REVIEW

Recent Advances in Primary Amoebic Meningoencephalitis: A Comprehensive Review of Therapeutic Compounds and Vaccine Prospects

Sabi Ur Rehman[1], Namira Islam[1], Saman Ali[1], Fazal Ur Rehman[2], Mian Waqar Mustafa[1], Sara Shoaib[3]

[1] Department of Pharmacy, Faculty of Natural Sciences, Forman Christian College (A Chartered University), Lahore, 54600, Pakistan
[2] Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan
[3] Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

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Corresponding author:
Sabi Ur Rehman
Address: Department of Pharmacy, Faculty of Natural Sciences, Forman Christian College (A Chartered University), Lahore, 54600, Pakistan
E-mail:
sabikhan19@gmail.com
sabirehman@fccollege.edu.pk

ORCID iDs

Sabi Ur Rehman
https://orcid.org/0000-0002-0880-0358
Namira Islam
https://orcid.org/0009-0001-3027-1659
Saman Ali
https://orcid.org/0000-0002-5962-3996
Fazal Ur Rehman
https://orcid.org/0000-0002-5923-4835
Mian Waqar Mustafa
https://orcid.org/0000-0002-1224-8826
Sara Shoaib
https://orcid.org/0009-0008-1886-8998

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ABSTRACT

This review article explores the latest developments in the study of primary amoebic meningoencephalitis. It emphasizes the importance of vaccinations as a possibly groundbreaking preventive measure that could revolutionize the fight against and elimination of primary amoebic meningoencephalitis. The importance of prospective applications of phytochemicals from different natural sources is further emphasized. This review provides an extensive overview of the frontiers in primary amoebic meningoencephalitis treatment and prevention by integrating the most recent research with potential future directions.

Keywords: *Naegleria fowleri*; Amebic Meningoencephalitis, Primary; Vaccines; Preventive Health Services; Public Health (Source: MeSH)

Avances recientes en la meningoencefalitis amebiana primaria: revisión exhaustiva de compuestos terapéuticos y perspectivas de vacunas

RESUMEN

Este artículo de revisión explora los últimos avances en el estudio de la meningoencefalitis amebiana primaria. Se destaca la importancia de las vacunas como posible medida preventiva innovadora que podría revolucionar la lucha contra la meningoencefalitis amebiana primaria y su eliminación. Además, se hace hincapié en la importancia de las aplicaciones prospectivas de los fitoquímicos procedentes de distintas fuentes naturales. Esta revisión ofrece un amplio panorama de las fronteras en el tratamiento y la prevención de la meningoencefalitis amebiana primaria, integrando las investigaciones más recientes con las posibles direcciones futuras.

Palabras clave: *Naegleria fowleri*; Meningoencefalitis Amebiana Primaria; Vacunas; Servicios Preventivos de Salud; Salud Pública (Fuente: DeCS)

INTRODUCTION

The amoeba *Naegleria fowleri* causes the uncommon but fatal brain infection known as primary amoebic meningoencephalitis (PAM). Swimming in warm freshwater rivers, lakes, and hot springs may spread *N. fowleri* through the nose into the body, leading to infection or disease. Once in the brain, the amoeba causes inflammation and brain tissue destruction by ascending the olfactory nerve (1).

PAM primarily affects children and young adults and is more prevalent in tropical and subtropical regions. Thirty-nine cases of PAM were reported globally between 2011 and 2020,

per a recent study by Güémez *et al.* (2). According to the place of origin, the United States was where most cases occurred, followed by Pakistan, Mexico, the Czech Republic, and India (2,3). Only a few individuals have ever survived PAM, with a mortality rate of over 98 % (4). Although there isn't a vaccine to protect against PAM in humans, researchers are working on various immunization approaches that may one day be utilized to stop this fatal illness (5).

Naegleria fowleri, commonly known as the "brain-eating amoeba," is a parasitic amoebic organism found in freshwater, ponds, and lakes. It belongs to the family Vahlkampfiidea, order Schizopyrenida and phylum Percolozoa. Among the 47 species within the Naegleria genus, *N. fowleri* is the sole pathogenic species. This parasite exhibits a global distribution on almost every continent except Antarctica. It thrives in temperatures exceeding 45 degrees Celsius and can endure temperatures ranging from 4 to over 50 degrees Celsius (6).

