ORIGINAL RESEARCH ARTICLE

Primary Amoebic Meningoencephalitis Caused by *Naegleria fowleri.*

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Keywords: Primary amoebic encephalitis; Brain-eating amoeba; *Naegleria fowleri*

ABSTRACT

Background: *Naegleria fowleri* is a free-living amoeba (FLA) commonly found in the environment. While it is harmless when ingested, it can enter the brain through the nasal passages, leading to a severe infection known as primary amoebic meningoencephalitis (PAM).

Methods: This literature review synthesizes existing literature from databases available on the National Library of Medicine (NCBI) platform to provide healthcare providers with essential information about the critical characteristics and pathogenesis of *N. fowleri.*

Results: The initial stage of PAM often presents vague symptoms and is frequently misdiagnosed as viral or bacterial meningitis. Unfortunately, once symptoms appear, patients typically experience rapid deterioration, regardless of the treatments and supportive care provided. Delayed and ineffective care, along with unnecessary tests and procedures, can result in irreversible brain damage and ultimately lead to death. Furthermore, the exact mechanism of treatment remains unclear. The standard therapeutic protocol recommended by the Centers for Disease Control and Prevention (CDC) involves an aggressive but unreliable combination of antibiotics.

Evidence-Based Care: PAM is a rare disease with a high fatality rate, underscoring the importance of prevention and early detection. Prompt diagnosis and treatment are crucial. A postmortem examination of brain tissue is usually required to confirm the diagnosis. Overall, raising awareness about the risks associated with *Naegleria fowleri* infections and the importance of prompt diagnosis and treatment is vital to reducing the incidence and impact of this deadly disease.

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What do we already know about this topic?
Primary amoebic meningoencephalitis (PAM) is a life-threatening illness caused by the free-living amoeba *Naegleria fowleri*, commonly found in freshwater and moist soil. Although PAM is rare, it is extremely deadly, with a mortality rate exceeding 97%. Between 1965 and 2022, 157 cases were reported in the United States.
N. fowleri enters the body through the nose and migrates to the brain via the olfactory nerves. Infection can occur during water-related activities or by inhaling dust-containing amoeba cysts. The incubation period lasts from 2 to 15 days, and death often occurs 3 to 7 days after the onset of symptoms. Patients typically succumb to complications such as cerebral herniation, severe edema, and increased intracranial pressure.
PAM is frequently misdiagnosed as viral or bacterial meningitis in its early stages. The Centers for Disease Control and Prevention (CDC) recommends an aggressive combination of antibiotics once *N. fowleri* is detected; however, treatment has generally been ineffective.

What is the main contribution to Evidence-Based Practice from this article?
This literature review provides essential, evidence-based knowledge for healthcare professionals, offering up-to-date insights to enhance clinical decision-making in diagnosing and managing Primary Amoebic Meningoencephalitis (PAM). It focuses on improving diagnostic precision by providing an overview of the organism's morphology, life cycle, and pathogenesis, which aids in better detection and treatment. Additionally, it seeks to increase understanding of how *Naegleria fowleri* invades and damages the central nervous system. Finally, the review highlights key areas for further research to strengthen diagnostic, therapeutic, and preventive strategies for PAM.

What are this research's implications for health policy?
This comprehensive literature review contributes to improved decision-making in clinical practices, public health policies, and future research related to *Naegleria fowleri* and primary amoebic meningoencephalitis (PAM).
Theoretical Implications: It highlights the unique evolutionary traits of *N. fowleri*, including its ability to shift between amoeboid and flagellate forms. The review also explores the interactions between the host and the pathogen, enhancing our understanding of the dynamics involved in amoebic invasion and the rapid progression of the disease.
Practical Implications: It defines current treatment protocols and their limitations, underscoring the necessity for more effective therapies. Furthermore, the review outlines *Naegleria fowleri*'s environmental preferences and transmission routes to inform public health measures and promote individual preventive actions.

Authors' Contributions Statement:
Ediane Gallo oversees every stage of the detailed research and writing process, highlighting her significant involvement and authorship in this literature review on *Naegleria fowleri* and primary amoebic meningoencephalitis (PAM).

Introduction

Pathogenic free-living amoebas that can incidentally infect and damage the cerebrovascular system of both humans and animals include *Naegleria fowleri*, *Acanthamoeba spp.*, *Balamuthia mandrillaris*, and *Sappinia pedata* (Visvesvara & Schuster, 2007). The immunosuppressed population is particularly vulnerable to developing chronic granulomatous amoebic encephalitis (GAE) caused by *Acanthamoeba spp.* or *Balamuthia mandrillaris*. Although *Sappinia pedate* is considered a potential cause of GAE, there has only been one reported case involving an immunocompromised patient who survived hospitalization and treatment (Lares-Jiménez et al., 2018). *Acanthamoeba spp.* and *Balamuthia mandrillaris* can enter the body through cuts on the skin, subsequently reaching the nervous system via the bloodstream (Guarner et al., 2007). In contrast, *N. fowleri* enters the nasal cavity directly and invades the brain.

