAC systems and humidifiers), brackish and sea waters, and the atmosphere.

Acanthamoeba is an aerobic organism but cysts have been isolated from anaerobic material such as feces and sewage. The trophozoites are killed by saline concentrations greater than 1%, although the more environmentally robust cysts have been isolated from marine environments. *Acanthamoeba* numbers in fresh-water habitats depend on temperature and bacterial food source. *Acanthamoeba* cysts have been isolated in marine sites, particularly those associated with sewage and waste effluent outlets.

Incidence /Disease risk

Granulomatous Amoebic Encephalitis due to *Acanthamoeba* is extremely rare, with about 200 cases reported worldwide; the survival rate is 2%.

Pathogenesis

Free-living amoebas have been isolated from human throats, suggesting that they are generally harmless in healthy individuals. *Acanthamoeba* spp. usually act as opportunistic pathogens, taking advantage of a loss of metabolic, physiologic, or immunologic integrity by the host. Among the most common factors predisposing an individual to *Acanthamoeba* infection are immunosuppressive therapy, treatment with broad-spectrum antibiotics, diabetes mellitus, various cancers, malnutrition, pregnancy, acquired immune

deficiency syndrome (AIDS), and chronic alcoholism. Surgical trauma, burns, wounds and radiation therapy can also promote infection.

The primary focus of infection for opportunistic *Acanthamoeba* is usually the lower respiratory tract or skin. The amebas may enter the respiratory tract by the inhalation of aerosols or dust containing cysts. The spread to the CNS is apparently hematogenous. The cerebral hemispheres in granulomatous amebic encephalitis may be edematous, with focal cortical softening, hemorrhage and abscesses. Uncal notching and cerebellar herniation may be present. Foci of hemorrhagic necrosis may be seen in the basal ganglia, midbrain, brainstem, and cerebellum. The histopathologic changes consist of a chronic, granulomatous encephalitis with multinucleated giant cells, mainly in the posterior fossa structures, basal ganglia, and cerebellum. Trophozoites and cysts may be found in the lesions.

Except in the case of amebic keratitis, the defenses of a healthy host seem sufficient to prevent *Acanthamoeba* or *B. mandrillaris* infection. Patients who contract GAE usually have impaired humoral and/or cell-mediated immunity. However, there are reports of patients with no demonstrable underlying disease or predisposing factor.

Semiology

Species of *Acanthamoeba* that are pathogenic to humans can cause two clinically distinct diseases:

1. Granulomatous amoebic encephalitis (GAE)
2. Keratitis (inflammation of the cornea)
A. polyphaga and *A. castellanii* are most frequently reported as causing keratitis, and *A. culbertsoni* is most frequently reported as causing GAE.

Granulomatous Amebic Encephalitis is a chronic disease of the immunosuppressed (chemotherapy, AIDS, drug or alcoholic abuse) host. GAE is subacute or chronic and invariably fatal. Symptoms include fever, headache, seizures, meningitis and visual abnormalities. The route of infection in GAE is unclear, although invasion of the brain may result from the blood following a primary infection elsewhere, possibly the skin or lungs. The precise source of such infections is unknown because of the almost ubiquitous presence of *Acanthamoeba* in the environment.

Infection usually mimics that of bacterial meningitis, tuberculous meningitis, or viral encephalitis. The misdiagnosis often leads to erroneous treatment that is ineffective.

Acanthamoeba keratitis affects previously healthy persons and is a severe and potentially blinding infection of the cornea. Untreated *Acanthamoeba* keratitis can lead to permanent blindness. Unilateral infection is the more common form. The disease is characterized by intense pain and ring-shaped infiltrates in the corneal stroma. Contact lens wearers are most at risk from the infection and account for approximately 90% of reported cases. Poor contact lens hygiene practices (notably ignoring recommended cleaning and disinfection procedures and rinsing or storing of lenses in tap water or non-sterile saline solutions) are recognized risk factors, although the wearing of contact lenses while swimming or participating in other water sports may also be a risk factor. In noncontact lens related keratitis, infection arises from trauma to the eye and contamination with environmental matter such as soil and water.

Acanthamoebic pneumonitis and dermatitis, characterized by the presence of cysts and trophozoites in alveoli or in multiple nodules or ulcerations of the skin, are opportunistic diseases that usually affect immunosuppressed or debilitated individuals. In *Acanthamoebic* pneumonitis, chest radiographs may show areas of consolidation.

Diagnostic

Timely diagnosis remains a very significant impediment to the successful treatment of infection, as most cases have only been discovered post-mortem.

In many cases, GAE is not diagnosed until after or, at best, shortly before death. Immunosuppression or other predisposing factors may provide important clues. The differential diagnosis includes space-occupying lesions such as tumors, abscesses, and even infarcts, as well as tuberculoma or fungal infection. Computed tomography and magnetic resonance imaging of the brain are important diagnostic tests, as is examination of cerebrospinal fluid and brain biopsy specimens. The diagnosis usually is made after examination of brain tissue with light a microscope.

Amebic “dermatitis” is often diagnosed by microscopic examination of a skin biopsy. Both trophozoites and cysts are usually visible.

In the case of amebic keratitis, scrapings of the corneal ulceration and biopsy specimens may contain amebic trophozoites and cysts. Both light and electron microscopy may be useful. Amebic cysts in the corneal stroma may be demonstrated by staining with hematoxylin and eosin, trichrome, calcofluor-white, or immunofluorescence techniques.

Culture

Acanthamoeba does grow on the standard medium for isolating free-living amebae, plain agar seeded with the bacterium *Escherichia coli*.

Treatment (see Page 17).

2-Balamuthia mandrillaris

Balamuthia mandrillaris: *B.mandrillaris* was first reported in 1990, isolated from the brain of a mandrill baboon that died in the San Diego Zoo Wild Life Animal Park. The amebae, causing cases of fatal encephalitis in humans and other primates, were found to be sufficiently distinct to be described as a new genus and species, *B.mandrillaris* (Visvesvara et al). Genetic analysis, however, revealed that *Balamuthia* is a close relative of *Acanthamoeba*. Since then *B.mandrillaris*. has been found in numerous animals, including humans (young and old, immunocompromised and immunocompetent persons), in countries around the world.

