N. fowleri: Epidemiology, Pathogenesis, and Current Treatment Options

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Abstract

N.fowleri is an amoeba that can cause a deadly disease in humans called Primary Amoebic Meningoencephalitis (PAM). Humans do not commonly contract this pathogen but when they do, its mortality rate is about 98%[1]. This pathogen is not confined to one region; it is spread out all around the world and primarily resides in warm freshwater bodies[2]. *N.fowleri* is the only species in the Naegleria genus that is pathogenic to humans, suggesting that it evolved to be pathogenic[3][4]. *N.fowleri* secretes certain molecules that damage the host cells, allowing the amoeba to evade the host's immune system and consume host cells. Treatment options are limited for this pathogen, but a few drugs like amphotericin B and chlorpromazine can help fight off the pathogen to some degree[5][6][7]. As *N.fowleri* is so deadly, prevention is better than cure. Some options to prevent contracting this life-threatening pathogen include not swimming in contaminated water bodies, or using nose plugs while swimming. This article will review the biology, pathogenesis, and treatment options of this pathogen.

Introduction

Naegleria fowleri (*N. fowleri)* is a brain-eating amoeba that causes an astonishing 98% mortality rate in infected humans. This high mortality rate is due to a lethal disease that *N. fowleri* precipitates: Primary Amoebic Meningoencephalitis (PAM). PAM is an infection of the central nervous system that involves the destruction of brain tissue, leading to death[1].

How does one contract *N. fowleri*? Typically, *N. fowleri* lives in warm freshwater bodies and can infect someone through the nose when swimming in a freshwater body[2]. Although science and technology have rapidly developed in recent years, the treatment options for *N. fowleri* are still limited[7]. Therefore, an important step towards reducing the risk posed by *N. fowleri* is educating more people about this subject. This article will conduct a systematic review of the basic biology, epidemiology, pathogenesis, and potential treatments for *N. fowleri*.

Biology and Epidemiology of *N. fowleri*

Basic Biology of N. fowleri

Naegleria fowleri is a thermophilic amoeba part of the Vahlkampfiidae family that has a three-phased life cycle—the trophozoite form, the flagellate form, and the cyst form. These phases largely depend on nutrient availability and environmental stressors. The main reproductive phase – trophozoites – is the most common form of the pathogen responsible for infections and is largely found in nutrient-rich, environmentally favorable conditions. During this stage, the amoeba is cylindrical with a bulging oval-shaped head approximately 22 µm long and 7 µm wide and primarily moves using hyaline semi-spherical pseudopodia. They divide through binary fission[1][7][8].

In nutrient-depleted conditions, the pathogen is capable of transitioning to a flagellate phase. Here the cell body takes a more oval shape, measuring from 10 to 16 µm in length, with two flagella of approximately the same length that allow the amoeba to move and swim towards more nutrient-dense and less stressful environments. If nutrients are found, it reverts to its trophozoite form. The flagellate thrives in temperatures between 27 and 37 degrees Celsius[1][7].

If environmental conditions continue to worsen – for example if freezing occurs – the

pathogen will enter a protective and dormant phase. In this state, the amoeba encysts, taking on a more rounded shape with a diameter that ranges from 7-12 μm, allowing it to resist environmental challenges such as colder temperatures or diminished food supplies. It has a thin ectocyst, which is a wall that protects the amoeba, and a thick endocyst, which is a thicker covering that helps protect the internal structures of the cyst. It also has mucoid-plugged pores, which are mucus-like substances that block harmful chemicals or other microbes from entering the cyst. Despite these elaborate structures, it appears to be sensitive to freezing. Nevertheless, once conditions improve, the pathogen can transition back to the trophozoite form[1][7].

Where is the pathogen found?