Approximately 50 species of the *Naegleria* genus are found in the environment, most non-pathogenic. However, *N. australiensis* and *N. italica* have been shown to cause neurological illness in laboratory mice, while *N. fowleri* (Tien & Singh, 2023) is the sole agent responsible for humans' deadly acute meningoencephalitis. Therefore, this literature review focuses on primary amoebic meningoencephalitis (PAM) caused by *Naegleria fowleri*, detailing its fundamental characteristics, morphology, life cycle, epidemiology, pathogenesis, diagnosis, current therapies, prevention strategies, and ongoing research and clinical trials related to this topic.

Methods

A literature review was conducted using the National Library of Medicine (NCBI) platform. The keywords chosen for the search included "brain-eating amoeba" OR "*Naegleria fowleri*" AND "*Naegleria*" AND "nervous system" OR "brain" OR "spinal cord" OR "central nervous system" AND "primary amoebic meningoencephalitis."

The target population consisted of all patients diagnosed with primary amoebic meningoencephalitis (PAM) caused by *Naegleria fowleri*, regardless of the disease stage, including postmortem cases. Papers focusing on other types of amoebic infections were excluded from the search. The review began with literature published in 1965, the first confirmed case of *N. fowleri*. Following this, abstracts of more recent articles were screened using specific eligibility criteria.

Discussion

The monocellular menace, *Naegleria fowleri,* was named after the Australian pathologist Malcolm Fowler. In 1965, Drs. Carter and Fowler officially isolated the first strain of *N. fowleri* while investigating four postmortem cases of acute pyogenic meningoencephalitis caused by this mysterious amoeba (Fowler & Carter, 1965). *Naegleria* is a thermophilic organism found in freshwater ecosystems and moist soil. It prefers temperatures between 80-115°F (27-46°C) and exhibits remarkable tolerance to environmental stressors (Visvesvara & Schuster, 2007). This organism is aerobic, relying on the oxygen dissolved in the water's surface.

Although multiple sources confirm that the parasite cannot survive in saltwater pools, it has been documented that the amoeboflagellate can endure up to 48 hours at a saline concentration of 1.4%, which is three to four times higher than the typical saline concentration in saltwater pools (Cope & Ali, 2016). Aside from the salt content in ocean water, *N. fowleri* can sustain its life in extreme environments as a cyst.

The cysts are spherical and measure 8-12 µm in diameter (Martinez & Visvesvara, 2003). Each cyst has a single, dense wall with one or two pores that allow trophozoites to emerge again when environmental conditions become favorable. Trophozoites (Figure 1) represent the organism's life cycle's active, feeding, and infective stage. They are slender, measuring approximately 22 µm long and 7 µm wide (Guemez & Garcia, 2021), and contain a single nucleus with a large, centrally located karyosome.