Using antiserum to the organism, the investigators were able to demonstrate that certain cases of GAE attributed to *Acanthamoeba* were in fact caused by *B.mandrillaris*.

Ecology- Source of Infection

Balamuthia has been isolated from a plant at the home of child that died in California and also from soil and dust particles deposited from the air in an urban environment (not related to any human case).

Balamuthia comes in contact with skin wounds and cuts, or when dust containing *Balamuthia* is breathed in or gets into the mouth. It can take weeks to months to develop the first symptoms of *Balamuthia* GAE after initial exposure to the amebas.

Since isolates of *Balamuthia* can grow at 37°C *in vitro*, perhaps this ameba can only survive in warm soils. The ability to grow at 37°C is a prerequisite for human pathogenicity.

Incidence and Disease Risk

Balamuthia: Approximately 200 cases of *B. mandrillaris* encephalitis have been described worldwide; approximately 85 of these cases were confirmed, some 50% were from the United States. At least ten cases have occurred in patients with human immunodeficiency virus (HIV). Other cases have been identified from Argentina, Australia, Canada, Czechoslovakia, Japan, Mexico and Peru. Only two cases are known to have survived.

Cases of amebic encephalitis are probably underdiagnosed. Data from the California Encephalitis Project (CEP) suggested that *Balamuthia* accounted for approximately 0.1% of total encephalitis cases in the otherwise healthy population. A review of all cases of encephalitis reported in Louisiana from 1999 to 2008 (10-year span) showed an average annual number of 180 cases among the population not immunocompromised. Applying the CEP estimate, this number would represent two cases for each 10-year period. The same review showed that there are about 60 cases of this type of encephalitis where etiology was undetermined.

Under the Radar: *Balamuthia* Amebic Encephalitis

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Background. We present data from 9 years (1999–2008) of tests for *Balamuthia mandrillaris*, an agent of amebic encephalitis that were conducted as part of the California Encephalitis Project.

Methods. Specimens obtained from patients with encephalitis were sent to the California Encephalitis Project for diagnostic testing; a subset of these specimens were tested for *Balamuthia* species. Tests included indirect immunofluorescent staining of sections for amebae, fluorescent antibody staining and enzyme-linked immunosorbent assay for serum titers, and polymerase chain reaction for *Balamuthia* 16S mitochondrial DNA. Cerebrospinal fluid (CSF) samples obtained from patients with diverse types of encephalitis were also tested for a broad range of cytokines.

Results. Of 13500 cases referred to the California Encephalitis Project, 10 were found to be amebic encephalitis on the basis of serologic and CSF tests and examination of stained tissue sections. Most of these cases would have been described as

“encephalitis of unknown origin” if it were not for the California Encephalitis Project. Nine of the 10 patients were male; ages ranged from 1.5 to 72 years. All patients had abnormal neuro-imaging findings and abnormal CSF composition. The more common symptoms at presentation included headache, seizures, cranial nerve palsies, and lethargy. CSF specimens from patients with *Balamuthia* infection had significant elevations in the levels of cytokines IL-6 and IL-8, compared with specimens obtained from persons with viral or noninfectious encephalitides.

Conclusions. Balamuthiasis is difficult to diagnose, and it is likely that cases go unrecognized because clinicians and laboratorians are unfamiliar with the disease. Alerting the medical community to this disease may lead to earlier diagnosis and improve the chances of survival.

Other Modes of Transmission:

According to a Morbidity and Mortality Weekly Report - Centers for Disease Control and Prevention (MMWR-CDC) report published in September 2010, two confirmed cases of *Balamuthia* transmission occurred through organ transplantation in December 2009 in Mississippi. Two kidney recipients, a 31-year old woman and a 27-year old man, suffered from post-transplant encephalitis due to *Balamuthia*. The woman died in February, 2010; the man survived with partial paralysis of his right arm. The CDC was notified by a physician on December 14, 2009 about possible transplant transmission in these two patients. Histopathologic testing of donor and recipient tissues confirmed the transmission. Two other patients who received heart and liver transplants from the same donor, but in different hospitals were placed on preemptive therapy and remain unaffected. A second cluster of transplant-transmitted *Balamuthia* in Arizona was reported in the same weekly report. There were four recipients; two from Arizona- liver and kidney-pancreas, one from California- kidney and one from Utah-heart. The recipients from Arizona, two males, a 56-year old and a 24-year old, both succumbed to encephalitis within a span of 40 days from transplantation. The other two were placed on preemptive therapy after the first two were reported and remain unaffected.

Pathogenesis

Balamuthia: Infection may originate in the skin or lungs and disseminate to a hematogenous route. It then penetrates the blood–brain barrier to gain access to the brain. The amoeba seems to grow at a rate that is limited by the antibodies in the serum. This serum not only holds the amoeba population in check but possibly prevents amoebae from binding to the endothelia of the blood–brain barrier, and may neutralize toxins such as the proteases that are presumed to take part in the destruction of the cells. The chronic phase of the infection is likely to be at the stage where the blood–brain barrier is intact, but as soon as the amoeba breaks through into the brain, the amoebae, being free of antibody, are able to multiply and to destroy the brain rapidly.

The defenses of a healthy host seem sufficient to prevent *B. mandrillaris* infection. Patients who contract granulomatous amoebic encephalitis usually have impaired humoral and/or cell-mediated immunity. However, there are reports of patients with no demonstrable underlying disease or predisposing factor.

Semiology

Like *Acanthamoeba* GAE, *B. mandrillaris* encephalitis is largely a disease of the immunocompromised host and infects either sex, and any age. However, cases are being recognized in persons with no underlying immunosuppression and with no history of contact or swimming in water.

The clinical course of the disease in humans ranges from 14 days to six months, with a mean of 75 days. Infection is invariably fatal. Clinical symptoms and histopathological findings are consistent with necrotizing or granulomatous amoebic encephalopathy (GAE) or "brain abscess". Cysts are also found in the tissues.