N. fowleri primarily exists in the trophozoite form (Figure 1), reproducing rapidly and mainly feeding on gram-negative bacteria. Under incredibly unfriendly circumstances, the trophozoite changes into a cyst stage, which is profoundly safe and fit for enduring temperatures as low as 4 degrees Celsius (7). Consequently, this parasite can thrive in various locations worldwide, excluding polar regions. When faced with unfavorable conditions, the amoeba transitions into a flagella-like shape that retains amoebic characteristics but is neither reproductive nor infective. Under normal conditions, the parasite reverts to the trophozoite stage. In this stage, it has a cup-shaped structure that enables it to feed on bacteria. This phase is when the parasite is most reproductive and infective, becoming pathogenic and easily entering the human body through the nasal passage during recreational or religious activities in warm freshwater bodies (8).

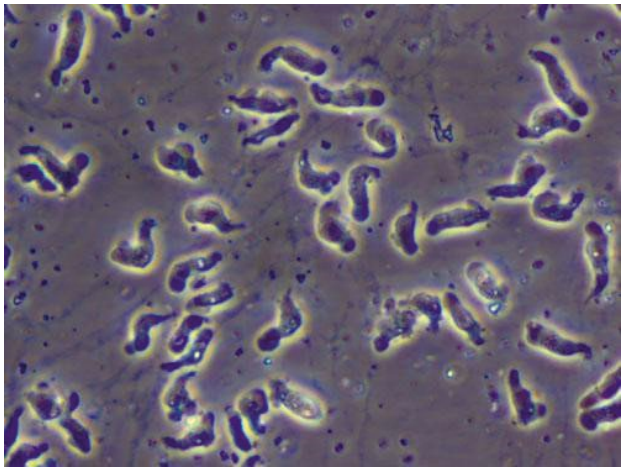

Figure 1. A wet mount of *N. fowleri* trophozoites cultured from the CSF of a patient with PAM (9). *Source: Centers for Disease Control and Prevention CDC), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED).*

Moreover, the genome of *Naegleria fowleri* has been sequenced, revealing insights into its evolutionary history and potential targets for intervention. This hereditary data has prepared for developing novel inoculation procedures to prevent the devastating impact of *Naegleria fowleri* infections (10).

PATHOPHYSIOLOGY

Naegleria fowleri, generally known as the "brain-eating amoeba," initiates its pathophysiological course when individuals encounter warm freshwater contaminated with the amoebae, which get sufficient access through the nasal entries (11). Using the olfactory nerve, these amoebae navigate the ethmoid bone's cribriform plate, bypassing the blood-brain barrier (BBB) (12).

Once in the brain, they predominantly target the olfactory bulb and frontal lobes, primarily in the trophozoite form, releasing cytotoxic compounds such as pore-forming proteins, proteases, and phospholipases. These compounds contribute to host cell damage by forming membrane pores, breaking down proteins, and disrupting phospholipids within cell membranes (13). The damage inflicted by *N. fowleri* involves several mechanisms: *N. fowleri* amoebae secrete proteins that can form pores or holes in host cell membranes. This disrupts the integrity of host cells and allows the entry of ions and molecules, leading to cell dysfunction and death. Within host cells, proteases can degrade proteins, disintegrating cellular components and eventual cell death (14). Furthermore, phospholipases are enzymes that degrade phospholipids, which are critical components of cell membranes, hence contributing to cell death by altering the structure of cell membranes (15).

In addition, the body's immune response to *Naegleria fowleri* releases inflammatory molecules and activates immune cells, which can further damage nerve cells (13). Those infected with this "brain-eating amoeba" experience severe neurological manifestations and an elevated mortality rate because of the immune system's inflammatory responses contributing to the damage (16). Diagnosing and treating PAM early improves survival chances (17).

Clinical Manifestations

Naegleria fowleri is an amoeba found in warm freshwater that causes PAM, a rare but severe disease. After exposure to the amoeba, people usually develop symptoms that resemble the flu, such as headaches, fever, nausea, and vomiting within a week. As the disease progresses, symptoms can include loss of appetite, irritability, neck stiffness, tiredness, sensitivity to light, and seizures. In extreme cases, patients can fall into a coma. Diagnosing PAM is difficult due to its generic initial symptoms, and sometimes confirmation only comes after a post-mortem examination that reveals the amoeba's impact on the brain (18,19).