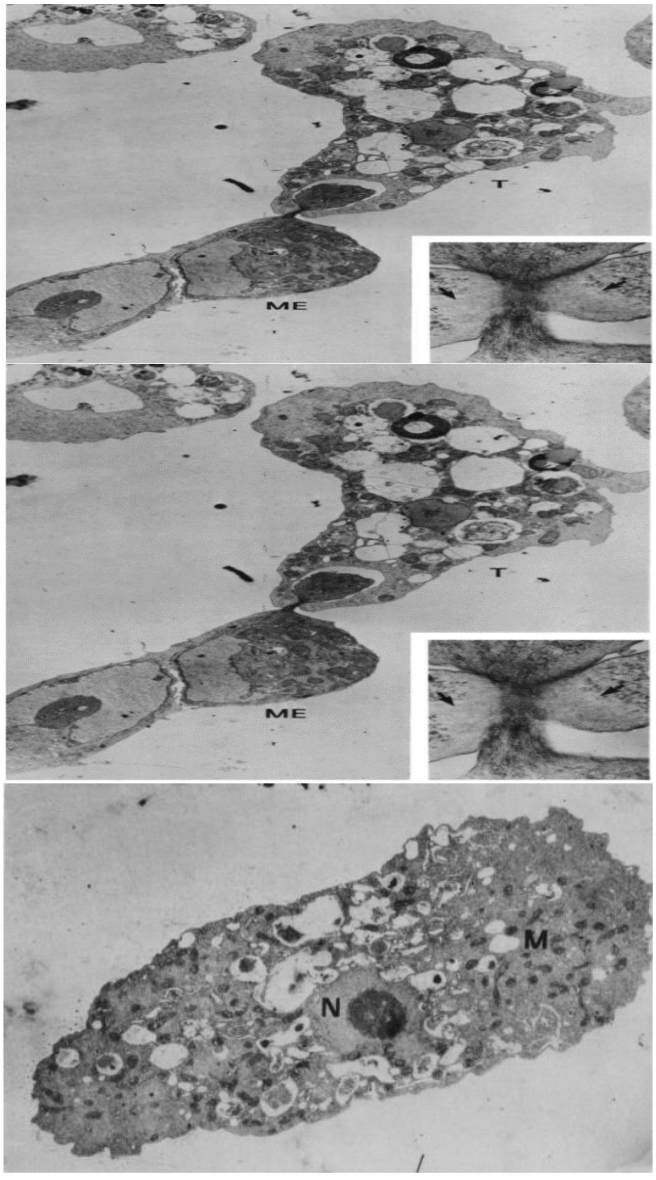

Figure 1: *Naegleria fowleri trophozoite. Inset: the tips of the pseudopodia at higher magnification (× 32 800) showing microfilament-like structures within the ribosome-free cytoplasm (arrows). The constricted ME-cell cytoplasm between the pseudopodia contains prominent microtubules. EM × 4800 (Brown, 1979).*

Naegleria reproduces through binary fission during its amoeboid stages (Siddiqui, 2016) and can live for many years in laboratory settings as actively reproducing (asexually) amoebas or dormant cysts. Unlike other pathogenic free-living amoebas (FLAs), *Naegleria fowleri* has an additional phase in its lifecycle. This phase features a transient, pear-shaped flagellate stage (Tien & Singh, 2023) characterized by two flagella of equal length (Guemez & Garcia, 2021). The amoeboflagellate form of the trophozoite is the most common type found in cerebrospinal fluid during an infection. *N. fowleri* exhibits remarkable mobility and adaptability as a flagellate, particularly when nutrients are scarce. Its ability to undergo morphological changes is linked to extra mitotic alpha and beta tubulins (Velle et al., 2022), which could be targeted to develop enzymes that interfere with microbial cell division and growth. One set of tubulins is used exclusively during mitosis, while the other set facilitates transient shapeshifting, which can occur in five hours or less.

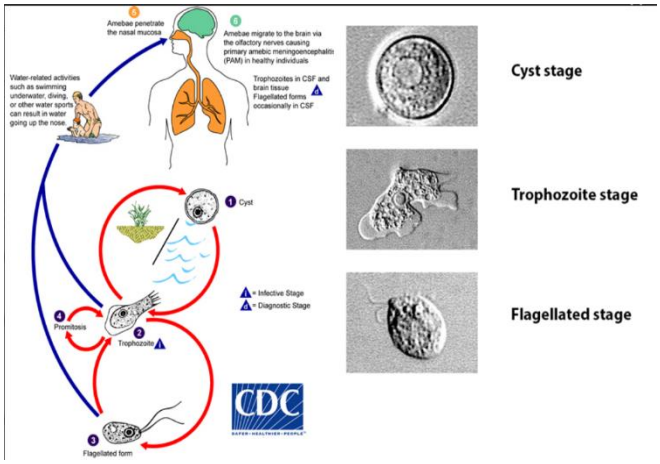

Figure 2: *The life cycle of N. fowleri, including cyst, trophozoite, and flagellate forms (CDC).*

Epidemiology

The infamous brain-eating amoeba is a significant concern in the United States during the year's hottest months. Water-based activities are common in the summer, which increases the likelihood of exposure to this pathogen. Although it is not officially classified as a nationally notifiable disease, the CDC collaborates with state health departments to maintain registries of primary amoebic meningoencephalitis (PAM) infections. From its first identification in 1965 until 2022, the CDC surveillance team reported 157 cases of amoebic meningoencephalitis in the country (Tien & Singh, 2023).

Primary amoebic meningoencephalitis is fatal but not contagious; it does not spread from person to person. Instead, this opportunistic pathogen can quickly enter the human body through the nose during activities like swimming in contaminated water or inhaling dust particles containing cysts (Martinez & Visvesvara, 2003). *Naegleria fowleri*, the amoeba responsible for PAM, is a facultative saprophyte, meaning it obtains nourishment from the environment until it accidentally contaminates a host (Figure 2). Consequently, *Naegleria fowleri* is neither a true saprophyte nor a true parasite; it exists in the environment and infects humans incidentally.

In contrast, protists can have facultative bacterial symbionts (Wang et al., 2023). There is also evidence of non-predatory relationships between *Naegleria* and various species such as *Stenotrophomonas*, *Legionella*, *Protochlamydia*, *Simkania*, *Neochlamydia*, *Acidovorax*, and *Flavobacterium* (Shi et al., 2021).

Pathogenesis

The severity and duration of the incubation period depend on the amount and specific strain of the pathogen involved. Trophozoites rapidly damage cell monolayers using their feeding cups, leading to a noticeable cytopathic effect (CPE) (Visvesvara & Schuster, 2007). The amoebastomes gradually consume red blood

cells and cells from the neurovascular unit of the blood-brain barrier (BBB), including microglia, astrocytes, and oligodendrocytes, through a process known as trogocytosis, which is a form of phagocytosis.

