Naegleria fowleri
Free-living Pathogenic Amoeba

Review Article

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By:

Fatima Faisal Khidhr

Supervised by:

Asst. Lecturer. Chreska Nooraldin Ahmed

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Supervisor’s Certification

I certify that this review article was prepared under my supervision at University of Salahaddin as a partial fulfillment of the requirements for the degree of BSc. in Biology.

Signature:

Supervisor: Asst. Lecturer. Chreska Nooraldin Ahmed

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Abstract

*Naegleria fowleri* is a free-living amoeba commonly found in warm freshwater environments such as hot springs, lakes, natural mineral water, and resort spas frequented by tourists. *N. fowleri* is the etiologic agent of primary amoebic meningoencephalitis (PAM), an acute fatal disease of the central nervous system that results in death in approximately seven days. Infection can occur when water containing the ameba enters the body through the nose, usually during recreational water activities such as swimming or diving. The PAM Previously thought to be a rare condition, the number of reported cases is increasing each year. PAM is difficult to diagnose because the clinical signs of the disease are similar to bacterial meningitis. Thus, the key to diagnosis is physician awareness and clinical suspicion, once amoebae are identified in CSF wet mount, treatment must be initiated immediately with high dose administration of systemic and intrathecal amphotericin B with or without miconazole, rifampin, and sulfisoxazole. *N. fowleri* proliferation can be controlled by adequate chlorination (one part per million), of heavily used swimming pools and in high-risk areas, monitoring of recreational waters for *N. fowleri* should be considered by local public health authorities particularly during the hot summer months. This review aimed to pay attention for its important and shows how to understand the effect of *Naegleria fowleri* infections on the health of human, its Pathogenesis, Clinical symptoms and the mechanisms that associate with the disease, as well as treatment and preventative ways.

**Keywords:** Free-living amoebae, Primary Amoebic meningitis, *Naegleria*, Diagnosis, Pathogen, Treatment.
1. Introduction

*Naegleria fowleri* is an ameboflagellate, known as "the brain-eating amoeba". (Gyori, 2003) it is a thermophilic amoeba that grows well in tropical and subtropical climates. Raised temperature during hot summer months or warm water from power plants facilitates its growth (Martinez and Visvesvara, 1997). This amoeba causes an acute, fulminant and rapidly fatal central nervous system (CNS) infection termed primary amoebic meningoencephalitis (PAM). The infection occurs from inhalation of trophozoites (Gyori, 2003). It enters the CNS, after insufflation of infected water, by attaching itself to the olfactory nerve, then migrating through the cribriform plate of the ethmoid bone along the fila olfactoria and blood vessels, and into the anterior cerebral fossae. It feeds on nerve tissue and causes extensive inflammation, necrosis and hemorrhage leading to death (Control and Prevention, 2008).

Infection occurs in healthy children and young adults with a recent history of exposure to warm fresh water (polluted water in ponds, swimming pools and man-made lakes). The ameba has been also detected in artificially heated industrial water sources (Huizinga and McLaughlin, 1990), and in domestic water supplies (Marciano-Cabral et al., 2003). About 310 cases have been reported with a high case fatality rate of approximately 95% (Cervantes-Sandoval et al., 2008, Gautam et al., 2012). The number of reported cases of PAM has increased worldwide in recent years. The increased incidence may be due to greater awareness of the disease or due to the development of more rapid, highly sensitive and specific diagnostic assays such as PCR. In addition, changes in environmental conditions, thermal pollution of water from industry and the development of industrialized areas with nuclear power plants and cooling towers that allow for concomitant greater growth of amoeba and their bacterial food source may afford greater opportunities for infection (Marciano-Cabral and Cabral, 2007).

