Naegleria fowleri (commonly referred to as the "brain-eating amoeba" or "brain-eating ameba"), is a free-living microscopic ameba, (single-celled living organism). It can cause a rare and devastating infection of the brain called primary amebic meningoencephalitis (PAM). The ameba is commonly found in warm freshwater (e.g. lakes, rivers, and hot springs) and soil. *Naegleria fowleri* usually infects people when contaminated water enters the body through the nose. Once the ameba enters the nose, it travels to the brain where it causes PAM, which is usually fatal. Infection typically occurs when people go swimming or diving in warm freshwater places, like lakes and rivers. In very rare instances, *Naegleria* infections may also occur when contaminated water from other sources (such as inadequately chlorinated swimming pool water or heated and contaminated tap water) enters the nose.

Illness & Symptoms:

You **cannot** get infected from **drinking** water contaminated with *Naegleria*. You can only be infected when contaminated water goes up into your nose.

Primary amebic meningoencephalitis (PAM), is a disease of the central nervous system. PAM is caused by *Naegleria fowleri*, a free-living ameba. It is a rare disease that is almost always fatal; only 3 people in the U.S. out of 132 have survived infection from 1962 to 2013. Signs and symptoms of *Naegleria fowleri* infection are clinically similar to **bacterial meningitis**, which lowers the chances of initially diagnosing PAM. Humans become infected when water containing *Naegleria fowleri* enters the nose and the ameba migrates to the brain along the olfactory nerve'. People do not become infected from **drinking** contaminated water. Symptoms start 1-7 days (median 5 days) after swimming or other nasal exposure to *Naegleria*-containing water. People die 1-12 days (median 5.3 days) after symptoms begin. PAM is difficult to detect because the disease progresses rapidly so that diagnosis is usually made after death. Signs and symptoms of the infection include:

- **Stage 1**
  - Severe frontal headache
  - Fever
  - Nausea
  - Vomiting

- **Stage 2**
  - Stiff neck
  - Seizures
  - Altered mental status
  - Hallucinations
  - Coma

The disease is generally fatal; among well-documented cases, there are only four known survivors in North America: one from the U.S. in 1978, one from Mexico in 2003, and two from the U.S. in 2013. The original U.S. survivor's condition gradually improved during a one-month hospitalization. The only reported side effect to treatment was a reduction in leg sensation for two

months after discharge, which gradually improved. There was also no detection of *Naegleria fowleri* 3 days post-treatment. It has been suggested that the original survivor's strain of *Naegleria fowleri* may have been less virulent, which contributed to the patient's recovery. In laboratory experiments, the California survivor's strain did not cause damage to cells as quickly as other strains, suggesting that it is less virulent than strains recovered from other fatal cases.

The Mexico survivor's condition did not begin to improve until 40 hours after hospital admission. On day 22 of admission, there was no abnormality shown in the brain scan and the patient was discharged the next day. The patient was followed up for the next 12 months without any recurrence of disease.

After 35 years without a *Naegleria* survivor in the United States, during the summer of 2013, two children with *Naegleria fowleri* infection survived. The first, a 12-year-old girl, was diagnosed with PAM approximately 30 hours after becoming ill and was started on the recommended treatment within 36 hours. She also received the investigational drug miltefosine, and her brain swelling was aggressively managed with treatments that included therapeutic hypothermia (cooling the body below normal body temperature). This patient made a full neurologic recovery and returned to school. Her recovery has been attributed to early diagnosis and treatment and novel therapeutics including miltefosine and hypothermia.

A second 8-year-old child is also considered a PAM survivor, although he has suffered what is likely permanent brain damage. He was also treated with miltefosine but was diagnosed and treated several days after his symptoms began. Therapeutic hypothermia was not used in this case.

Overall, the outlook for people who get this disease is poor, although early diagnosis and new treatments might increase the chances for survival.