There is an active route for the efflux of cerebrospinal fluid (Spera et al., 2023) along the olfactory nerves that cross the perforated cribriform plate in the ethmoidal bone. This pathway allows the parasite to travel up the olfactory system and spread to other areas of the central nervous system. The progression of the infection (Figure 3) begins with trophozoites entering a person's nasal cavity upon exposure, and they then migrate to the central nervous system (CNS) via the subarachnoid space, which takes approximately 7 to 10 days. After this period, the pathogen invades the frontal lobes, deteriorating brain tissue within 3 to 6 days and ultimately leading to the host's death. Most patients succumb to a combination of neurological injuries related to cerebral herniation (Ahmad et al., 2023), severe edema, and increased intracranial pressure.

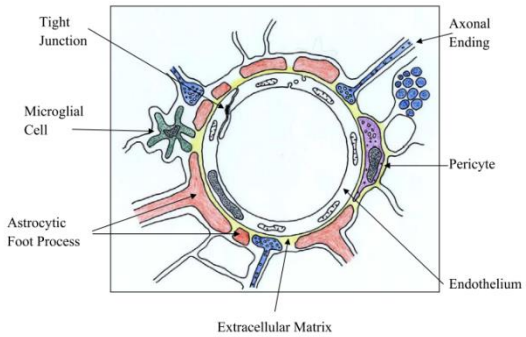

Figure 3: *Diagram depicting the neurovascular unit/cell association forming the BBB. The cerebral endothelial cells come together at their edges, forming tight junctions that seal entirely off any paracellular pathways between them (Begley, 2004).*

Primary amoebic meningoencephalitis can increase the risk of co-infections with other microorganisms. In 2016, a seemingly healthy 15-year-old boy from India died from chronic meningoencephalitis caused by multiple infectious agents, including *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, and *Naegleria fowleri* (Ravinder et al., 2016). Additionally, several confirmed cases of PAM co-infections have been confirmed, including a recent case involving *Naegleria fowleri* and *Streptococcus pneumoniae* (Ghanchi et al., 2023).

Immunology

Contrary to expectations, PAM-afflicted patients seldom develop nasal irritation or inflammation (Baig, 2016) as an immediate reaction to the amoebic infiltration. While our innate immunity swiftly recognizes bacterial and viral particles as foreign (Moseman, 2020), *amoebas* are unicellular eukaryotic organisms that can pass through our recognition receptors nearly undetected. Due to *N. fowleri* trophozoites' peculiar chemotaxis (Baig, 2016) and migration to the neural tissues, the areas within the brain parenchyma are the most impaired. Parenchyma is the functional part of the brain responsible for cognition, and it is made up of the flagellate's main target: neurons and glial cells (Begley, 2004).

Trogocytic *Naegleria* uses a tactical immunomodulatory approach by incorporating material acquired from target cells into their own (Schriek & Villadangos, 2023). Like phagocytosis, the mechanism of trogocytosis is an actin-dependent process detected in T, B, and major histocompatibility complex (MHC) bound antigen-presenting cells (Sohn et al., 2010). The consequences of active trogocytosis can be mild, with transient exchanging of information between cells, or fatal (trogoptosis). The discovery of trogocytosis has revolutionized the notion that cellular structures could only perform their genetically predisposed functions (Schriek & Villadangos, 2023).

Because of the short period between symptom onset and mortality, there is not enough time to accurately examine the impact of the host's immunity. Hence, serology tests are not

included in the initial assessment for PAM diagnosis. The innate immune system, which serves as the body's primary defense mechanism, may induce a cytokine storm. Leukocytes produce cytotoxic effects by releasing reactive oxygen species (ROS) to kill microbes inside the cells via oxidative stress (Pugh & Levy, 2016). Unfortunately, it is too late to control the parasite once an intense humoral reaction finally arises. Though studies are being conducted to investigate the role of proinflammatory cytokines (Chen & Moseman, 2023), there still needs to be an evident relationship between *Naegleria fowleri's* pathophysiology and the host's innate or adaptive immunity dynamics.

To breach through the blood-brain barrier (Guemez et al., 2021), the amoeboflagellate secretes hydrolase enzymes that modify actin filaments and degrade tight junction proteins (TJP) in the cerebral endothelium. Amoebic cysteine proteases play an important role in their acute and chronic infectious mechanisms in CNS. Alongside proteolytic enzymes, extracellular vesicles (EVs) could be involved in *Naegleria* trophozoites' pathogenesis, communication, and immunomodulation (Lertjuthaporn et al., 2022). Extracellular vesicles (EVs) are signaling molecules that are released by all prokaryotic and eukaryotic cells. These molecules transport various biomolecules in exosomes or intraluminal vesicles (ILVs). For instance, IL-8 chemokines released by macrophages in response to injury trigger inflammation by rapidly recruiting neutrophils to the cerebrum, contributing to the acute form of PAM. (Lertjuthaporn et al., 2022). Another EV recently identified in *N. fowleri* is the eukaryotic elongation factor 1-alpha (eeEF1 α). Interestingly, this protein is equally found in the exosomes of *Leishmania donovani* (Moreira et al., 2022). Electron-dense granules (EDGs) are minuscule components with robust proteolytic activity. They are known for effectively breaking down proteins and facilitating numerous essential physiological processes (Kothandan et al., 2020). Also called GRA proteins, they are smaller than the alpha granules primarily found in platelets (thrombocytes) to regulate the coagulation cascade. *Naegleria fowleri* trophozoites exhibit contact-related secretion of EDGs when they interact with collagen substrates in the host cells (Chavez-Munguia et al., 2014). Interestingly, these EDGs are potential exosome reservoirs (Figure 4) in a process dependent on filamentous actin and an unconventional myosin motor named *TgMyoF*.