DIAGNOSIS AND TREATMENT

PAM is a severe infection caused by *Naegleria fowleri* and is typically diagnosed in advanced stages due to overt clinical symptoms. Diagnostic procedures include brain imaging through CT scans or MRIs to detect signs of brain swelling or inflammation (Figure 2). The definitive diagnosis is confirmed via a brain biopsy, which can detect the amoeba directly. CSF analysis through lumbar puncture also plays a role in the diagnostic process. Treatment of PAM involves a multidisciplinary approach with a combination of antifungal and antibiotic medications, including miltefosine, amphotericin B, rifampicin, fluconazole, azithromycin, and dexamethasone. Despite these efforts, PAM has a high mortality rate, and treatment success varies (20,21). Recent studies have found that a drug combination has led to the successful treatment of a small number of patients, suggesting that posaconazole may be more effective than other antifungals like ketoconazole or fluconazole, pointing toward the necessity of personalized treatment regimens (22,23).

Table 1 outlines the medication regimen administered used in a successful treatment of PAM in the United States in 2013. Along with this treatment, the patient underwent regular cerebrospinal fluid (CSF) drainage and hyperosmolar therapy using Mannitol. Despite achieving patient survival, significant mental deficits were observed 18 months post-discharge, as indicated in Table 1 (24).

Table 1. Successful treatments: the dosage regimen of the drugs used to treat PAM

Sr. Nº	DRUG	REGIMEN
1	Amphotericin B	1 mg/kg/day; IV (19 days-0.1 mg); IT (5 days)
2	Rifampin	12 mg/kg/day PO for 19 days
3	Fluconazole	12 mg/kg/day loading dose, then 9 mg/kg/day IV for 19 days
4	Azithromycin	10 mg/kg/day PO for 19 days
5	Miltefosine	150 mg PO in 3 divided doses for 19 days
6	Dexamethasone	0.6 mg/kg/day IV as adjuvant therapy

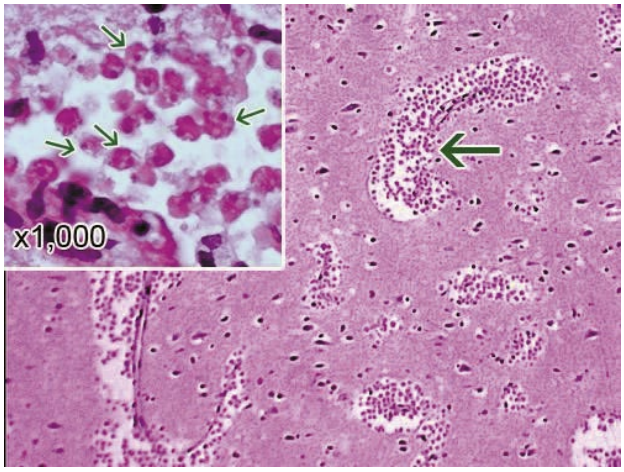

Figure 2. A hematoxylin and eosin-stained cerebral section of a PAM patient's brain indicates a substantial number of *N. fowleri* trophozoites and damage to normal brain tissue (9). *Source: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED).*

Phytochemical Therapy: Novel Therapeutics

Plants have long been recognized as valuable sources of medicinal compounds with the potential to treat various diseases. Compared to synthetic compounds, more bioactive compounds exist in natural products. Research has focused on identifying phytochemicals with antifungal and anti-parasitic properties for treating diseases caused by *N. fowleri*. In a recent study evaluating plant-derived compounds' potential against amoebas, specifically *N. fowleri* and B. mandrillaris, three compounds demonstrated noteworthy anti-amoebic activity. After a 24-hour treatment period with these compounds, ursolic acid left 49.30 % of *N. fowleri* viable, betulinic acid left 28.19 %, and betulin resulted in 14.82 % viable amoebas. These findings underscore the therapeutic potential of plant-based compounds, particularly ursolic acid, betulinic acid, and betulin, in targeting *N. fowleri* infections (25,26). A study analyzed ten chamigrane-type sesquiterpenes extracted from the Laurencia dendroidea found on the southeastern Brazilian coast. Of these, three compounds, (+)-elatol, (-)-elatol, and (-)-rogiolol, displayed significant activity against *N. fowleri* strains, outperforming the reference drug miltefosine. Remarkably, (+)-elatol demonstrated potency comparable to amphotericin B and effectively targeted the amoeba's trophozoite and cyst stages without toxicity at low concentrations (27).

Six lignan compounds from Larrea tridentata displayed activity against various pathogens, including *Naegleria fowleri*. Notably, nordihydroguaiaretic acid (NDGA) and 3'-O-methyl-NDGA showed significant efficacy against *N. fowleri*, with EC50 values of 36 μM and 38 μM, respectively. These results outperformed the standard drug miltefosine, which had an EC50 of 54.5 μM. The action mechanism for these compounds against *N. fowleri* is hypothesized to involve the modulation of cysteine protease activity in the trophozoites (28).