Pathogen & Environment:

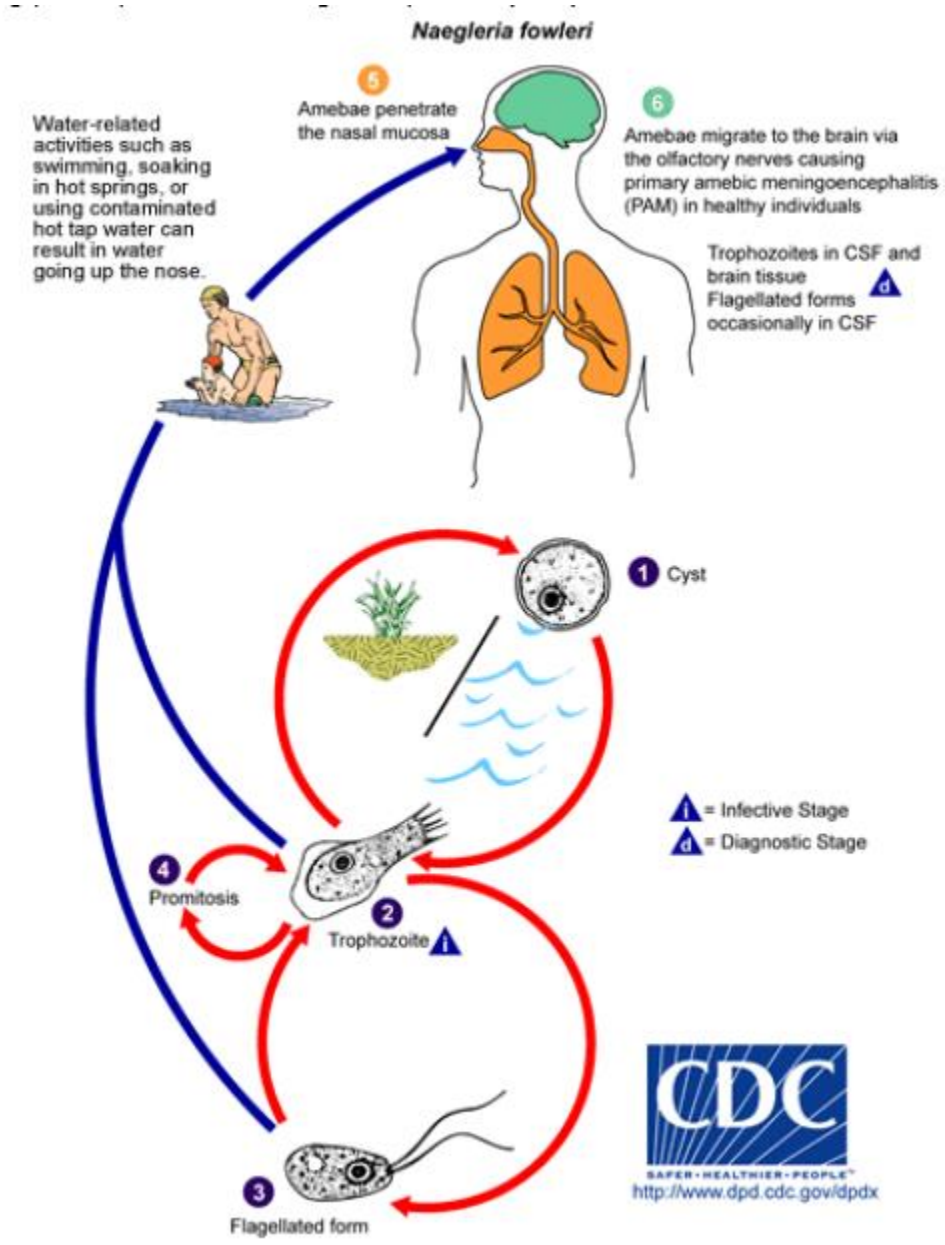
Naegleria fowleri is a heat-loving (thermophilic), free-living ameba (single-celled microbe), commonly found around the world in warm fresh water (like lakes, rivers, and hot springs) and soil. *Naegleria fowleri* is the only species of *Naegleria* known to infect people. Most of the time, *Naegleria fowleri* lives in freshwater habitats by feeding on bacteria. However, in rare instances, the ameba can infect humans by entering the nose during water-related activities. Once in the nose, the ameba travels to the brain and causes a severe brain infection called primary meningoencephalitis (PAM), which is usually fatal