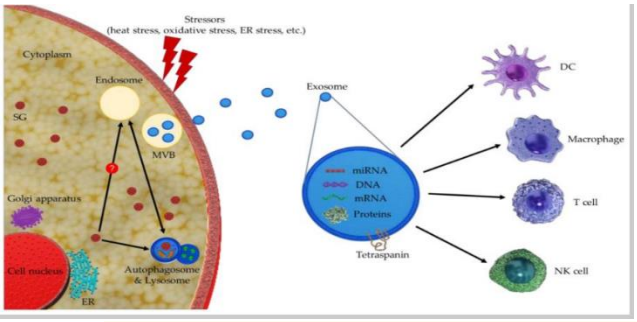

Figure 4: *Crosstalk between stress granules (SGs), exosomes, and immune cells. ER: endoplasmic reticulum; MVB: multivesicular body; miRNA: microRNA; DC: dendritic cell; NK: natural killer cell (Kothandan et al., 2020).*

Clinical Presentation

The onset of symptoms for PAM typically occurs within a week of exposure to the pathogen. However, some patients may show signs as soon as two days after contact (Ahmad et al., 2023). A statistical analysis of 381 confirmed cases of *Naegleria fowleri* worldwide from 1962 to 2018 showed that 75% of the victims were young and biologically male (Gharpure et al., 2021). Of these cases, only 256 had enough information on disease manifestation (Figure 5). Initial symptoms were mostly non-specific and flu-like, including fever, headache, nausea, and fatigue. As the disease advanced, symptoms worsened, leading to altered mental status, neck stiffness, seizures, light sensitivity, and coma. Infants exhibited early signs like high fever and feeding difficulties (Zhou et al., 2022).

Fever	226 (88)	113 (86)	68 (91)	45 (90)
Headache	209 (82)	111 (85)	64 (85)	34 (68)
Nausea/vomiting	147 (57)	80 (61)	41 (55)	26 (52)
Fatigue/lethargy	65 (25)	44 (34)	17 (23)	4 (8)
Respiratory	19 (7)	7 (5)	7 (9)	5 (10)
Late (central nervous system involvement)	215 (84)	104 (79)	67 (89)	44 (88)
Altered mental status	128 (50)	70 (53)	34 (45)	24 (48)
Nuchal rigidity	90 (35)	38 (29)	34 (45)	18 (36)
Seizures	55 (21)	25 (19)	23 (31)	7 (14)
Coma	34 (13)	10 (8)	14 (19)	10 (20)
Photophobia	29 (11)	20 (15)	4 (5)	5 (10)
Drowsiness	22 (9)	12 (9)	8 (11)	2 (4)
Kernig's/Brudzinski's sign	22 (9)	3 (2)	6 (8)	13 (26)
Extremity weakness	11 (4)	5 (4)	3 (4)	3 (6)
Blurred vision	10 (4)	3 (2)	6 (8)	1 (2)
Abnormal gait	8 (3)	4 (3)	0 (0)	4 (8)
Cranial nerve abnormalities	8 (3)	2 (2)	4 (5)	2 (4)
Sensory abnormalities	7 (3)	1 (1)	2 (3)	4 (8)

Figure 5: *Clinical signs on initial and late presentation to a healthcare facility for reported cases of PAM classification (N = 256) (Gharpure et al., 2021).*

Laboratory Testing

Neural infections with viruses are the most common, followed by bacteria, which are the most severe. Other known agents of meningitis are *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Streptococcus agalactiae* (Group B Strep), and Escherichia coli. Chronically immunosuppressed individuals may acquire neural infections with *Cryptococcus neoformans* and *Actinomyces odontolyticus* (Huang et al., 2023). On rare occasions, parasitic infections with free-living amoebas can occur. PAM is the deadliest form of all, so it is highly advisable to seek emergency care if non-specific symptoms suddenly arise following recent water-associated activities.

The number of medical facilities within the United States equipped with molecular testing for efficient identification of amoebic meningoencephalitis is limited. Thus, healthcare providers must use clinical acumen when treating people with acute neurological conditions. When meningoencephalitis is suspected, physicians may suggest a computed tomography (CT) scan as a standard diagnostic tool. Still, CT scans are generally inconclusive and may not provide a definitive diagnosis.