This review aimed to highlight the role of *N. fowleri* as a causative agent of meningitis to draw special attention for its diagnosis and management.
2. Ecology and Morphology of *Naegleria fowleri*

Members of the genus *Naegleria* are distributed worldwide in soil and water (De Jonckheere, 2012) and have been isolated from fresh and warm-water lakes, streams, spas, heated but non-chlorinated swimming pools, hot springs, hydrotherapy and remedial pools, aquaria, sewage and even the nasal passages and throats of healthy individuals (Rodríguez-Zaragoza, 1994, Trabelsi et al., 2012). This amoeba has also been isolated from various animals, including reptiles, amphibians and fishes (Pantchev and Tappe, 2011). However, this micro-organism has not been recovered from seawater, suggesting its sensitivity to elevated osmolality. The vertical distribution of *N. fowleri* in water has been correlated with the presence of cyanobacteria and eubacteria; therefore, it is possible that the natural function of Naegleria genus is regulating bacterial populations (Kyle and Noblet, 1985). Additionally, the distribution of Naegleria has been associated with the concentrations of manganese and iron in the water column (Martínez-Castillo et al., 2015) *N. fowleri* is thermophilic and can survive temperatures of up to 45°C (Kyle and Noblet, 1987). Therefore, these amoebae proliferate mainly during the summer months, when the environmental temperature is likely to be high (Sifuentes et al., 2014).

There are three distinct morphological stages in the life cycle of *N. fowleri*: trophozoite, flagellate, and cyst (Figure 1A-C). The trophozoite is the infective stage of the amoeba. They are ~10-20mm long and contain a nucleus with a large karyosome surrounded by a halo. Trophozoites reproduce by binary fission and are motile due to round processes filled with granular cytoplasm called lobopodia. *N. fowleri* is a thermophilic organism and can tolerate temperatures up to 45°C; the ideal growth temperature for trophozoites is 42°C. When free-living, trophozoites use a structure called a food-cup (Figure 1D) to ingest bacteria and yeast – in a human host this same structure is used to ingest red blood cells, white blood cells, and tissue. Another important structure is the contractile vacuole. This vacuole
ruptures, empties, and reforms in a rapid process and is valuable in recognizing amebic trophozoites among other tissue cells.

The flagellate stage is entered as a response to a change in pH or ion concentration of the amoeba’s environment. In just minutes to a few hours trophozoites differentiate into bi-flagellated cells. This change can be induced by placement of trophozoites from culture into distilled water. Additionally, in unfavorable conditions (low nutrient, crowding, cold temperatures, desiccation), *N. fowleri* can form cysts. These cysts are ~8-15mm long and if they are introduced to the favorable environment of the human nasal passages can revert to the trophozoite stage and become infective (Martinez, 1985).

![Figure 1. Stages of *N. fowleri*. (A) Trophozoite (B) cyst (C) Flagellate (D) EM of food cup (Martinez, 1985).](image)

3. Life Cycle of *Naegleria fowleri*

*N. fowleri* has three stages, cysts, trophozoites, and flagellated forms, in its life cycle. The trophozoites replicate by promitosis (nuclear membrane remains intact). *Naegleria*
fowleri is found in fresh water, soil, thermal discharges of power plants, heated swimming pools, hydrotherapy and medicinal pools, aquariums, and sewage, 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 (Yoder et al., 2010) (Figure 4).

Figure 2. Life cycle of Naegleria fowleri (David and AP, 2006).

4. Pathogenesis

N. fowleri infections have been documented in healthy children and adults following recreational water activities, including swimming, diving, and water skiing. And it has been thought to infect the human body by entering the host through the nose when water
is splashed or forced into the nasal cavity. Infectivity occurs first through attachment to the nasal mucosa, followed by locomotion along the olfactory nerve and through the cribriform plate (which is more porous in children and young adults) to reach the olfactory bulbs within the CNS (Jarolim et al., 2000). Once *N. fowleri* reaches the olfactory bulbs, it elicits a significant immune response through activation of the innate immune system, including macrophages and neutrophils. *N. fowleri* enters the human body in the trophozoite form. Structures on the surface of trophozoites known as food cups enable the organism to ingest bacteria, fungi, and human tissue (Marciano-Cabral and Cabral, 2007).

In addition to tissue destruction by the food cup, the pathogenicity of *N. fowleri* is dependent upon the release of cytolytic molecules, including acid hydrolases, phospholipases, neuraminidases, and phospholipolytic enzymes that play a role in host cell and nerve destruction (De Jonckheere, 2011). The combination of the pathogenicity of *N. fowleri* and the intense immune response resulting from its presence results in significant nerve damage and subsequent CNS tissue damage, which often result in death.