History:

The first PAM infections were reported in 1965 in Australia. The ameba identified caused a fatal infection in 1961 and turned out to be a new species that has since been named *Naegleria fowleri* after one of the original authors of the report, M. Fowler. The first infections in the U.S., which occurred in 1962 in Florida, were reported soon after. Subsequent investigations in Virginia using archived autopsy tissue samples identified PAM infections that had occurred in Virginia as early as 1937

The Pathogen and Life Cycle:

Naegleria fowleri has 3 stages in its life cycle: ❶, ameboid trophozoites ❷, flagellates, and ❸ cysts. The only infective stage of the ameba is the ameboid trophozoite. Trophozoites are 10-35 µm long with a granular appearance and a single nucleus. The trophozoites replicate by binary division during which the nuclear membrane remains intact (a process called promitosis) ❹. Trophozoites infect humans or animals by penetrating the nasal tissue ❺ and migrating to the brain ❻ via the olfactory nerves causing primary amebic meningoencephalitis (PAM).



Trophozoites can turn into a temporary, non-feeding, flagellated stage (10-16 µm in length) when stimulated by adverse environmental changes such as a reduced food source. They revert back to the trophozoite stage when favorable conditions return. *Naegleria fowleri* trophozoites are found

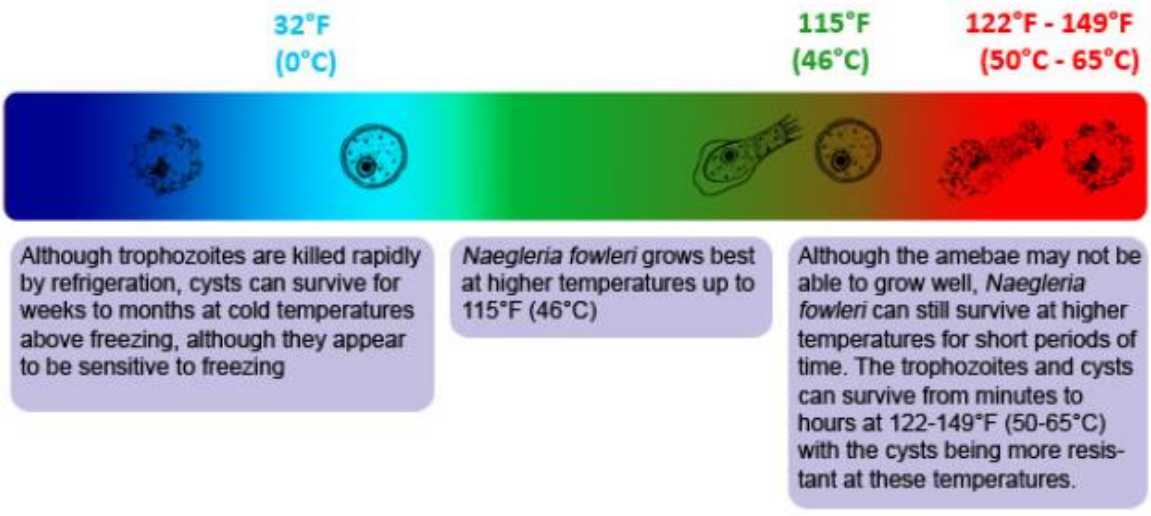
in cerebrospinal fluid (CSF) and tissue, while flagellated forms are occasionally found in CSF. Cysts are not seen in brain tissue. If the environment is not conducive to continued feeding and growth (like cold temperatures, food becomes scarce) the ameba or flagellate will form a cyst. The cyst form is spherical and about 7-15 µm in diameter. It has a smooth, single-layered wall with a single nucleus. Cysts are environmentally resistant in order to increase the chances of survival until better environmental conditions occur

Environmental Resistance:

Naegleria fowleri is normally found in the natural environment and is well adapted to surviving in various habitats, particularly warm-water environments. Although the trophozoite stage is relatively sensitive to environmental changes, the cysts are more environmentally hardy. There are no means yet known that would control natural *Naegleria fowleri* levels in lakes and rivers.