A pivotal discovery in this regard is the identification of heat shock proteins Hsp90 in *N. fowleri* as essential drug targets. Bergenin and epigallocatechin gallate have emerged as the most potent binders to Hsp90, making them promising candidates for phytochemical therapy (29). Kaempferol, a flavonoid in various edible plants such as tea, broccoli, and tomatoes, has garnered attention due to its diverse therapeutic properties, including anti-cancer, antioxidant, anti-inflammatory, neuroprotective, and cardioprotective effects. A recent study conducted by Le *et al.* (30) demonstrated that kaempferol induces apoptosis in *Naegleria fowleri*, suggesting its potential as a promising drug molecule for the treatment of primary amoebic encephalitis caused by *N. fowleri*.

Sesquiterpene lactones, derived from plants and aquatic algae, have exhibited notable anti-ameboid properties. Among these compounds, Anhydroartemonin stands out due to its enhanced blood-brain barrier penetration compared to miltefosin and a superior selectivity index. These attributes, coupled with its low molecular weight, position Anhydroartemonin as a promising therapeutic agent for central nervous system diseases (31). Furthermore, debromolaurinterol, derived from sesquiterpene cyclouranes found in the marine source Laurencia, has shown remarkable specificity in targeting ATPases. This compound exhibits an impressive ATP inhibition rate of 99.98 % and demonstrates potent amoebicidal activity against *Naegleria fowleri* (32). Additionally, in silico studies predict that salicylic acid, carvacrol, curcumol, curcumenol, thymol, and dehydroxyisocalamendiol are natural inhibitors of the CYP51 protein in *N. Fowleri* (33).

Prevention and Control

Naegleria fowleri infects people when water contaminated with the amoeba enters their nasal passages. This infrequent contamination typically occurs during activities such as swimming or diving in warm freshwater areas like lakes and rivers. It's important for people to avoid submerging their heads completely underwater, to abstain from cleaning their noses during religious practices, and to refrain from irrigating their sinuses with tap water that may be contaminated. *Naegleria fowleri* cultivates itself in pipes, water heaters, and closed water systems, including public drinking sources water systems. You cannot get sick from drinking contaminated water, but your health can be affected only when contaminated water goes up your nose. It is enigmatic why specific individuals get infected with the amebae, whereas many other people who swam in the same warm water as the infected people did not get sick. This includes people who were swimming right next to the infected people. Scientists have attempted to determine the threshold of *Naegleria fowleri* in the environment that poses a significant risk (34). In any case, no strategy exists to precisely and reproducibly gauge the quantities of amebae in the water. This makes it indistinct how a standard may be set to safeguard human well-being and how general well-being authorities would quantify and uphold such a norm. In any case, the gamble of *Naegleria fowleri* disease is exceptionally reduced. Between 2013 and 2022, 29 cases of water contamination were reported in the US, despite numerous water sports events annually (3,35).

The primary preventive measure is to avoid activities that involve open water sources, such as swimming in lakes, ponds, rivers, and even hot springs. This is because *N. fowleri* is mainly found in warm freshwater environments. Individuals can take steps to ensure safe washing practices, particularly when using water for nasal irrigation or religious rituals. It is crucial to avoid using water sources that may be contaminated with *N. fowleri*. Instead, using sterile or treated water is recommended (3,7).

VACCINE MODELS: A DETAILED ANALYSIS

A vaccine, i.e., a biological product, is used to safely induce protection against pathogens by initiating an immune response and forming memory cells. The cutting-edge advancements in vaccine development involve viral vector vaccines, nucleic acid vaccines, bacterial vaccines, and the use of antigen-presenting cells, like dendritic cells and T-cells. Researchers are exploring some of these technologies and using different models to develop a vaccine against PAM (36).

Model 1: DNA Vaccines and Nfa1 Gene Expression

The significance of DNA vaccines has been escalating in recent times. They have showcased efficacy against various pathogens. However, their potential against parasites is an area of ongoing exploration. A DNA vaccine was developed using viral vectors expressing the nfa1 gene, which augmented the expression of multiple IgG subclasses, specifically IgG1 and IgG2 (37). The Nfa1 protein is predominantly present on the pseudopodia of the parasite, playing an instrumental role in its phagocytic activity, essential for its survival and proliferation (38). To ascertain the effectiveness of this vaccine, mice were administered intra-peritoneally and intra-nasally doses. The latter proved more effective, resulting in heightened expression of IgG antibodies and induction of cytokines such as Th1, IFN-γ, and the regulatory cytokine IL-10 (37). Moreover, in another experimental setup, it was discerned that the anti-Nfa1 antibody attenuated the cytotoxic impact of *N. fowleri* trophozoites on CHO cells (38).