N. fowleri are thermophiles usually found in warm freshwater bodies, and are often associated with contaminated soil[1][2]. While there is no strict geographical range for the pathogen, recent infections have been reported worldwide. As of 2021, approximately 381 cases of PAM have been documented across the world, with the highest number recorded in the United States, followed by Pakistan and Mexico. In most cases, the pathogen was contracted through swimming or diving in infected water bodies (58%), resulting in the pathogen traveling up the victims' noses. Lakes, ponds, and reservoirs constituted the highest of these infected water bodies (45%).

Interestingly, cases of infections are not restricted to large bodies of water. Other infections have been reported by people swimming in untreated swimming pools and also people using tap water for various purposes, suggesting that the pathogen can thrive in various environments[2]. Regardless of where the pathogen is found, the mortality rate of *N. fowle*ri infection is an astonishing 98%, meaning that only about 2% of those who have contracted this pathogen have survived[1].

Genetics of N. fowleri

There are at least 44 different *Naegleria* species, of which 3 species are pathogenic. *Naegleria fowleri* is the only known pathogen from this genus to infect humans; the other two species, *Naegleria australiensis* and *Naegleria italica*, are only pathogenic to mice (there are no known reported human infections that involve these two species). Interestingly, *N. fowler*i is not closely related to the other two species, as they belong to different clusters on the phylogenetic tree. Instead, the pathogen is most closely related to a non-pathogenic relative, *N. lovaniensis* (Figure 1) [3]. This suggests that *N. fowleri* independently evolved to be pathogenic or *N. lovaniensis* evolutionarily lost its ability to be infectious[4].

Research conducted by Bexkens et al. shows that *Naegleria gruberi*, a distant relative of *N. fowleri*, metabolizes lipids as its preferred nutrient source. While many aerobes metabolize glucose through glycolysis, *N. gruberi* primarily performs beta-oxidation, utilizing fatty acids to produce acetyl-CoA, which can then be used in the Krebs cycle to produce ATP. Though distantly related, it is still predicted that *N. fowleri* has a similar food preference for lipids. Notably, the white matter of the brain is rich in myelin sheaths, which surround the axons of neurons and are lipid-rich. The researchers predict that the reason *N. fowleri* has a similar food preference to *N.gruberi* is related to why *N. fowleri* can infect the human brain, suggesting it breaks down myelin sheath lipids and uses these lipids to perform beta-oxidation to obtain energy in the form of ATP[9].

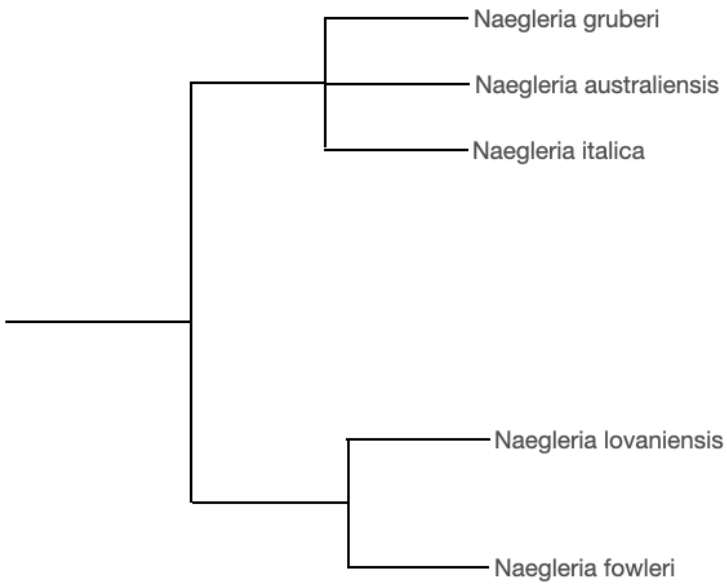

Figure 1: Family tree of different species found in the Naegleria genus. As shown, N. gruberi is evolutionarily distinct from *N. fowleri*. The 2 species that are pathogenic to mice but not humans, *N.australiensis* and *N.italica*, are closely related to each other and to *N.gruberi*. Figure adapted from [3].