Diagnostic

Timely diagnosis remains a very significant impediment to the successful treatment of infection, as most cases have only been discovered post-mortem.

In many cases, granulomatous amebic encephalitis is not diagnosed until after or, at best, shortly before death. Immunosuppression or other predisposing factors may provide important clues. The differential diagnosis includes space-occupying lesions such as tumors, abscesses, and even infarcts, as well as tuberculoma or fungal infection. Computed tomography and magnetic resonance imaging of the brain are important diagnostic tests, as is examination of cerebrospinal fluid and brain biopsy specimens. The diagnosis usually is made after examination of brain tissue with light a microscope.

Culture

B. mandrillaris does not grow on the standard medium for isolating free-living amebae, plain agar seeded with the bacterium *Escherichia coli*. *B. mandrillaris* has been cultured from only a few cases of infection using mammalian tissue culture cell lines. As a consequence of the difficulties in growing the organism, there have been no reports of the isolation of *B. mandrillaris* from water or other environmental samples.

Treatment (see Page 17).

3-Naegleria fowleri

History

Fowler and Carter first described human disease caused by amoeba-flagellates in Australia in 1965. Their work on amoeba-flagellates has provided an example of how a protozoan can effectively live both freely in the environment, and in a human host. A number of cases of infection occurred in towns served by unchlorinated water delivered through long above-ground pipelines. About half of the cases had swum in warm freshwater, and the other half had sniffed or squirted water from the town supply into their noses. There have been no cases in South Australia since 1981, with chloramination of the water supply and a public education campaign.

Since 1965, more than 144 cases have been confirmed in a variety of countries. In 1966, Fowler termed the infection resulting from *N. fowleri* primary amoebic meningo-encephalitis (PAM) in order to distinguish this CNS invasion from other secondary invasions caused by other true amoebas such as *Entamoeba histolytica*. A retrospective study determined that the first documented case of PAM possibly occurred in Ireland in 1909.

Biology

Naeglerias exist in three forms:

Trophozoite stage: Reproductive stage;

Transforms near 25°C/77°F and grows fastest 42°C/106.7°F,

Proliferates by binary fission

Travel by pseudopodia

Feed on bacteria when free. In tissues, they phagocytize RBC and WBC and destroy tissue.

Flagellate stage: Biflagellate form

When trophozoites exposed to change in ionic concentration (in distilled water)

Cyst stage when conditions become unfavorable such as food deprivation, crowding, desiccation, accumulation of waste products, cold temperatures (below 10°C/50°F)

Ecology- Source of Infection

Naegleria: There are several species of *Naegleria*, but *Naegleria fowleri* is the only species that has been shown to cause disease in humans.

Sources of *Naegleria* include:

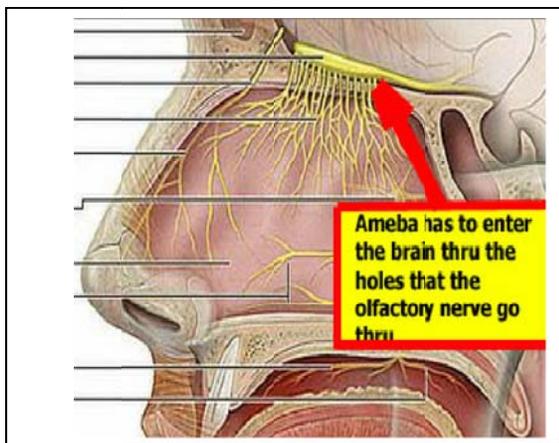
- **Lakes, ponds, ditches, puddles, rivers**: A comprehensive survey to document the presence of free-living amoebae was conducted along 58 km of James River, near Richmond, Virginia, U.S.. Sites included tidal and non-tidal freshwater areas, near 40 combined sewer outflows, three municipal wastewater treatment plant release sites, and thermal discharge from a coal-fired power plant. Amoebae were present on all collection dates, spring through autumn, and at all sites ($n=330$). Five genera, *Naegleria*, *Vannella*, *Acanthamoeba*, *Vahlkampfia*, and *Hartmannella* were present in both the water column and sediment. The most common isolates from the water column were *Naegleria* and *Vannella*. Water conditions conducive to the presence of large quantities of fecal coliform bacteria were correlated with the prevalence of free-living amoebae. Some of the amoebae in this complex ecosystem can act as opportunistic pathogens, may play a role in diseases of aquatic organisms in this heavily urbanized river, and may present a risk to human health (Ettinger M et al. 2002. Distribution of free-living amoebae in James River, Virginia, USA. Parasitology Research Volume 89, Issue 1, pp 6-15)