Model 2: cDNA Library and Mp2CL5 Expression

The immunoprotective response of two vaccine antigens against the causative agent of primary amoebic meningoencephalitis, *Naegleria fowleri*, has been previously reported (39). In the study published by Gutiérrez-Sánchez *et al.* (39), mice were given the antigen 19 kDa polypeptide, an immunogenic peptide from membrane protein MP2CL5 intranasally, and cholera toxin as an adjuvant. The nasal cavity and serum showed increased immune response markers like T and B lymphocytes, integrin α4β1, and immunoglobulins, along with significant protection (up to 100 % with the 19 kDa polypeptide and CT). Based on these results, these antigens may be helpful as vaccine candidates to prevent infections caused by *N. fowleri*.

A novel membrane protein called Mp2CL5 has been discovered by Reveiller *et al.* (40) and is only expressed specifically in pathogenic strains. Produced in *Escherichia*

coli, this 23-kDa recombinant protein matches the natural 17-kDa protein on the plasma membrane of *N. fowleri* trophozoites, indicating that it plays a crucial role in the pathogenicity of the organism. Interestingly, Mp2CL5 is only expressed in pathogenic *N. fowleri*, while it is not expressed in non-pathogenic Naegleria species or Acanthamoeba. The increased production of the protein during the amoeba's logarithmic to stationary growth phases is highlighted in the study, which may indicate that it is a virulence factor. To further highlight the adaptive mechanisms of *N. fowleri* in avoiding host defenses and establishing infection, pathogenic *N. fowleri* also demonstrates chemotactic responses to nerve cell components, a feature lacking in non-pathogenic counterparts. These results provide important new information about the pathogenesis and treatment of primary amoebic meningoencephalitis by highlighting the possible role of Mp2CL5 as a virulence factor.

Model 3: Cholera toxin with amoeba lysates

Carrasco-Yepez *et al*. (41) inoculated mice intranasally with cholera toxin and *N. fowleri* lysates, which resulted in enhanced survival, production of antibodies (IgA, IgG), and polymorphonuclear cell activation. This response was improved by up-regulating genes for antibody formation and particular cytokines (IL-10, IL-6, IFN-γ) while decreasing TNF-α production. The results indicated that this vaccination approach successfully blocks *N. fowleri* infection by establishing a robust immunological atmosphere in the nasal cavity.

Optimal Route of Administration: The Nasal Pathway

The exploration for the optimal vaccine delivery route has encompassed diverse animal models, including mice, guinea pigs, and rabbits. Among these, farm animals were found to have the closest anatomical resemblance to humans, making them prime candidates for testing (42,43). Given that the parasite in question infiltrates the human body through the nostrils, progressing to the brain via the olfactory nerves, researchers have pivoted towards understanding the human olfactory system. This system, in fact, is susceptible to various infectious agents, including influenza, herpes, and coronavirus. A pivotal study by Carrasco-Yepez M *et al*. (41) highlighted that nasal mucosa vaccination resulted in heightened levels of specific IgA and IgG antibodies. These antibodies, once bound to the trophozoite surfaces, inhibited their movement. The associated polymorph nucleotides (PMNs) prevented the parasite's entry and subsequent infection of the olfactory epithelium, positioning this mechanism as a primary protective barrier against the parasitic infection. Consequently, the nasal route emerges as the most effective mode of vaccination to counteract PAM (44).

Future Horizons in the Treatment and Prevention of PAM

PAM has persistently posed significant challenges to the global health community. A principal reason behind this is the low permeability of most drugs across the blood-brain barrier, which impedes effective treatment (45). Furthermore, the late manifestation of its symptoms often results in delayed diagnosis and treatment, exacerbating its impact (46). Recent strides in research have uncovered potential therapeutic avenues sourced from marine and terrestrial flora. Compounds such as bergenin, kaempferol, and sesquiterpenes, extracted from select marine sources and plants, have demonstrated promising results in preliminary studies (30). However, a comprehensive understanding of their therapeutic potential and pharmacokinetics is essential to establish them as primary therapeutic agents. As with any promising therapy, rigorous research is needed to delineate their safety, efficacy, and potential side effects. Parallel to therapeutic advancements, prophylactic measures, particularly vaccines, stand out as the most promising avenue for the prevention and potential eradication of PAM. Vaccines are inherently proactive, aiming to pre-emptively defend the host against the causative agent, *Naegleria fowleri*. Their utilization can simplify the complexity associated with PAM treatment regimens and considerably decrease the overall disease burden.