**Figure 3.** Head anatomy with the olfactory nerve and the olfactory bulb highlighted. The amoebas primarily invade the brain along this nerve pathway (Gillen et al., 2019).
5. Primary Amoebic Meningoencephalitis (PAM)

Primary amebic meningoencephalitis (PAM) is a rapidly progressive and often fatal condition caused by the free-living ameba *Naegleria fowleri*. The earliest infection of PAM was reported in 1965 in Australia (Fowler and Carter, 1965). To date, few hundred cases of PAM have been reported worldwide. Incidentally, most cases have been described from the United States, Australia, and Europe. Trophozoites are the only form found in the lesions. *N. fowleri* overrun the olfactory bulbs following spread to the posterior areas of the brain. *N. fowleri* has been isolated from the cerebrospinal fluid (CSF) of infected patients (Cain et al., 1981). Initial symptoms include serious headache and fever (38.5 – 41°C), and then nausea, vomiting, and signs of meningeal irritation. Notably, involvement of the olfactory lobes may lead to disturbances in the sense of smell or taste and may be prominent early in the progression of the disease, whilst visual instabilities may also ensue. The patient may experience confusion, irritability, and may behave irrationally prior to lapsing into seizures and coma (John, 1982). The disease is referred to as “primary” to differentiate it from infection produced by other parasitic amoebae, which attack the CNS following dissemination through blood.

PAM is accompanied by strong inflammation, often made up of neutrophils, eosinophils, macrophages and lymphocytes. The incubation period from being exposed to the parasite until the development of the disease varies from one to 16 days (Martinez, 1977). People contract the parasite through exposure to contaminated water. Following infection, *N. fowleri* infiltrate the cribiform plate and the nasal mucosa then pass along the olfactory neuroepithelial route to gain entry to the brain to produce meningoencephalitis with quick cerebral edema, leading to cerebellar herniation and death (Martinez, 1977, Hecht et al., 1972). The acute hemorrhagic necrotizing meningoencephalitis that follows invasion of the CNS generally results in death 7–10 days post infection (Martinez and Visvesvara, 1997).
Figure 4. A section of the cerebral portion of the brain from PAM patient, stained with hematoxylin and eosin, showing large cluster of Naegleria fowleri trophozoite and destruction of normal brain tissue architecture. Cysts are not seen. Magnification: 100x. Inset: Higher magnification (1000x) of Naegleria fowleri trophozoites (arrows) with characteristic nuclear morphology (Control and Prevention, 2014). http://www.cdc.gov/parasites/naegleria/naegleria-fowleri-images.html

6. Host Immune Responses to N. fowleri Infection

Naegleria fowleri reaches the upper nasal mucosa using water as a vehicle. It then reaches the sub olfactory region of the cribiform plate, above which the olfactory bulb is located. As it ascends through the pores of this plate and makes its first contact with the olfactory epithelium/bulb and tissues surrounding this specific region (Baig and Khan, 2014). The understanding of the host immune response against Naegleria fowleri comes from the microscopic patterns that emerge and the cytokines that are released in patients with PAM. With Naegleria fowleri infection the macrophages of the olfactory region tend to recruit intense neutrophil influx. With all the acute inflammatory cytokines that are produced by the local and leukocytes that are recruited, the release of proteases, collagenase, and several other tissue destructive
lysosomal products by the inflammatory leukocytes an extensive cerebral edema and neuronal tissue damage occurs as seen at autopsy (Baig, 2015). After BBB gets breached the neural tissue gets infiltrated with leukocytes, with neutrophils and macrophages that predominate these lesions. Complement activation and tissue destructive cytokines along with cytotoxicity caused by proteases produced by the Naegleria trophozoites, evolve a lesion which is made up of fibrino-suppurative exudates, in PAM, The damage to the microvasculature leads to hemorrhage that is seen in gross and microscopic slides prepared from the autopsied brains (Cerva, 1989).