In contrast, a lumbar puncture (spinal tap) for spinal fluid's microbial and chemical analysis can greatly influence PAM diagnostics. Intracranial pressure (ICP) or hypertension can be noticed immediately upon sample collection. Spinal fluid is normally colorless but may appear turbid, milky, or xanthochromic. These changes are indicators of increased white blood cells, proteins, and microorganisms present during the early stages of the infection (Guemez & Garcia, 2021). Glucose levels typically decrease due to the combination of elevated proteins and leukocytes, suggesting the presence of glucose-catabolizing agents. Pleocytosis is marked by multinucleated or polymorphonuclear leukocytes (PMNs), a defining feature of meningococcal meningitis, except neither intracellular nor extracellular bacteria can be found (Figure 6).

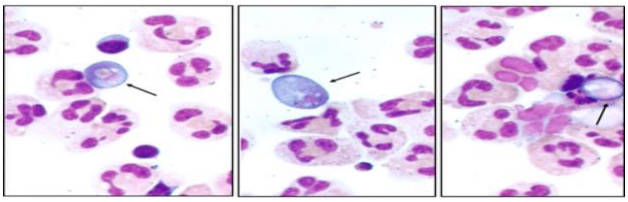

Figure 6: *N. fowleri* on Wright–Giemsa-stained slides from patient's CSF (1000x). Black arrows point to trophozoites with a background of neutrophils (Pugh & Levy, 2016).

Most hospital facilities' meningoencephalitis workup only requests a microbial Gram stain to scan for potential pathogens. Regrettably, the staining process damages the amoeba's cell structure and morphology, making them resemble macrophages or lining cells.

The CDC recommends microscopically scanning the sample immediately after collection to increase the chances of detecting parasites in the CSF. Microscopic screening of the CSF can be done by concentrating the cells using a centrifuge (cytospin), by loading the specimen directly onto a hemocytometer (hematology cell counting chamber), or by placing a droplet of the sample directly on a glass slide and adding a coverslip (wet mount) (Figure 7). It is crucial to avoid refrigeration specimens that might

contain *N. fowleri*. The amoebas' unique limacine, slug-like forward movement can only be visible at room temperature. Experienced laboratorians slightly warm the sample by leaving the slide or counting chamber under the microscope light for 5-10 minutes before the examination to encourage distinctive trophozoite locomotion. See the supplementary material for *Naegleria* identification from the *Amoeba Summit of 2019*.

If an initial microscopic scan for parasites turns positive, the organisms can be identified by staining fixed slide preparations with either Hematoxylin and Eosin (H&E), Trichrome, or Wright-Giemsa stains. Periodic Acid-Schiff (PAS) stains can also highlight trophozoites due to their considerable glycogen content. *Naegleria* can be distinguished from white blood cells because of the amoebas' large nucleus and centrally located karyosome.

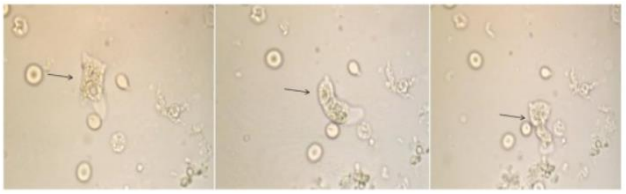

Figure 7: *N. fowleri trophozoites seen on the slide via direct microscopic examination of the patient's CSF on a wet mount (Zhang et al., 2018).*

Culture

Primary amoebic meningoencephalitis requires immediate attention, and culturing specimens for diagnosis is inadequate since *Naegleria fowleri* trophozoites take time to grow (Guemez & Garcia, 2021). For research purposes, axenic cultures should be used with non-nutrient agar or agar media that contain low concentrations of nutrients (for example, peptone at 0.05%, yeast extract at 0.05%, and glucose at 0.1%). The growth of trophozoites can be stimulated by inoculating the culture media with either live or inactivated gram-negative bacteria, which serve as a food source for these organisms.

Molecular Diagnostics

The Centers for Disease Control and Prevention (CDC) considers the polymerase chain reaction (PCR) test the gold standard for detecting Naegleria fowleri infections because of its high sensitivity and reliability. PCR is a type of nucleic acid amplification test (NAAT) that identifies the organism's genus and species (Guemez & Garcia, 2021). While PCR testing is relatively quick, typically taking about 2 to 3 hours, not all healthcare facilities have access to the advanced analyzers needed for this test. Consequently, some facilities are required to send specimens to reference laboratories for analysis.

Amoeba "omics"

Metagenomics has become a valuable tool for exploring non-pathogenic and pathogenic free-living amoebae from an epidemiological perspective (Chung, 2022). Researchers have classified the parasite into eight genotypes by comparing variations in the 5.8S rRNA, ITS-1, and ITS-2 gene sequences (T1-T8). Specifically, the Nf genotypes T1, T2, and T3 have been identified in the Americas, while genotypes 3T, 4T, 6T, 7T, and 8T have been found in Europe. Nf genotypes 2T, 3T, and 5T have been recorded in Asia. To date, only Nf genotype 5T has been reported in Oceania (Rodrigues-Anaya et al., 2021).