Conclusion

Primary Amoebic Meningoencephalitis represents a profound medical challenge due to its clinical complexities and the limitations of current therapeutic interventions. However, recent advancements spotlight a promising horizon. The exploration of marine and terrestrial-derived compounds offers potential novel therapeutic avenues that could revolutionize the treatment of PAM. Meanwhile, the research and development of vaccines against the causative agent, *Naegleria fowleri*, offer an optimistic avenue for not just the treatment but potentially the eradication of PAM. Moving forward, integrated research approaches, combining both therapeutic and preventive strategies, will be pivotal in curbing the impact of this disease and enhancing global health outcomes.

Author's contribution

Conceptualization: SUR; data collection, management, and curation: SUR, NI, FUR, MWM, SS; original version's redaction: SUR, NI, FUR, MWM, SS; results interpretation: SUR, NI, FUR, MWM, SS; final version's review and redaction: SUR, NI, FUR, MWM, SS.

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Ethical aspects

Not applicable.

Interests' conflicts

The authors have no conflict of interest associated with the material presented in the manuscript.

REFERENCES

1. Visvesvara GS. Infections with free-living amebae. Handb Clin Neurol. 2013;114:153-68. doi: 10.1016/B978-0-444-53490-3.00010-8
2. Güémez A, García E. Primary amoebic meningoencephalitis by Naegleria fowleri: pathogenesis and treatments. Biomolecules. 2021;11(9):1320. doi: 10.3390/biom11091320