Drying: Drying appears to make trophozoites nonviable instantaneously and cysts nonviable in <5 min.

Temperature: *Naegleria fowleri* is a heat-loving (thermophilic) ameba able to grow and survive at higher temperatures, such as those found in hot springs and in the human body, even under fever temperatures. *Naegleria fowleri* grows best at higher temperatures up to 115°F (46°C). Although the amebae may not be able to grow well, *Naegleria fowleri* can still survive at higher temperatures for short periods of time. The trophozoites and cysts can survive from minutes to hours at 122-149°F (50-65°C) with the cysts being more resistant at these temperatures. Although trophozoites are killed rapidly by refrigeration, cysts can survive for weeks to months at cold temperatures above freezing, although they appear to be sensitive to freezing. As a result, colder temperatures are likely to cause *Naegleria fowleri* to encyst in lake and river sediment where the cyst offers more protection from freezing water temperatures



Disinfection: *Naegleria fowleri* trophozoites and the more resistant cysts are sensitive to disinfectants like chlorine and monochloramine which are used for disinfection of drinking water and swimming pools if adequate levels are maintained and monitored. The chlorine sensitivity is

moderate and in the same range as the cysts from ***Giardia intestinalis***, another waterborne pathogen. Specifically, chlorine at a concentration of 1 ppm (1 mg/L) added to 77°F (25°C) clear water at a pH of 7.5 will reduce the number of viable *Naegleria fowleri* trophozoites by 99.99% (4 logs) in 12 minutes. The more resistant *Naegleria fowleri* cysts are reduced by 99.99% (4 logs) in 53 minutes under the same conditions

To make your water safe for sinus rinsing and ritual nasal rinsing and ablution using chlorine bleach, follow these instructions.

If the water is clear:

- Add 1/8 teaspoon (or 8 drops; about 0.625 milliliters) of unscented liquid household chlorine (5-6%) bleach for each gallon of clear water (or 2 drops of bleach for each liter or each quart of water),
  - Stir the mixture well.
  - Let it stand for 30 minutes or longer before you use it.
  - Store the disinfected water in clean, sanitized containers with tight covers.

If the water is cloudy:

- Filter it through a clean cloth, paper towel, or coffee filter OR allow any sediment to settle.
- Draw off the clear water.
- Add 1/8 teaspoon (or 8 drops; about 0.625 milliliters) of unscented liquid household chlorine (5-6%) bleach for each gallon of clear water (or 2 drops of bleach for each liter or each quart of water),
  - Stir the mixture well.
  - Let it stand for 30 minutes or longer before you use it.
  - Store the disinfected water in clean, sanitized containers with tight covers.

Sources of Infection & Risk Factors:

Naegleria fowleri is a free-living ameba that causes primary amebic meningoencephalitis (PAM), a disease of the central nervous system [1, 2]. PAM is a rare disease* that is almost always fatal. In the United States**, there have been 132 PAM infections from 1962 through 2013 with only three survivors. These infections have primarily occurred in 15 southern-tier states, with more than half of all infections occurring in Texas and Florida. PAM also disproportionately affects males and children. The reason for this distribution pattern is unclear but may reflect the types of water activities (such as diving or watersports) that might be more common among young boys

Spread or Transmitted:

Humans become infected when water containing *Naegleria fowleri* enters the nose, usually while swimming. People do not get infected by **drinking** contaminated water. The ameba migrates to the brain along the olfactory nerve, through a bony plate in the skull called the cribriform plate, where it reaches the brain and begins to destroy the brain tissue. The ameba has never been shown to have spread from one person to another

Transplantation of organs from donors infected by *Naegleria fowleri* has been recorded, although none of the organ recipients became infected.However, the occurrence of *Naegleria fowleri* outside the brain has been observed; *Naegleria fowleri* has been documented in tissue sections of lung, kidney, heart, spleen, and thyroid from two deceased PAM cases. As a result, although the risk of transmission of *Naegleria fowleri* by donor organs is still unknown, it is unlikely to be zero so the risks of transplantation with an organ possibly harboring *Naegleria fowleri* should be carefully weighed for each individual organ recipient against the potentially greater risk of delaying transplantation while waiting for another suitable organ. This warrants continued study of the benefits and risks of transplanting organs or tissues from people infected by *Naegleria fowleri*.