- **Naturally hot water such as hot springs**: In 2008 a child swam in a bath fed with geothermal water and developed PAM due to *Naegleria fowleri*. A total of 73 water samples, 48 sediments and 54 swabs samples were collected from six sampling points between June 2011 and July 2012. Thermophilic amoebae were present at nearly all collection sites. The pathogenic *N. fowleri* was the most frequently encountered thermophilic species followed by *N. lovaniensis*. The concentration of *N. fowleri* was rather low in most water samples, ranging from zero to 22 per liter. Sequencing revealed that all *N. fowleri* isolates belonged to a common Euro-American genotype, the same as detected in the human case in Guadeloupe. (Mirna Moussa et al. 2013. Survey of *Naegleria fowleri* in Geothermal Recreational Waters of Guadeloupe (French West Indies. PLoS One. 2013; 8(1): e54414. Published online 2013 January 18. doi: 10.1371/journal.pone.0054414).
- Poorly maintained and under-chlorinated or unchlorinated swimming pools, recreational water facilities
- Warm water discharge from industrial plants
- Domestic water supply
- Soil, however water is the only known source of human infection.
- The majority of cases of PAM have been reported in the United States, and these cases have occurred in previously healthy young adults and children **associated with water sports** (Wellings, F. et al. 1977. Isolation and identification of pathogenic *Naegleria* from Florida lakes. Appl. Environ. Microbiol. 34:661–667; DeNapoli, T. et al. 1996. Primary amoebic meningoencephalitis after swimming in the Rio Grande. Tex. Med. 92:59–63; Dingley, D. 1996. Commentary: safe water practices can lower risk of contracting primary amoebic meningoencephalitis. Tex. Med. 92:28–29).
- In a study of **home water supplies associated with the deaths of two children from PAM in Arizona**, 19 samples were collected from sources in their homes (bathroom, bathtub water, kitchen pipes, sink traps); 17 of these 19 samples were positive for *N.fowleri* by PCR (Marciano-Cabral F et al. 2003. Identification of *Naegleria fowleri* in domestic water sources by Nested PCR. Applied and environmental microbiology, p 5864-5869).
- Sniffing water into the nasal passages as **a religious ritual prior to prayer** (Lawande, R. V., J. T. Macfarlane, W. R. Weir, and C. Awunor-Renner. 1980. A case of primary amebic meningoencephalitis in a Nigerian farmer. Am. J. Trop. Med. Hyg. 29:21–25).
- **Full-submersion baptism** in a domestic water supply (Barnett, N. et al. 1996. Primary amoebic meningoencephalitis with *Naegleria fowleri*: clinical review. Pediatr. Neurol. 15:230–234).
- Immersion of the head in a **trough of water in a school playground** (. Dorsch, M. et al.1983. The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia. Trans. R. Soc. Trop. Med. Hyg. 77:372–377.
- **Total immersion in bathwater** (Miller, G., G. Cullity, I. Walpole, J. O’Connor, and P. Masters. 1982. Primary amoebic meningoencephalitis in Western Australia. Med. J. Aust. 1:352–357).
- **Indoor swimming pools** have been the source of PAM in Czechoslovakia (Cerva, L., K. Novak, and C. G. Culbertson. 1968. An outbreak of amoebic meningoencephalitis. Am. J. Epidemiol. 88:436–444)
- In Mexico, five cases of PAM were associated with swimming in **shallow water in an artificial canal** (Lares-Villa, F et al. 1993. Five cases of primary amebic meningoencephalitis in Mexicali, Mexico: study of the isolates. J. Clin. Microbiol. 31:685–688).

- In Great Britain, PAM was acquired from **mud puddles** in which children played after a heavy rainstorm (Apley, J et al. 1970. Primary amoebic meningoencephalitis in Britain. Br.Med. J. 1:596–599).
- In South Australia, cases of PAM have occurred through the **domestic water supply**. These cases occurred during the summer months in children submerged in **bathtubs and wading pools**. Household water was delivered via overland pipelines during a prolonged period of hot weather (Anderson, K., and A. Jamieson. 1972. Primary amoebic meningoencephalitis. Lancet i: 902–903; Anderson, K., and A. Jamieson. 1972. Primary amoebic meningoencephalitis. Lancet ii: 379; 3. Anderson, K., et al. 1973. Primary amoebic meningoencephalitis. Lancet ii: 672–673; Dorsch, M. et al. 1983. The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia. Trans. R. Soc. Trop. Med. Hyg. 77:372–377; Miller, G et al. 1982. Primary amoebic meningoencephalitis in Western Australia. Med. J. Aust.1:352–357).
- In Australia, tap water from **homes unoccupied for long period of time during the summer** (Carter, R. F. 1972. Primary amoebic meningo-encephalitis: an appraisal of present knowledge. Trans. R. Soc. Trop. Med. Hyg. 66:193–213; Miller, G. 1982. Primary amoebic meningoencephalitis in Western Australia. Med. J. Aust. 1:352–357).
- Use of **Neti pot** for sinus irrigation (Yoder J. 2012. Primary Amebic Meningoencephalitis Deaths Associated With Sinus Irrigation Using Contaminated Tap Water. Clinical Infectious Diseases 55: 79-85).
- **Well water:** The recent association in Arizona between unchlorinated drinking water and the transmission of *N. fowleri* suggests that groundwater has been an unrecognized source of this organism. PCR detected *N. fowleri* DNA in 11 (7.7%) of 143 wells. Of 185 total samples, 30 (16.2%) tested positive for *N. fowleri*. The organism was most often detected after the wells had been purged (17.9% purged vs. 10.0% initial samples), suggesting that *N. fowleri* was present in the aquifer or was released from the well casing or column during pumping. The wells testing positive for *N. fowleri* ranged in temperature from 21.9°C to 37.4°C (average 29.0°C; median 29.5°C) (Blair B.2008. *Naegleria fowleri* in Well Water. Emerg Infect Dis. 2008 September; 14(9): 1499–1501).

The presence of *N. fowleri* in environmental water has been linked to temperature, pH, coliforms, and the amount of organic matter present. Iron and iron-containing compounds in water favor growth of *N. fowleri*.

They grow best in warm water, especially between 25°C and 40°C. Any water body that seasonally exceeds 30°C or continually exceeds 25°C can support the growth of *N. fowleri*.



Naegleria infects people by entering the body when water containing the ameba goes up the nose. This may occur:

- when people swim, dive or fall into warm freshwater containing *Naegleria*.
- following domestic bathing, for example, when young children fall or slip in a bath of water containing *Naegleria*.

Naegleria infections do not occur as a result of drinking contaminated water, swimming in the sea, or from swimming in a properly cleaned, maintained and chlorinated swimming pool.