Point-of-Care Testing (POCT)

The U.S. Food and Drug Administration (FDA) has not approved any CLIA-waived point-of-care tests (POCT) for screening and diagnosing Primary Amoebic Meningoencephalitis. However, a promising candidate for POCT is known as loop-mediated isothermal amplification (LAMP). This type of nucleic acid amplification assay can detect *N. fowleri* in the early stages of the disease (Figure 8). During the 2019 pandemic caused by SARS-CoV-2, the FDA issued emergency authorization for various

LAMP-based COVID-19 test kits in the United States (Das & Chuang, 2022).

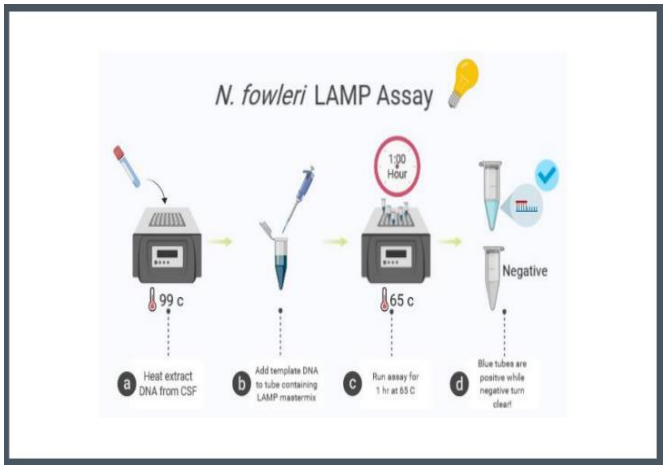

Figure 8: *Loop-mediated isothermal amplification assay (Georgacopoulos, 2019).*

Histopathology

To postmortem diagnose amoebic meningoencephalitis, it is essential to collect samples from brain and tissue biopsies that have been preserved in 10% neutral buffered formalin (NBF) for a minimum of 72 hours (Guarner et al., 2007). After fixation in NBF, trophozoite detection can be performed using hematoxylin and eosin (H&E) staining or an immunoperoxidase (IP) staining technique. Immunohistochemical assays utilize antibodies specific to the amoeba, which help visualize trophozoites in necrotic areas rich in microglial cells. PAM is characterized by fibrinous exudate in the inner membrane of the meninges (Guarner et al., 2007) and is associated with coagulative necrosis resulting from hypoxia due to ruptured blood vessels and brain edema. Notably, histopathological examinations of brain or tissue biopsies do not reveal cysts of *Naegleria fowleri* (Visvesvara & Schuster, 2007).

Management & Treatment

Survivor case records indicate that the extensive list of antimicrobials used for treating PAM includes amphotericin B, azoles (antifungal agents), rifampin (antimycobacterial), azithromycin (antibacterial), miltefosine (antileishmanial), dexamethasone (corticosteroid), and prophylactic anticonvulsants (Gharpure et al., 2021). The rationale for using this aggressive combination of drugs is to target the pathogen through multiple mechanisms of action (Figure 9).

In addition to pharmacological treatment, adjunctive procedures may include the placement of temporary intraventricular shunts, hyperosmolar therapy (using 3% saline or mannitol), moderate hyperventilation (maintaining PaCO2 at 30-35 mm Hg), and inducing hypothermia (lowering body temperature to between 32°C and 34°C) to help reduce cerebral edema.

This combined therapeutic approach is intended to decrease levels of reactive oxygen species (ROS), nitrogen species (NOS), neural apoptosis, and pro-inflammatory cytokines (Chen & Moseman, 2022), thereby mitigating hyperinflammation in the patient's brain. The treatment may also include inducing a pentobarbital coma and administering miltefosine through a nasogastric tube.

Prompt initiation of therapeutic interventions is crucial to improving the chances of a positive outcome.

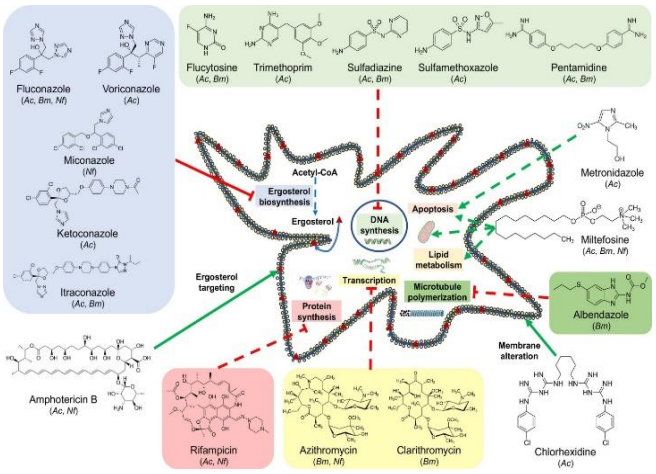

Figure 9: *Common cellular pathways and mechanisms of action of drugs used to treat FLA infections (Taravaud et al., 2021).*

Clinical Trials

PAM is a rare but rapidly progressing disease,

limiting the opportunities to test potential therapies for opportunistic FLA infections in humans (Chung et al., 2022). As a result, pharmacology, toxicology, and biochemical research are primarily conducted through experiments using *Naegleria* in murine models in vivo, human tissue culture cells in vitro, and computational screening for feasible ligand-receptor binding models in silico.