3. Centers for Disease Control and Prevention. Prevention and Control of Naegleria fowleri in Recreational Water. Atlanta: CDC; 2023.
4. Lopez C, Budge P, Chen J, Bilyeu S, Mirza A, Custodio H, et al. Primary amebic meningoencephalitis: a case report and literature review. Pediatr Emerg Care. 2012;28(3):272-6. doi: 10.1097/PEC.0b013e3182495589
5. Kim JH, Lee SH, Sohn HJ, Lee J, Chwae YJ, Park S, et al. The immune response induced by DNA vaccine expressing nfa1 gene against Naegleria fowleri. Parasitol Res. 2012;111(6):2377-84. doi: 10.1007/s00436-012-3093-5
6. Siddiqui R, Ali IKM, Cope JR, Khan NA. Biology and pathogenesis of Naegleria fowleri. Acta Trop. 2016;164:375-394. doi: 10.1016/j.actatropica.2016.09.009.
7. Pugh JJ, Levy RA. Naegleria fowleri: Diagnosis, Pathophysiology of Brain Inflammation, and Antimicrobial Treatments. ACS Chem Neurosci. 2016;7(9):1178-9. doi: 10.1021/acschemneuro.6b00232
8. Chalmers RM. Chapter Twenty–Naegleria. In: Percival SL, Yates MV, Williams DW, Chalmers RM, Gray NF, editors. Microbiology of Waterborne Diseases (Second Edition) [Internet]. London: Academic Press; 2014 [cited 2024 Mar 23]. p. 407–16. Available from: https://www.sciencedirect.com/science/article/pii/B9780124158467000202
9. Prevention CfDCa. Naegleria fowleri–Primary amebic meningoencephalitis (PAM)–Amebic encephalitis [Internet]. 2021 [cited 2024 Mar 3]. Available from: https://www.cdc.gov/parasites/naegleria/naegleria-fowleri-media.html.
10. Dereeper A, Allouch N, Guerlais V, Garnier M, Ma L, De Jonckheere JF, et al. Naegleria genus pangenome reveals new structural and functional insights into the versatility of these free-living amoebae. Front Microbiol. 2023;13:1056418. doi: 10.3389/fmicb.2022.1056418
11. Mungroo MR, Anwar A, Khan NA, Siddiqui R. Brain-eating Amoebae Infection: Challenges and Opportunities in Chemotherapy. Mini Rev Med Chem. 2019;19(12):980-87. doi: 10.2174/1389557519666190313161854
12. Fillatre P, Crabol Y, Morand P, Piroth L, Honnorat J, Stahl J, et al. Infectious encephalitis: Management without etiological diagnosis 48 hours after onset. Med Mal Infect. 2017;47(3):236-51. doi: 10.1016/j.medmal.2017.02.004
13. Siddiqui R, Khan NA. Primary amoebic meningoencephalitis caused by Naegleria fowleri: an old enemy presenting new challenges. PLoS Negl Trop Dis. 2014;8(8):e3017. doi: 10.1371/journal.pntd.0003017
14. Schultz DR, Harrington WJ Jr. Apoptosis: programmed cell death at a molecular level. Semin Arthritis Rheum. 2003;32(6):345-69. doi: 10.1053/sarh.2003.50005
15. Cummings BS, McHowat J, Schnellmann RG. Phospholipase A2s in cell injury and death. Journal of pharmacology and experimental therapeutics. 2000;294(3):793-9.
16. Betanzos A, Bañuelos C, Orozco E. Host Invasion by Pathogenic Amoebae: Epithelial Disruption by Parasite Proteins. Genes (Basel). 2019;10(8):618. doi: 10.3390/genes10080618
17. Cope JR, Ali IK. Primary Amebic Meningoencephalitis: What Have We Learned in the Last 5 Years? Curr Infect Dis Rep. 2016;18(10):31. doi: 10.1007/s11908-016-0539-4
18. Jahangeer M, Mahmood Z, Munir N, Waraich UeA, Tahir IM, Akram M, et al. Naegleria fowleri: Sources of infection, pathophysiology, diagnosis, and management; a review.Clin Exp Pharmacol Physiol. 2020;47(2):199-212. doi: 10.1111/1440-1681.13192
19. Kofman A, Guarner J. Infections Caused by Free-Living Amoebae. J Clin Microbiol. 2022;60(1):e0022821. doi: 10.1128/JCM.00228-21
20. Deresinski S. Amebic Meningoencephalitis. Infectious Disease Alert. 2021;40(11).
21. Centers for Disease Control and Prevention. Primary Amebic Meningoencephalitis (PAM)–Diagnosis & Treatment. Atlanta: CDC; 2021.
22. Russell AC, Kyle DE. Differential growth rates and in vitro drug susceptibility to currently used drugs for multiple isolates of Naegleria fowleri.Microbiol Spectr. 2022;10(1):e0189921. doi: 10.1128/spectrum.01899-21
23. Russell AC, Bush P, Grigorean G, Kyle DE. Characterization of the extracellular vesicles, ultrastructural morphology, and intercellular interactions of multiple clinical isolates of the brain-eating amoeba, Naegleria fowleri. Front Microbiol. 2023;14:1264348. doi: 10.3389/fmicb.2023.1264348
24. Cope JR, Conrad DA, Cohen N, Cotilla M, DaSilva A, Jackson J, et al. Use of the novel therapeutic agent miltefosine for the treatment of primary amebic meningoencephalitis: report of 1 fatal and 1 surviving case. Clin Infect Dis. 2016;62(6):774-6. doi: 10.1093/cid/civ1021
25. Singh P, Kochhar R, Vashishta RK, Khandelwal N, Prabhakar S, Mohindra S, Singhi P. Amebic meningoencephalitis: spectrum of imaging findings. AJNR Am J Neuroradiol. 2006;27(6):1217-21.
26. Atanasov AG, Zotchev SB, Dirsch VM; International Natural Product Sciences Taskforce; Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021;20(3):200-16. doi: 10.1038/s41573-020-00114-z
27. Arberas-Jiménez I, Nocchi N, Chao-Pellicer J, Sifaoui I, Soares AR, Díaz-Marrero AR, et al. Chamigrane-Type Sesquiterpenes from Laurencia dendroidea as Lead Compounds against Naegleria fowleri. Mar Drugs. 2023;21(4):224. doi: 10.3390/md21040224
28. Morales-Ubaldo AL, Rivero-Perez N, Valladares-Carranza B, Madariaga-Navarrete A, Higuera-Piedrahita RI, Delgadillo-Ruiz L, et al. Phytochemical compounds and pharmacological properties of larrea tridentata. Molecules. 2022;27(17):5393. doi: 10.3390/molecules27175393
29. Basharat Z, Zaib S, Yasmin A. Computational study of some amoebicidal phytochemicals against heat shock protein of Naegleria fowleri. Gene Rep. 2017;6:158-62. doi: 10.1016/j.genrep.2016.09.003
30. Lê HG, Kang JM, Võ TC, Na BK. Kaempferol induces programmed cell death in Naegleria fowleri. Phytomedicine. 2023;119:154994. doi: 10.1016/j.phymed.2023.154994
31. Arberas-Jiménez I, Rizo-Liendo A, Nocchi N, Sifaoui I, Chao-Pellicer J, Souto ML, et al. Sesquiterpene lactones as potential therapeutic agents against Naegleria fowleri. Biomed Pharmacother. 2022;147:112694. doi: 10.1016/j.biopha.2022.112694
32. Arberas-Jiménez I, García-Davis S, Rizo-Liendo A, Sifaoui I, Morales EQ, Piñero JE, et al. Cyclolauranes as plausible chemical scaffold against Naegleria fowleri. Biomed Pharmacother. 2022;149:112816. doi: 10.1016/j.biopha.2022.112816
33. Daniel N, Ferdinand F, Aditya PA. In silico targeting CYP51 of Naegleria fowleri using bioactive compounds from Indonesian plants. J Pharm Pharmacogn Res. 2023;11(5):841-62. doi: 10.56499/jppres23.1693_11.5.841
34. Cabanes PA, Wallet F, Pringuez E, Pernin P. Assessing the risk of primary amoebic meningoencephalitis from swimming in the presence of environmental Naegleria fowleri. Appl Environ Microbiol. 2001;67(7):2927-31. doi: 10.1128/AEM.67.7.2927-2931.2001
35. Bureau UC. Statistical Abstract of the United States. 2009.
36. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021;21(2):83-100. doi: 10.1038/s41577-020-00479-7
37. Lee YJ, Kim JH, Sohn HJ, Lee J, Jung SY, Chwae YJ, et al. Effects of immunization with the rNfa1 protein on experimental Naegleria fowleri-PAM mice. Parasite Immunol. 2011;33(7):382-9. doi: 10.1111/j.1365-3024.2011.01296.x