Who Gets Infected?

PAM infections have been reported from around the world. From 1962 through 2013, 132 infections have been documented in the U.S. Infections have occurred in all age groups, but 111 cases (84%) have occurred in children under 18 years of age (median age of 11.5 years; range 8 months to 66 years). Over three-quarters (>75%) of infections have been in males. Infected people were often reported to have participated in water-related activities such as swimming underwater, diving, and head dunking that could have caused water to go up the nose.

Naegleria fowleri has also been documented to infect animals such as cattle and a South American tapir. Experimental infection can be induced in other species including mice, which are used as the model system for studying *Naegleria fowleri* infections resulting from swimming

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When Infections Occur:

Infections linked to freshwater swimming mostly occur during the heat of summer in July and August when water temperatures peak and water levels are low. Infections can increase during heat wave years as water temperatures increase

Risk of Infection

No data exist to accurately estimate the true risk of PAM. Hundreds of millions of visits to swimming venues occur each year in the U.S. that result in 0-8 infections per year. The extremely low occurrence of PAM makes epidemiologic study difficult. It is unknown why certain persons

become infected with the amebae while millions of others exposed to warm recreational fresh waters, including those who were swimming with people who became infected, do not. Attempts have been made to determine what concentration of *Naegleria fowleri* in the environment poses an unacceptable risk. However, no method currently exists that accurately and reproducibly measures the numbers of amebae in the water. This makes it unclear how a standard might be set to protect human health and how public health officials would measure and enforce such a standard

Diagnosis & Detection:

Primary amebic meningoencephalitis (PAM) is a serious infection and inflammation of the brain caused by *Naegleria fowleri*. The disease is diagnosed using specific laboratory tests available in only a few laboratories in the United States. Because of the rarity of the infection and difficulty in initial detection, about 75% of diagnoses are made after the death of the patient.

PAM and *Naegleria fowleri* infection can be diagnosed in the laboratory by detecting:

1. *Naegleria fowleri* organisms in cerebrospinal fluid (CSF), biopsy, or tissue specimens, or
2. *Naegleria fowleri* nucleic acid in CSF, biopsy, or tissue specimens, or
3. *Naegleria fowleri* antigen in CSF, biopsy, or tissue specimens.

Naegleria fowleri test methods:

Direct visualization: The motile amebae can often be seen moving rapidly under a microscope when looking at a fresh sample of CSF. The amebae can also be stained with a variety of stains, such as Giemsa-Wright or a modified trichrome stain, for identification.

Antigen Detection: A specific antibody to *Naegleria fowleri* can be used in conjunction with another antibody that deposits a chemical (immunohistochemistry [IHC]) or glows under specific types of light (indirect immunofluorescence [IIF]) to directly stain the amebic antigens in tissue.

Polymerase Chain Reaction (PCR): Specific molecular tools can amplify DNA from the amebae in CSF or tissue to specifically identify if the amebae are present. Looking at strains or subtypes of *Naegleria fowleri* can be done, but little is known about the natural populations in the environment, which makes it difficult to interpret what the findings mean.

Ameba culture: The amebae can be grown in culture to increase the likelihood of detecting the ameba by direct visualization or PCR. The sample is added to a growth plate covered in bacteria that can serve as a food source for *Naegleria fowleri*. The initial screening is accomplished by incubating the plate at a higher temperature (108°F/42°C) that kills most free-living amebae, while selecting for heat-loving (thermophilic) amebae, such as *Naegleria fowleri* or other amebae. This initial screen shows up as tracks made by an ameba as it moves across the plate eating the bacteria. If there are no amebae on the plate grown at the higher temperature, then *Naegleria fowleri* is not present. If heat-loving (thermophilic) amebae are present on the plate grown at the higher temperature, then these amebae undergo further specific testing to determine whether *Naegleria fowleri* is present since other free-living amebae can also be heat-loving (thermophilic). (NOTE:

Amebae, including thermophilic amebae other than *Naegleria fowleri*, can be common in water systems but none of these other amebae cause primary amebic meningoencephalitis (PAM)).