Pathogenesis and evading the immune system

During infections, the pathogen, which is in the trophozoite form, enters a victim through the nose. From there, it travels up the olfactory nerve, crosses through the cribriform plate, and then travels into the olfactory bulb, from which it can easily access the brain[10]. Once infected, patients experience flu-like characteristics such as fever, nausea, headache, and vomiting. As the infection progresses, later symptoms include hallucinations, seizures, and altered mental status, often leading to death[11]. Autopsies on patients have revealed that the olfactory bulbs contain inflammatory exudates and hemorrhages. The cerebral hemispheres of the brain are soft, swollen, edematous, and gravely congested after an infection. The white matter of the brain and spinal cord display demyelination or a loss of myelin sheath, consistent with the claim that *N.fowleri* breaks down myelin sheath lipids to perform beta-oxidation to obtain energy[1].

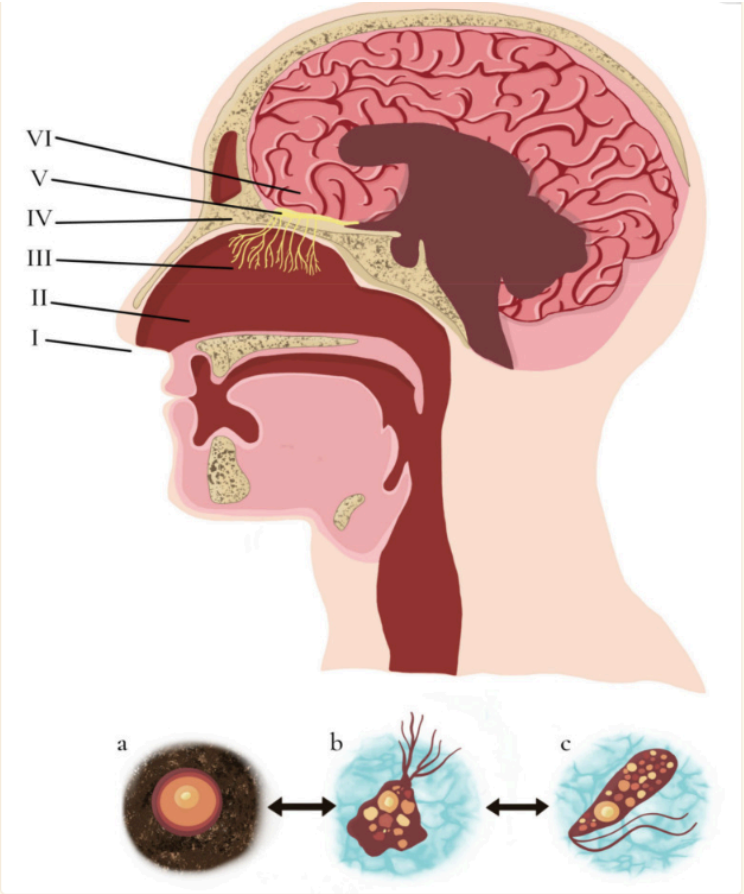

Figure 2: Diagram depicting the route *N. fowleri* takes to reach the brain. (a) the cyst form; (b) the flagellate form; and (c) the trophozoite form. Figure 2 was taken from[1].

N. fowleri's pathogenicity, as described by Herman et al., is considered to be a gain-of-function evolutionary change[4]. In other words, it's hypothesized that *N. fowleri's* pathogenicity is due to natural selection. *N. fowleri's* pathogenicity is determined by differences in its secreted proteases, lysosomal machinery, and motility compared to its close relative *N.gruberi*[4]. As described by Shibayama et al., *N. fowleri* secretes significant amounts of cysteine proteases that are capable of degrading tight junction proteins, ZO-1 and Claudin 1, between epithelial cells. Tight junction proteins are intracellular structures that play a crucial role in antimicrobial defense by not allowing incoming pathogens to cross epithelial cells. That said, *N.fowleri* can degrade these proteins, allowing it to easily penetrate the olfactory epithelium and invade the central nervous system. Surprisingly, the same experiment conducted with *N.gruberi* showed that it didn't degrade any tight junction proteins, preventing it from invading the CNS[12].