38. Kang SY, Song KJ, Jeong SR, Kim JH, Park S, Kim K, et al. Role of the Nfa1 protein in pathogenic Naegleria fowleri cocultured with CHO target cells. Clin Diagn Lab Immunol. 2005;12(7):873-6. doi: 10.1128/CDLI.12.7.873-876.2005
39. Gutiérrez-Sánchez M, Carrasco-Yépez MM, Correa-Basurto J, Ramírez-Salinas GL, Rojas-Hernández S. Two MP2CL5 Antigen Vaccines from Naegleria fowleri Stimulate the Immune Response against Meningitis in the BALB/c Model. Infect Immun. 2023;91(7):e0018123. doi: 10.1128/iai.00181-23
40. Réveiller FL, Suh SJ, Sullivan K, Cabanes PA, Marciano-Cabral F. Isolation of a unique membrane protein from Naegleria fowleri. J Eukaryot Microbiol. 2001;48(6):676-82. doi: 10.1111/j.1550-7408.2001.tb00208.x
41. Carrasco-Yepez M, Campos-Rodriguez R, Lopez-Reyes I, Bonilla-Lemus P, Rodriguez-Cortes AY, Contis-Montes de Oca A, et al. Intranasal coadministration of Cholera toxin with amoeba lysates modulates the secretion of IgA and IgG antibodies, production of cytokines and expression of pIgR in the nasal cavity of mice in the model of Naegleria fowleri meningoencephalitis. Exp Parasitol. 2014;145 Suppl:S84-92. doi: 10.1016/j.exppara.2014.04.002
42. Lai R, Ogunsola AF, Rakib T, Behar SM. Key advances in vaccine development for tuberculosis-success and challenges. NPJ Vaccines. 2023;8(1):158. doi: 10.1038/s41541-023-00750-7
43. Casadei E, Salinas I. Comparative models for human nasal infections and immunity. Dev Comp Immunol. 2019;92:212-222. doi: 10.1016/j.dci.2018.11.022
44. Rojas-Ortega DA, Rojas-Hernández S, Sánchez-Mendoza ME, Gómez-López M, Sánchez-Camacho JV, Rosales-Cruz E, et al. Role of FcγRIII in the nasal cavity of BALB/c mice in the primary amebic meningoencephalitis protection model. Parasitol Res. 2023;122(5):1087-1105. doi: 10.1007/s00436-023-07810-w
45. Nicolson GL. The Fluid-Mosaic Model of Membrane Structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. Biochim Biophys Acta. 2014;1838(6):1451-66. doi: 10.1016/j.bbamem.2013.10.019
46. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol Med Microbiol. 2007;50(1):1-26. doi: 10.1111/j.1574-695X.2007.00232.x