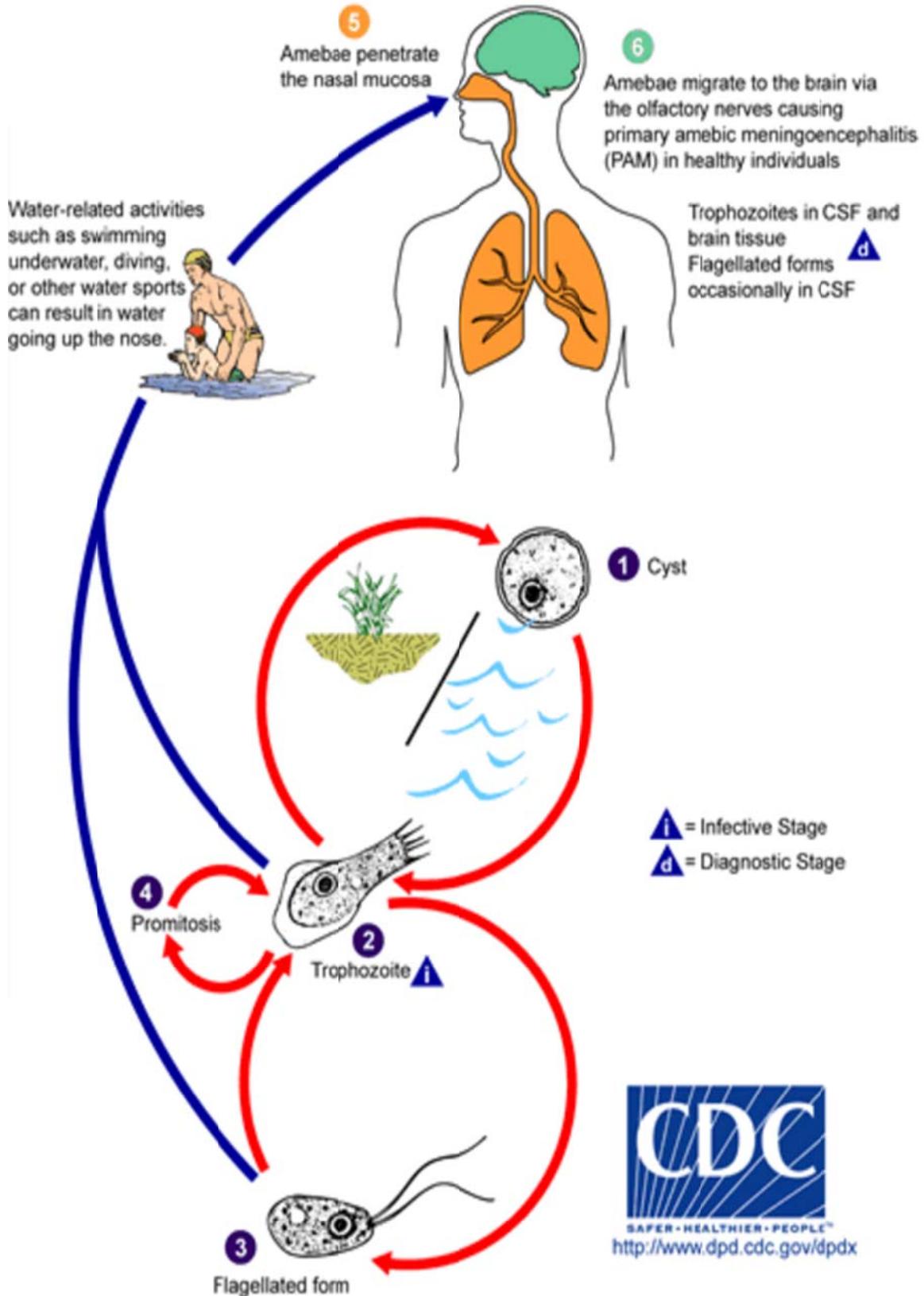
The amebae travel up the nose to the base of the brain where they infect brain tissue. The risk of infection is extremely small. Children and young adults appear to be more susceptible to infection than adults.

Persistence in the Environment

N.fowleri cannot survive in water that is clean, cool and chlorinated. Free chlorine or chloramines at 0.5mg/L or higher will control *N.fowleri*, provided that the disinfectant persists through the water supply system.

Infective cysts do persist in dust and aerosols.

Naegleria fowleri



Incidence /Disease Risk

Naegleria fowleri: Although *N.fowleri* can be commonly found in the environment, infection is rare. Cases of *Naegleria* meningoencephalitis have been recorded in South Australia, Western Australia, Queensland and New South Wales, and in many countries throughout the world. The risk of *Naegleria fowleri* infection is very low. There have been 30 reported infections in the U.S. in the ten years from 2000 to 2009, despite millions of recreational water exposures each year. By comparison, in the ten years from 1996 to 2005, there were over 36,000 drowning deaths in the United States.

In the U.S. in the ten years from 1998 to 2007, 33 infections were reported. Thirty-one had contact with recreational water and two had contact with water from a geothermal (naturally hot) water supply. It is estimated that the risk from recreational water activities (such as swimming/diving/waterskiing) in potentially contaminated freshwater in the U.S., is five cases of *N.fowleri* infection for every billion episodes of recreational water activity.

Semiology

Naegleria causes a very rare infection of the brain and brain coverings called primary amebic meningoencephalitis (PAM or PAME). Even with treatment, most people with *Naegleria fowleri* infection die.

In humans, *N. fowleri* can invade the central nervous system via the nose, more specifically through the olfactory mucosa and cribriform plate of the nasal tissues. The penetration initially results in significant necrosis of and hemorrhaging in the olfactory bulbs. From there, the amebae climbs along nerve fibers through the floor of the cranium via the cribriform plate and into the brain. It begins to consume cells of the brain piecemeal by means of a unique sucking apparatus extended from their cell surface. It then becomes pathogenic, causing PAM, which is a syndrome affecting the central nervous system. PAM usually occurs in healthy children or young adults with no prior history of immune compromise who have recently been exposed to bodies of fresh water.

Onset symptoms of infection start one to 14 days after exposure. The initial symptoms include, but are not limited to changes in taste and smell, also headache, fever, nausea, vomiting, and stiff neck. Secondary symptoms include confusion, hallucinations, lack of attention, ataxia and seizures. After the start of symptoms, the disease progresses rapidly in three to seven days, with death occurring in seven to 14 days.

Diagnostic

Timely diagnosis remains a very significant impediment to the successful treatment of infection, as most cases have only been discovered post-mortem.

In many cases, granulomatous amebic encephalitis is not diagnosed until after or, at best, shortly before death. Immunosuppression or other predisposing factors may provide important clues. The differential diagnosis includes space-occupying lesions such as tumors, abscesses, and even infarcts, as well as tuberculoma or fungal infection. Computed tomography and magnetic resonance imaging of the brain are important diagnostic tests, as is examination of cerebrospinal fluid and brain biopsy specimens. The diagnosis usually is made after examination of brain tissue with light a microscope.