In nerve cells, *N.* fowleri is known to release cytolytic molecules, such as phospholipases that destroy cell membranes, neuraminidases that facilitate the destruction of tissue cells, and phospholipolytic enzymes that break down phospholipids from cell membranes. This generates a variety of lipid products that the amoeba might use to perform beta-oxidation[10][13][14]. Additionally, *N.fowleri* contains genes named *Nfa1* and *Nf-actin* that produce Nfa1 and Nf-actin proteins respectively. The *Nfa1* gene is linked to the pathogen's movement and the formation of

amoebastomes— small food cups that the amoeba uses to phagocytose or ingest its food[1]. These food cups were not found in *N.gruberi*[15]. The *Nf-actin* gene increases the pathogen's adhesion, phagocytosis, and cytotoxicity[1].

Several papers have suggested *N. fowleri* is resistant to host cytokine-mediated pathways, such as tumor necrosis factor (TNF)-α and IL-1, and cytolytic molecules such as the membrane attack complex (MAC) C5b-C9 of complement which allows them to evade the immune system[16]. The complement system is part of the innate immune system that opsonizes pathogens and helps fight off infections; MAC is a group of proteins that performs this function[17]. *N.fowleri* can easily avoid complement-mediated lysis by MAC by producing complement-regulatory proteins that interact with protein kinases, resulting in vesiculation and removal of the MAC from its membrane surface, thus protecting itself. *N.fowleri* can also avoid complement-mediated lysis by shedding the MAC or endocytosing and degrading the MAC[16].

Treatment Strategies

While treatment options are currently limited[7], there are a few methods to block *N.fowleri* infections. According to an experiment conducted by Jarillo-Luna et al., intranasal immunization with a protein named Cry1Ac and *N.fowleri* lysates (broken down *N.fowleri* cells) provides some protection against the pathogen as they induce the production of a mucosal antibody called IgA that is capable of binding to and preventing the pathogen from infecting the body[18].

Other methods include the use of drugs, including the antifungal drug amphotericin B (AmB). An experiment done by Cárdenas-Zúñiga et al. showed that amoebic cells treated with AmB were less viable and shrunk in size. The shrinkage was primarily due to a decrease in intracellular potassium levels, which caused a loss of cytoplasmic fluid from the cells. It was reported that the *N.fowleri* cells lost a lot of vacuoles and experienced DNA degradation. At the end of the experiment, *N.fowleri* cells also had an irregular nuclear membrane. Together, these results suggest AmB induces apoptosis-like programmed cell death in *N.fowleri* cells[5].

That said, while AmB seems like a good option to treat PAM, it has significant adverse side effects, limiting its utility. Kim et al. suggest that chlorpromazine is a better option than using AmB; chlorpromazine was less toxic than AmB *in vivo* when measuring and comparing the blood urea nitrogen (BUN) levels in mice inoculated with AmB and chlorpromazine. Normal BUN levels for mice range between 15 and 40mg/dl (a high BUN level indicates renal toxicity). The BUN level for 20mg/kg chlorpromazine was 38.4mg/dl and the BUN level for 20mg/kg AmB was 44.1mg/dl, indicating that chlorpromazine was less toxic than AmB. They also observed that a higher proportion of mice inoculated with chlorpromazine survived than mice inoculated with Amphotericin B— 75% of mice treated 3 times with chlorpromazine after being infected with *N.fowleri* survived while only 40% of mice treated 3 times with Amphotericin B survived[6].