The United Nations (UN) recently acknowledged the need for an international platform dedicated to rare disease (RD) genotypes and standardized phenotypic nomenclature. To promote better policies and enhance the integration of genomic medicine (Chung et al., 2022), the UN has included RDs in the 2030 Sustainable Development Goals (SDGs) under the Leave No One Behind (LNOB) agenda.

Prevention

It is crucial to prioritize prevention by educating the public about the importance of avoiding tap water and using boiled, filtered, or sterile water for irrigation (Ahmad et al., 2023). To minimize the risk of exposure, it is strongly recommended that people refrain from participating in freshwater activities during July, August, and September. To further reduce the chances of contamination, individuals should keep their heads above water and use nose clips to limit fluid exposure to the nasal cavities.

Children are particularly susceptible to pathogenic free-living amoebae (FLAs) because they often play in and disturb sediments where these organisms thrive. Therefore, maintaining the safety of recreational waters requires a proactive approach that includes monitoring high-risk areas and promptly informing the public about the presence of *Naegleria fowleri*. Proper management and chlorination of swimming pools, spas, hot tubs, and water systems are essential for this effort (Gharpure et al., 2021). Both amoebic cysts and trophozoites can be completely inactivated with a chlorine concentration of at least 0.5 mg/L (ppm) (Cope & Ali, 2016). A convenient chlorine dilution calculator is provided in the supplementary materials. Additionally, research shows that ultraviolet (UV) light, combined with an appropriate amount of chlorine, can completely inactivate *Naegleria* under experimental conditions (Arberas-Jiménez et al., 2022).

Vaccination is the most effective way to stimulate immunoregulation, combat disease-causing agents, and prevent outbreaks. Immunoassays have determined that Nfa1, a recombinant protein found on the pseudopodia of *Naegleria fowleri* trophozoites, can provoke a mixed type of immunogenic response in murine models involving both Th1 and Th2 cells and regulatory T-cells (Kothandan et al., 2020). However, extensive research and advancements are necessary before advancing to traditional immunization development models for PAM caused by *Naegleria fowleri*.

Conclusions

Water-associated primary amoebic meningoencephalitis, caused by *Naegleria fowleri* amoeba, is a rare but severe infection that progressively affects the central nervous system. While the overall incidence of this condition is low, its mortality rate is exceptionally high, with only four documented survivors in the United States. In states such as Florida, Texas, and Louisiana, where reporting fatal lens-associated (FLA) infections is mandatory, every other state should be encouraged to send suspected samples to the CDC for confirmation. Without this, the true impact of *N. fowleri* pathophysiology could remain significantly underestimated.

A potential solution to this information gap is establishing an international database that collects relevant data from a global registry. The high fatality rate is largely attributable to a lack

of awareness regarding sporadic amoebiasis, delays in diagnosis, and the absence of specific antibiotics and treatment protocols. Healthcare providers, especially those working with pediatric patients, should include PAM in their differential diagnosis when evaluating meningitis-like symptoms during the summer months. Incorporating Wright-Giemsa staining as part of the routine analysis for cerebrospinal fluid (CSF) in states with higher incidence rates could prove beneficial. This practice would alert laboratory staff to screen for amoebas and use appropriate methods when examining specimens suspected to have meningitis.

Public health officials in high-risk regions should proactively enhance water management and sanitation practices. It is crucial to stay vigilant about parasitic and non-parasitic pathogenic agents in the environment. Additionally, monitoring the presence of FLAs in ecosystems is essential, as they can harbor other clinically significant microorganisms.

Since clinical judgment is vital for survival, the faster the pathogen is identified, the sooner treatment and intervention can begin. Consequently, healthcare practitioners will be better positioned to improve recovery outcomes and prevent increased PAM-related morbidity and mortality.

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Supplementary Material

Naegleria fowleri strain Nf 69 (ATCC 30215) was originally isolated by Fowler and Carter from a 9-year-old boy in Adelaide, Australia, who died of PAM in 1969.
https://www.atcc.org/products/30215

Cyanomethyl Vinyl Ethers Against *Naegleria fowleri* (Research Article)
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CDC - *Naegleria fowleri* – Primary Amoebic Meningoencephalitis (PAM)
https://www.cdc.gov/parasites/naegleria/index.html

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Chlorine Dilution Calculator
https://www.publichealthontario.ca/en/health-topics/environmental-occupational-health/water-quality/chlorine-dilution-calculator