Culture

N. fowleri can be grown in several kinds of liquid axenic media or on non-nutrient agar plates coated with bacteria. *Escherichia coli* can be used to overlay the non-nutrient agar plate with a drop of CSF sediment added to it. Plates are then incubated at 37°C and checked daily for clearing of the agar in thin tracks, which indicate that the trophozoites have fed on the bacteria.

Detection in water is performed by centrifuging a water sample with *Escherichia coli* added, and then applying the pellet to a non-nutrient agar plate. After several days, the plate is microscopically inspected and *Naegleria* cysts are identified by their morphology.

Final confirmation of the species' identity can be performed by various molecular or biochemical methods. Confirmation of *Naegleria* presence can be done by a so-called flagellation test, when the organism is exposed to a hypotonic environment (distilled water). *Naegleria* in contrast to other amoebae, differentiates within two hours into the flagellar state. Pathogenicity can be further confirmed by exposition to high temperature (42°C); *Naegleria fowleri* is able to grow at this temperature, but the non-pathogenic *Naegleria gruberi* is not.

PCR testing

The nested-PCR assay constitutes a highly sensitive tool for discriminating *N. fowleri* from other *Naegleria* species as well as from amoebae of the genus *Acanthamoeba* and other free-living amoebae commonly found in the environment. Pathogenic *N. fowleri* and nonpathogenic *Naegleria lovaniensis* are antigenically related. Therefore, a sensitive and specific nested-PCR assay is necessary to identify *N. fowleri* in water and soil samples. The nested-PCR assay can detect as few as five amoebae in 50 ml of water.

Recognition of *N. fowleri* in water requires specialized testing. Testing to identify the amoeba is not done routinely and is expensive.

Immunologic techniques:

Samples may be analyzed by Western immunoblotting to confirm the presence of *Naegleria* amoebae. However, the immunological technique *using polyclonal antiserum is genus specific and cannot distinguish the species N. fowleri from N. lovaniensis.*

Animal pathogenicity tests have been used to distinguish pathogenic from nonpathogenic *Naegleria* spp. Although *Naegleria australiensis* is pathogenic for mice, it has not been isolated from a human case of PAM. However, animal pathogenicity tests do not distinguish *N. fowleri* from *N. australiensis*.

Treatment (see Page 17).

Lab Diagnosis in the CDC

Since time is a crucial factor in the suspected *Naegleria* case, it is okay to ship specimens directly from the hospital without going through the public health laboratory.

Followings are some general guidelines as to how to ship specimens for diagnostic testing.

Diagnostic Assistance Provided by CDC.

Specimens can be sent to CDC for diagnostic assistance. If possible, please send the following specimens:

- **Fresh, unfixed CSF or tissue**
 - o If the specimen has been previously frozen or is preserved in formalin, CDC will still accept the specimen but the full range of testing methodologies might not be available
- **Paraffin-embedded and formalin-fixed tissue**
 - o Preferably a few H&E-stained slides and a few [about 6] unstained slides, or
 - o Paraffin-embedded tissue block
- **Sera** (two samples taken 2 weeks apart)

Fresh, unfixed specimens (i.e., CSF and tissue) should be sent at **ambient temperature** by overnight priority mail. Please ship these specimens separately from other chilled or frozen samples being shipped. The free-living amoebae are heat-loving and can be killed by cold temperatures (either refrigeration or freezing).

If the specimen (i.e., CSF or tissue) has been previously frozen or is preserved in formalin, the CDC will still accept the specimen but the full range of testing methodologies might not be available. Please send these specimens by overnight priority mail on **ice packs** (if tissue is frozen) (do **NOT** ship on dry ice) and ambient temperature if the tissue is fixed in formalin.

Care should be taken to pack glass slides securely, as they can be damaged in shipment if not packed in a crush-proof container.

Serum specimens can be collected from the patient in a red-top tube (plain vacuum tube with no additive) or a serum-separator tube (tiger top) tube (red/gray speckled top with gel in the tube). Please centrifuge the specimen, and if possible, send serum only. If using a plain red-top tube, you must separate the serum before shipping and send the serum only. Serum samples should be shipped refrigerated or frozen and packed with **cold packs**.

Please include the CDC specimen submission form 50.34, which can be found at this link:

<http://www.cdc.gov/laboratory/specimen-submission/form.html>. Be sure to add the pertinent travel history as well as relevant clinical history and any results from previous infectious disease testing. **Please arrange Monday–Friday delivery only. Packages cannot be accepted on weekends or federal holidays.**

Send packages by overnight express to:

CDC
SMB/STAT Lab
Attn: Unit 53
1600 Clifton Road
Atlanta, GA 30329
USA
Phone: 404-718-4157; 404-718-4174

The following link contains additional information on submitting specimens to CDC and diagnostic tests available at CDC. <http://www.cdc.gov/laboratory/specimen-submission/form.html>

Treatment

Treatments such as amphotericin-B, rifampicin, trimethoprim-sulfamethoxazole, ketokonazole, fluconazole, sulfadiazine, albendazole are only tentatively successful.

Amphotericin B is effective against *N. fowleri* in vitro, but the prognosis remains bleak for those that contract PAM, and survival rate remains less than 1%. On the basis of the in vitro evidence alone, the CDC currently recommends treatment with Amphotericin B for PAM, but there is no evidence that this treatment affects outcome. Treatment combining miconazole, sulfadiazine, and tetracycline has shown limited success only when administered early in the course of an infection.

Miltefosine had therapeutic effects during an in vivo study in mice. Miltefosine is now available for treatment of free-living amoeba infections caused by *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Acanthamoeba* species. Contact the CDC Emergency Operations Center at (770) 488-7100 to consult with a CDC expert regarding the use of this drug.

Miltefosine (INN, trade names Impavido and Miltex) is a phospholipid drug originally developed as an antineoplastic (and licensed for topical use), it is finding use as an antiprotozoal drug. It can be administered orally and topically.