Environmental Detection: Water samples can be collected, concentrated, and put into culture to grow and select for *Naegleria fowleri*. Samples can be tested using the serologic or molecular methods described above.

Treatment:

Although most cases of primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri* infection in the United States have been fatal (129/132 in the U.S.,), there have been four well-documented survivors in North America: one in the U.S. in 1978, one in Mexico in 2003, and two additional survivors from the U.S. in 2013. It has been suggested that the original U.S. survivor's strain of *Naegleria fowleri* was less virulent, which contributed to the patient's recovery. In laboratory experiments, the original U.S. survivor's strain did not cause damage to cells as rapidly as other strains, suggesting that it is less virulent than strains recovered from other fatal infections.

Recently an investigational breast cancer and anti-leishmania drug, miltefosine, has shown some promise in combination with some of these other drugs. Miltefosine has shown ameba-killing activity against free-living amebae, including *Naegleria fowleri*, in the laboratory. Miltefosine has also been used to successfully treat patients infected with *Balamuthia* and disseminated *Acanthamoeba* infection. CDC now has a supply of miltefosine for treatment of *Naegleria fowleri* infection. If you are a clinician and have a patient with suspected *Naegleria* or other free-living ameba infection, please contact the CDC Emergency Operations Center at 770-488-7100 to consult with a CDC expert regarding the use of this drug.

After 35 years without a *Naegleria* survivor in the United States, during the summer of 2013, 2 children with *Naegleria fowleri* infection survived. The first, a 12-year-old girl, was diagnosed with PAM approximately 30 hours after becoming ill and was started on the recommended treatment within 36 hours. She also received the investigational drug miltefosine and her brain swelling was aggressively managed with treatments that included cooling the body below normal body temperature (therapeutic hypothermia). This patient made a full neurologic recovery and returned to school. Her recovery has been attributed to early diagnosis and treatment and novel therapeutics including miltefosine and hypothermia.

A second child, an 8-year-old male, is also considered a PAM survivor, although he has suffered what is likely to be permanent brain damage. He was also treated with miltefosine but was diagnosed and treated several days after his symptoms began. Cooling of the body below normal body temperature was not used.

Prevention & Control:

Naegleria fowleri infects people when water containing the ameba enters the body through the nose. Infection is rare and typically occurs when people go swimming or diving in warm freshwater places, like lakes and rivers. Very rarely, infections have been reported when people submerge their heads, cleanse their noses during religious practices, or irrigate their sinuses (nose) using

contaminated tap or faucet water. *Naegleria fowleri* can grow in pipes, hot water heaters, and water systems, including treated public drinking water systems. You **cannot** get infected from **drinking** water contaminated with *Naegleria.* You can only be infected when contaminated water goes up into your nose.

Risk of Infection:

Naegleria fowleri is found naturally in freshwater lakes, rivers, and hot springs in the United States, particularly in southern-tier states, but has recently caused infections as far north as Minnesota. No data exist to accurately estimate the true risk of PAM. Hundreds of millions of visits to swimming venues occur each year in the U.S. that result in 0-8 infections per year. It is unknown why certain persons become infected with the amebae while millions of others exposed to warm recreational fresh waters do not, including those who were swimming with people who became infected. Attempts have been made to determine what concentration of *Naegleria fowleri* in the environment poses an unacceptable risk. However, no method currently exists that accurately and reproducibly measures the numbers of amebae in the water. This makes it unclear how a standard might be set to protect human health and how public health officials would measure and enforce such a standard. However, the risk of *Naegleria fowleri* infection is very low. There have been 34 reported infections in the U.S. in the 10 years from 2004 to 2013, despite hundreds of millions of recreational water exposures each year. By comparison, in the ten years from 2001 to 2010, there were more than 34,000 drowning deaths in the United States.