Discussion & Conclusion

N.fowleri is an amoeba that causes a deadly disease named PAM (Primary Amoebic Meningoencephalitis). Humans do not commonly contract this pathogen, but its mortality rate is about 98%[1]. *N.fowleri* secretes certain molecules that damage the host cells, allowing the amoeba to evade the host's immune system and consume host cells. Treatment options are limited for this pathogen, but a few drugs like amphotericin B and chlorpromazine can help fight the pathogen to some degree[5][6][7]. As *N.fowleri* is deadly, prevention is better than cure. Not swimming in contaminated water bodies, or using nose plugs while swimming are some options

to prevent contracting this life-threatening pathogen.

Given the rarity of *N. fowleri* infections, one might question whether it constitutes a major public health concern. The low incidence rate suggests that the general population is at minimal risk, and the pathogen's presence does not necessarily translate to a widespread threat. However, the severity of the infections when they do occur cannot be ignored. The infection is understudied and often misdiagnosed — usually identified as bacterial meningitis[14]. While it may not be a significant public health concern based on the number of infections alone, its ability to cause severe disease necessitates continued awareness to prevent and manage cases that do arise. Research on better diagnostics and the pathogen itself may additionally be fruitful, especially with global warming expanding the pathogen's presence in the environment. Drugs that inhibit the expression of genes that produce cytotoxic molecules could prevent invasion of the host epithelial cells. Alternatively, targeting *N. fowleri's* ability to form amoebastomes could effectively starve the pathogen by preventing it from ingesting nutrients from the host's body. Both strategies may prove effective in defeating *N.fowleri* and should be further investigated.

Works Cited

1. Güémez, A., & García, E. (2021). Primary Amoebic Meningoencephalitis by Naegleria fowleri: Pathogenesis and Treatments. *Biomolecules*, *11*(9), 1320. https://doi.org/10.3390/biom11091320

2. Gharpure, R., Bliton, J., Goodman, A., Ali, I. K. M., Yoder, J., & Cope, J. R. (2021). Epidemiology and Clinical Characteristics of Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri* : A Global Review. *Clinical Infectious Diseases*, *73*(1), e19–e27. https://doi.org/10.1093/cid/ciaa520

3. de Jonckheere, J. F. (2011). Origin and evolution of the worldwide distributed pathogenic amoeboflagellate Naegleria fowleri. *Infection, Genetics and Evolution*, *11*(7), 1520–1528. https://doi.org/10.1016/j.meegid.2011.07.023

4. Herman, E. K., Greninger, A., van der Giezen, M., Ginger, M. L., Ramirez-Macias, I., Miller, H. C., Morgan, M. J., Tsaousis, A. D., Velle, K., Vargová, R., Záhonová, K., Najle, S. R., MacIntyre, G., Muller, N., Wittwer, M., Zysset-Burri, D. C., Eliáš, M., Slamovits, C. H., Weirauch, M. T., … Dacks, J. B. (2021). Genomics and transcriptomics yields a system-level view of the biology of the pathogen Naegleria fowleri. *BMC Biology*, *19*(1), 142. https://doi.org/10.1186/s12915-021-01078-1

5. Cárdenas-Zúñiga, R., Silva-Olivares, A., Villalba-Magdaleno, J. D. A., Sánchez-Monroy, V., Serrano-Luna, J., & Shibayama, M. (2017). Amphotericin B induces apoptosis-like programmed cell death in Naegleria fowleri and Naegleria gruberi. *Microbiology*, *163*(7), 940–949. https://doi.org/10.1099/mic.0.000500

6. Kim, J.-H., Jung, S.-Y., Lee, Y.-J., Song, K.-J., Kwon, D., Kim, K., Park, S., Im, K.-I., & Shin, H.-J. (2008). Effect of Therapeutic Chemical Agents In Vitro and on Experimental Meningoencephalitis Due to *Naegleria fowleri*. *Antimicrobial Agents and Chemotherapy*, *52*(11),

4010–4016. https://doi.org/10.1128/AAC.00197-08

7. Pervin, N., & Sundareshan, V. (2022). *Naegleria*.
Sarma, J. V., & Ward, P. A. (2011). The complement system. *Cell and Tissue Research*, *343*(1), 227–235. https://doi.org/10.1007/s00441-010-1034-0