Miltefosine main use now is for the treatment of visceral and cutaneous leishmaniasis, and is undergoing clinical trials for this use in several other countries since a 2005 survey concluded that Miltefosine is the only effective oral treatment for both forms of leishmaniasis. It is also an investigatory antiprotozoal and antifungal usage. It may also be effective against *Trypanosoma cruzi*, the parasite responsible for Chagas' disease. Studies have found the drug to be effective against *Cryptococcus neoformans*, *Candida*, *Aspergillus* and *Fusarium*. An in-vitro study found that Miltefosine is effective against metronidazole-resistant variants of *Trichomonas vaginalis*, a sexually transmitted protozoal disease.

The main side effects reported with miltefosine treatment are nausea and vomiting. Miltefosine has exhibited teratogenicity, and should not be administered to pregnant women

Prevention

Environmental persistence:

There have been numerous reports in the literature of the isolation of pathogenic free-living amoebae (PFLA) in chlorinated domestic and recreational waters (Cerva 1971; Anderson & Jamieson 1972; Cerva & Huldt 1974; De Jonckheere & van de Voorde 1976; Lyons & Kapur 1977). Consequently, public health authorities have expressed concern over the possible contraction of primary amoebic meningo-encephalitis (PAM) from these water sources. However, Cerva (1971) stated that "the strictest observations of all routine safety measures applied to water systems of swimming pools cannot prevent the constant presence of amoebae of the limax group in these treated water systems."

Free living amoebas in water systems

Thomas JM, Ashbolt NJ 2011. Do free living amoebae in treated water systems present an emerging health risk? Environ. Sci. Technol. 45: 860-869

- 19 water systems studied at point of use (Tap in dwelling - 16)
- 14 countries: BG, BR, CDN, CH, D, E, GB, HK, IL, K, MEX, NIC, PL, USA
- Number of samples in each survey: three to 283

- % Pos: range 7%-100%, mean 45%
- *Acanthamoeba* (most common), *Cochliopodium*, *Echinamoeba*, *Filamoeba*, *Hartmannella*, *Limax*, *Mayorella*, *Naegleria*, *Parvamoeba*, *Platyamoeba*, *Rosculus*, *Saccamoeba*, *Vannella*, *Vahlkampfia*, *Vexillifera*
- Most common in summer
- Increase with prolonged storage before distribution, roof tanks (22%), water cisterns (49%), main supply (13%).

TABLE 4. Detection of FLA in Treated Drinking Water at Point of Use

location	% positive (no. samples)	density (amoebae · L ⁻¹)	diversity (no. genera)	FLA genera identified (in order of frequency)	ref
tap water					
Bulgaria	18% (60)		>2	<i>Hartmannella</i> , <i>Acanthamoeba</i>	82
Germany	66% (3)	>0–100	>4	<i>Acanthamoeba</i> , <i>Hartmannella</i>	16
Poland	58% ^a (31)		1 ^a	<i>Acanthamoeba</i> ^a	100
Spain	60% ^a (148)		1 ^a	<i>Acanthamoeba</i> ^a	89
Switzerland	7.5% (200)		3	<i>Hartmannella</i> , <i>Acanthamoeba</i> , Unknown eukaryote	12
United Kingdom	89% (27) ^b		5	<i>Acanthamoeba</i> , <i>Hartmannella</i> , <i>Naegleria</i> , <i>Vahlkampfia</i> , <i>Vannella</i>	95
United Kingdom (London)	48% ^{Tank} (50) 26% ^{Mains} (50)		7	<i>Acanthamoeba</i> , <i>Hartmannella</i> , <i>Vahlkampfia</i> , <i>Vannella</i> , <i>Platyamoeba</i> , <i>Filamoeba</i> <i>Nuclearia</i>	94
Israel	92% ^a (26) 0% ^{Desal}		>1 ^a	<i>Acanthamoeba polyphaga</i> ^a	93
Canada	100% (18)	940 – 5300	5	<i>Vannella</i> , <i>Hartmannella</i> , <i>Vahlkampfia</i> , <i>Acanthamoeba</i> , <i>Naegleria</i>	87
Mexico	49% ^{Cistern} = 22% ^{Tank} = (27)		1 ^a	<i>Acanthamoeba</i> ^a	98
U.S.			3	<i>Vahlkampfia</i> , <i>Acanthamoeba</i> , <i>Naegleria</i>	86
U.S. (Florida)	19% ^{Toilet} (283)		12	<i>Vexillifera</i> , <i>Hartmannella</i> , <i>Acanthamoeba</i> , <i>Vahlkampfia</i> , <i>Vannella</i> , <i>Cochliopodium</i> , <i>Limax</i> , <i>Platyamoeba</i> , <i>Mayorella</i> , <i>Echinamoeba</i> , <i>Parvamoeba</i> , <i>Saccamoeba</i>	83
U.S. (Atlanta)	70% (207) ^c		4	<i>Hartmannella</i> , <i>Acanthamoeba</i> , <i>Vahlkampfia</i> , <i>Rosculus</i>	84
Nicaragua	23% ^a (74)		2 ^a	<i>Acanthamoeba</i> , ^a <i>Naegleria</i> ^a	101
West Indies	36% ^a (180)		1 ^a	<i>Acanthamoeba</i> ^a	92
Brazil	35% (135) ^c		>1 ^a	<i>Acanthamoeba</i> ^a	96
Hong Kong, China	10% ^a (100)		1 ^a	<i>Acanthamoeba</i> ^a	90
Hong Kong, China	8% ^a (90)		>1 ^a	<i>Acanthamoeba</i> ^a	91
Korea	47% (207)		>1 ^a	<i>Acanthamoeba</i> ^a	97

^a Data only reported for a single FLA genera or species. ^b Known sampling bias for certain FLA. ^c Data aggregated and some non-drinking water samples.