7. Clinical and Laboratory Diagnosis

Clinical symptoms and signs of infection with *N. fowleri* usually present within 2-8 days of infectivity, though some have been reported within 24 hours (Visvesvara et al., 2007, Goyal et al., 1979) Despite the absence of specific signs and symptoms indicating *N. fowleri* infection, the most common symptoms include severe headache, fever, chills, positive Brudzinski sign, positive Kernig sign, photophobia, confusion, seizures, and possible coma. In addition, cardiac rhythm abnormalities and myocardial necrosis have been observed in some cases (Martinez, 1985). Perhaps most importantly, increases in intracranial pressure and cerebral spinal fluid (CSF) pressure have been directly associated with death. CSF pressures of 600 mm H₂O have been observed in patients with *N. fowleri* infection. The motile amebae can often be seen moving rapidly under a microscope when looking at a fresh sample of CSF (Visvesvara et al., 2007). The amebae can also be stained with a variety of stains, such as Giemsa-Wright or a modified trichrome stain, for identification (Visvesvara, 2010). Magnetic resonance imaging (MRI) of the brain often shows abnormalities in various regions of the brain, including the midbrain and subarachnoid space (Visvesvara et al., 2007).
Figure 5. A cytospin of fixed CSF showing a *N. fowleri* trophozoite (arrow) stained with Giemsa-Wright amidst polymorphonuclear leukocytes and a few lymphocytes. Within the trophozoite, the nucleus and nucleolus can be seen. Magnification 1000x (Control and Prevention, 2014). http://www.cdc.gov/parasites/naegleria/naegleria-fowleri-images.html

Figure 6. Specimen from a 12-year-old Caucasian girl. *Naegleria fowleri* on Wright-Giemsa-stained cerebrospinal fluid (CSF) cytospin slides (original magnification, ×1000; oil immersion). Arrows indicate Naegleria organisms (Dunn et al., 2016).

8. Treatments

It is important to highlight that an appropriate diagnosis is the key to choosing an appropriate treatment. However, PAM is not commonly confirmed during the early stages of infection, and most people infected with this organism die. Because of the high mortality rate, more effective drugs are urgently needed. Drug discovery research has improved since the first report of PAM (Fowler and Carter, 1965, Carter, 1969, Rice et al., 2015).
Due to the rarity of *N. fowleri* infections in humans, there are no clinical trials to date that assess the efficacy of one treatment regimen over another. Most of the information regarding medication efficacy is based on either case reports or *in vitro* studies. Perhaps the most-agreed-upon medication for the treatment of *N. fowleri* infection is amphotericin B, which has been studied *in vitro* and also used in several case reports. Amphotericin B, a polyene antifungal, is considered to be the foundation of treatment. It acts by binding to ergosterol in the cell membrane, forming pores which alter membrane permeability and lead to cell death. (Grace et al., 2015). The current study suggests that the quinazolines alone as well as conjugated with silver nanoparticles may serve as potent therapeutics against brain-eating amoebae.

**9. Prevention and Control**

*Naegleria fowleri* is a thermophilic amoeba and hence proliferates in water when the ambient temperature increases above 30°C. Steps which can be taken by individuals who participate in water-related sports in warmer climates include avoidance of exposure to freshwater bodies such as lakes, rivers, and ponds, especially during the summer months when the water temperature is higher. Both chlorinated and salt water significantly decrease the risk of *N. fowleri* infection due to the inability of *N. fowleri* to survive in such environments. If freshwater activities cannot be avoided, it is recommended that individuals avoid jumping into the body of water, splashing, or submerging their heads under the water in order to avoid *N. fowleri* entering the nasal passages. If such activities cannot be avoided, individuals should use nose clips to decrease the chance of contaminated water entering the nose (Cogo et al., 2004).

*N. fowleri* amoebae should be considered by local public health authorities and appropriate warnings posted, particularly during the hot summer months. Warning children not to immerse their heads in suspect waters is recommended (Schuster and Visvesvara, 2004).
10. Conclusions

*Naegleria fowleri* is a dangerous parasite that is responsible for primary amoebic meningoencephalitis with a death rate approximately 95% in human. It is a wide spread in the countries where summer months are very hot. The parasite causes the disease when human is in contact with polluted water. The infection begins with the entrance of the infective stage "Trophozoites" to the nasal passage, reaches the brain, causing the infection to the central nervous system and finally death in 3 – 7 days. Even there is a medication this serious infection such as Amphotericin B but it is still killing. Finally, it could be concluded that it is important to make further studied about this parasite and how to protect the immune system by controlling this pathogen.
References