8. Soontrapa, P., Jitmuang, A., Ruenchit, P., Tiewcharoen, S., Sarasombath, P. T., & Rattanabannakit, C. (2022). The First Molecular Genotyping of Naegleria fowleri Causing Primary Amebic Meningoencephalitis in Thailand With Epidemiology and Clinical Case Reviews. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.931546
Visvesvara, G. S. (2013). *Infections with free-living amebae* (pp. 153–168). https://doi.org/10.1016/B978-0-444-53490-3.00010-8

9. Bexkens, M. L., Zimorski, V., Sarink, M. J., Wienk, H., Brouwers, J. F., de Jonckheere, J. F., Martin, W. F., Opperdoes, F. R., van Hellemond, J. J., & Tielens, A. G. M. (2018). Lipids Are the Preferred Substrate of the Protist Naegleria gruberi, Relative of a Human Brain Pathogen. *Cell Reports*, *25*(3), 537-543.e3. https://doi.org/10.1016/j.celrep.2018.09.055

10. Grace, E., Asbill, S., & Virga, K. (2015). Naegleria fowleri: Pathogenesis, Diagnosis, and Treatment Options. *Antimicrobial Agents and Chemotherapy*, *59*(11), 6677–6681. https://doi.org/10.1128/AAC.01293-15

11. Capewell, L. G., Harris, A. M., Yoder, J. S., Cope, J. R., Eddy, B. A., Roy, S. L., Visvesvara, G. S., Fox, L. M., & Beach, M. J. (2015). Diagnosis, Clinical Course, and Treatment of Primary Amoebic Meningoencephalitis in the United States, 1937-2013. *Journal of the Pediatric Infectious Diseases Society*, *4*(4), e68–e75. https://doi.org/10.1093/jpids/piu103

12. Shibayama, M., Martínez-Castillo, M., Silva-Olivares, A., Galindo-Gómez, S., Navarro-García, F., Escobar-Herrera, J., Sabanero, M., Tsutsumi, V., & Serrano-Luna, J. (2013). Disruption of MDCK cell tight junctions by the free-living amoeba Naegleria fowleri. *Microbiology*, *159*(Pt_2), 392–401. https://doi.org/10.1099/mic.0.063255-0

13. Balboa, M. A., & Balsinde, J. (2021). Phospholipases: From Structure to Biological Function. *Biomolecules*, *11*(3), 428. https://doi.org/10.3390/biom11030428

14. Visvesvara, G. S. (2013). *Infections with free-living amebae* (pp. 153–168). https://doi.org/10.1016/B978-0-444-53490-3.00010-8

15. John, D. T., Cole, T. B., & Marciano-Cabral, F. M. (1984). Sucker-like structures on the pathogenic amoeba Naegleria fowleri. *Applied and Environmental Microbiology*, *47*(1), 12–14. https://doi.org/10.1128/aem.47.1.12-14.1984

16. Marciano-Cabral, F., & Cabral, G. A. (2007). The immune response to *Naegleria fowleri* amebae and pathogenesis of infection. *FEMS Immunology & Medical Microbiology*, *51*(2), 243–259. https://doi.org/10.1111/j.1574-695X.2007.00332.x

17. Sarma, J. V., & Ward, P. A. (2011). The complement system. *Cell and Tissue Research*, *343*(1), 227–235. https://doi.org/10.1007/s00441-010-1034-0

18. Jarillo-Luna, A., Moreno-Fierros, L., Camps-Rodriguez, R., Rodriguez-Monroy, M.A., Lara-Padilla, E., Rojas-Harnadez, S. (2008). Intranasal immunization with *Naegleria fowleri* lysates and Cry1Ac induces metaplasia in the olfactory epithelium and increases IgA secretion. *Parasite Immunology*, *30*(1), 31–38. https://doi.org/10.1111/j.1365-3024.2007.00999.x