Mark W. Dawson & Tim J. Brown (1987); The effect of chlorine and chlorine dioxide on pathogenic free-living amoebae (PFLA) in simulated natural conditions: The presence of bacteria and organic matter, New Zealand Journal of Marine and Freshwater Research, 21:1, 117-123

Chlorine in the range of 0.5 to 1 mg/L is cysticidal for *Naegleria fowleri*

Derreumaux et al. (1974. Action du chlorure sur les amibes de l'eau. Annales de la Societe Belge de Medecine Tropicale 54[4/5] 415-428.) demonstrated that 0.5 mg/L of HOCl, the active disinfecting component of chlorine treatment, was able to destroy *Naegleria* spp. De Jonckheere & van de Voorde (1976: De Jonckheere JF et al. Differences in destruction of cysts of pathogenic and non-pathogenic *Naegleria* and *Acanthamoeba* by chlorine. Applied and Environmental Microbiology 31[2] 294-297) found that an initial chlorine concentration in the range 0.5-1.0mg/L was cysticidal for *Naegleria* spp., but that 40 mg/L was insufficient to inactivate *Acanthamoeba* cysts.

Cursons et al. (1980. Effect of disinfectants on pathogenic free-living amoebae: in axenic conditions.

Appl Environ Microbiol. July; 40[1]: 62–66) found that *Naegleria* spp. were more sensitive to chlorine than *Acanthamoeba* spp. in axenic conditions at pH 7.0, at which the concentrations required were 0.79 and 1.25 mg/L, respectively.

Chlorine dioxide has not received as much attention as chlorine and, though Cursons et al. (1980) showed it to be amoebicidal, the concentrations of chlorine dioxide tested, of up to 2.0 mg /L for *Naegleria* spp. and 3.4 mg /L for *Acanthamoeba* spp., did not totally eliminate the organisms. *Acanthamoeba* spp. were shown to be more resistant to chlorine dioxide than *Naegleria* spp.

Drinking water at 0.5 mg/L (ppm) of residual chlorine is safe.
A swimming pool at 1 to 3 mg/L (ppm) of residual chlorine is safe.

Personal actions to prevent infection:

Naegleria fowleri is found in many warm freshwater lakes and rivers in the United States, particularly in southern tier states. It is likely that a low risk of *Naegleria fowleri* infection will always exist with recreational use of warm freshwater lakes, rivers, and hot springs. The low number of infections makes it difficult to know why a few people have been infected compared to the millions of other people using the same or similar waters across the U.S. The only certain way to prevent a *Naegleria fowleri* infection is to refrain from water-related activities in warm, untreated, or poorly-treated water. The following list of measures may reduce risk for those planning to take part in water-related activities.

- Natural bodies of water:
 - Avoid jumping, diving or submerging your head in bodies of warm fresh water or thermal pools
 - Keep head above water in spas, thermal pools and warm fresh water bodies
 - Hold the nose shut or use nose clips when taking part in water-related activities in bodies of warm freshwater
 - Use nose clips
 - Avoid digging in or stirring up the sediment while taking part in water-related activities in shallow, warm freshwater areas
- Artificial bodies of water: pools
 - Empty and clean small collapsible wading pools and let them dry in the sun after each use
 - Use chlorine to disinfect the water
 - Keep head above water in spas, thermal pools
 - Ensure swimming pools and spas are adequately chlorinated and well maintained
 - Flush stagnant water from hoses before allowing children to play with hoses or sprinklers
- At home:
 - Don't allow water to go up your nose when bathing, showering or washing your face
 - Supervise children playing with hoses or sprinklers and teach them not to squirt water up their nose
 - Potentially contaminated water should not be used for any form of nasal irrigation or nasal lavage including Neti (an Ayurvedic practice of nasal cleansing).

- Private wells

N.fowleri has been identified where bore water is rested in above-ground dams and then piped over distances in above-ground pipes to private homes. The presence of *N.fowleri* will vary with ambient temperature, the distance water is piped, and the length of time the water is at temperatures favorable to the ameba while in storage and pipework. This length of time may be related to the rate of water use. In

such circumstances, measures to prevent infection should be observed. Seek specialist advice regarding the pros and cons of water treatment processes (eg chlorination, chloramination filtration, UV treatment).

Posting warning signs around recreational water bodies is not useful

Recreational water users should assume that there is always a low level of risk whenever they enter warm freshwater (for example, when swimming, diving, or waterskiing) in southern-tier states. Posting signs is unlikely to be an effective way to prevent infections. This is because the location and number of amebae in the water can vary over time. In addition, posted signs might create a misconception that bodies of water without signs are *Naegleria fowleri*-free.

Emergency Disinfection of Drinking Water

http://www.cdc.gov/healthywater/drinking/travel/emergency_disinfection.html

You can disinfect water by using household bleach. Bleach will kill some, but not all, types of disease-causing organisms that may be in the water (chlorine and iodine may not be effective in controlling more resistant organisms like Cryptosporidium).

Add 1/8 teaspoon (or 8 drops) of regular, unscented, liquid household bleach for each gallon of water, stir it well, and let it stand for 30 minutes before you use it. Store disinfected water in clean containers with covers.

Bleach	Container
8 Drops or 1/8 teaspoon	1 Gallon
2 ½ Teaspoons	20 Gallon container
5 to 9 Teaspoons	Average tub 40 to 70 gallons
18 Teaspoons or ½ Cup	Kiddie pool (depend on size) about 140 Gallons

- To calculate the volume of a tub:
 - Measure the length, the width and the height of water in inches,
 - Volume in cubic inches = Length * Width * Height of water
 - Divide by 233 to calculate Gallons
 - Example for a tub 60” long, 24” wide and water at 12” height:
 $60 \times 24 \times 12 = 17,280$ Cubic inches, $17,280 / 233 = \text{about } 75$ Gallons”.

- To calculate the volume of a kiddie pool:
 - Measure the diameter of the circular pool and the height of the water in inches
 - Volume of water in cubic inches $3.14 * (\text{Diameter}/2) * (\text{Diameter}/2) * \text{Height}$
 - Divide by 233 to calculate Gallons
 - Example for a kiddie pool with 48” diameter and water at 18” height:
 $3.14 * (48/2) * (48/2) * 24 = 3.14 * 24 * 24 * 18 = 32,555$ cubic inches $32,555 / 233 = 140$ Gallons