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Review Article

Brain-Eating Amoeba Naegleria fowleri: Global Epidemiology, Diagnosis, Therapeutic Challenges, and Strategies to Combat Primary Amoebic Meningoencephalitis

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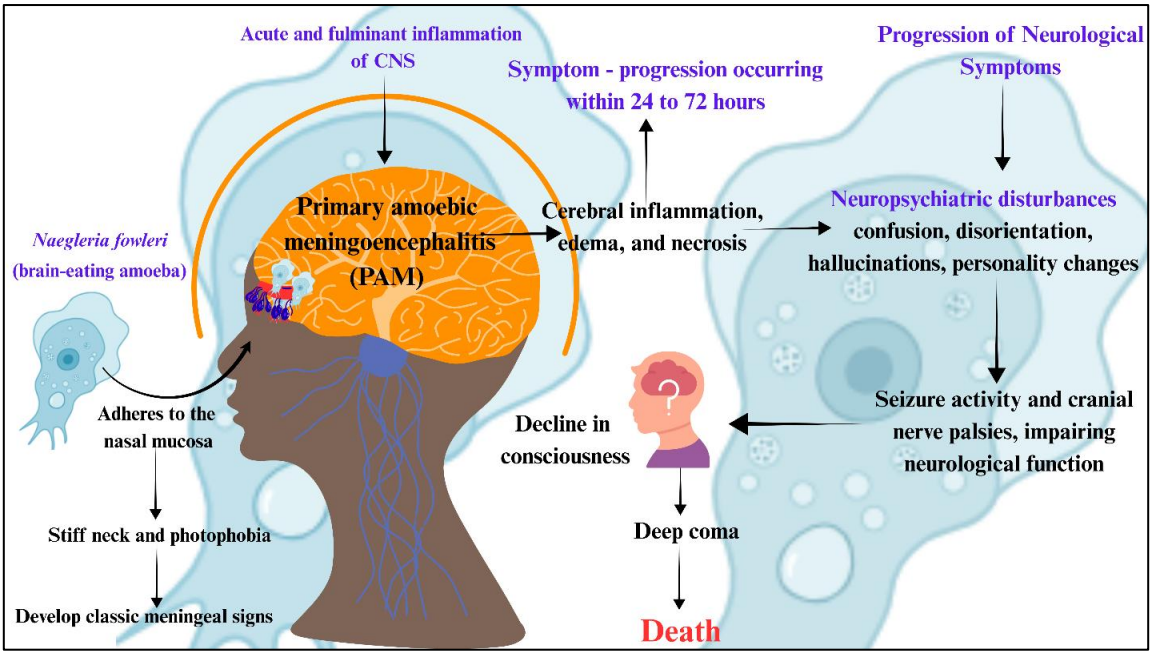
Abstract

Naegleria fowleri, colloquially known as the "brain-eating amoeba," is a free-living protozoan that causes the fulminant and often fatal disease primary amoebic meningoencephalitis (PAM). Although considered rare, the global case fatality rate exceeds 95%, making it one of the deadliest human infections. Traditionally associated with warm freshwater environments in tropical and subtropical regions, recent cases from temperate areas suggest climate change and water resource mismanagement are contributing to an expanding epidemiological footprint. Despite advances in molecular biology and phylogenetics, timely diagnosis remains elusive, as early clinical manifestations mimic bacterial or viral meningitis, often leading to misdiagnosis and delayed treatment. Current therapeutic regimens, largely based on amphotericin B, azoles, rifampin, and miltefosine, demonstrate limited success, and no standardized treatment protocol has been universally adopted. This review synthesizes the latest insights into the biology and pathogenicity of *N. fowleri*, outlines the global epidemiological trends and phylogenetic diversity, and discusses diagnostic challenges and therapeutic interventions. Furthermore, it highlights the pathogen's emerging public health threat in the context of climate change and globalization, and proposes multi-pronged strategies for prevention, early detection, and therapeutic innovation. Strengthening surveillance systems, integrating genomic tools, and fostering international collaborations are essential to mitigate the devastating burden of PAM and to prepare for the potential global spread of this lethal pathogen.

Keywords: Brain-eating amoeba, emerging infections, global health, *Naegleria fowleri*, primary amoebic meningoencephalitis.

Graphical abstract



Highlights:

- ✓ *Naegleria fowleri*, the "brain-eating amoeba," causes primary amoebic meningoencephalitis (PAM), a rapidly progressive CNS infection with >95% mortality.Infection occurs via nasal exposure to warm freshwater, with trophozoites migrating through the olfactory nerve to the brain.
- ✓ Global epidemiology is expanding, with cases reported in traditional tropical regions and emerging temperate areas due to climate change, urbanization, and recreational water use.Early clinical manifestations are nonspecific, mimicking bacterial or viral meningitis, leading to frequent misdiagnosis and delayed treatment.
- ✓ Diagnostic advances include PCR, next-generation sequencing, and emerging point-of-care assays, but accessibility remains limited.Therapeutic interventions (amphotericin B, miltefosine, azoles, combination therapy) show limited efficacy; experimental approaches like nanoparticles and immunotherapy are under investigation.
- ✓ N. fowleri poses a growing global public health threat, exacerbated by underreporting, inadequate water treatment, and low public awareness.Strategic interventions require integrated approaches: preventive water safety measures, rapid diagnostics, therapeutic innovation, surveillance, and a One Health framework for global preparedness.

1. Introduction

Naegleria fowleri is an opportunistic free-living amoeba (FLA) that inhabits freshwater bodies such as lakes, rivers, hot springs, and inadequately chlorinated swimming pools. First described in 1965 in Australia, the organism has since been recognized as the causative agent of primary amoebic meningoencephalitis (PAM), a rapidly progressive infection of the central nervous system (CNS) with a near-universal fatality rate.[1] Although infection is exceedingly rare, the catastrophic outcomes, coupled with its evocative label as the "brain-eating amoeba," have placed *N. fowleri* under significant scientific and public scrutiny.The disease typically occurs following recreational water exposure, when contaminated water enters the nasal cavity and trophozoites migrate via the olfactory nerve to the brain.[2] The infection progresses within days, with initial symptoms including headache, fever, and nausea, rapidly advancing to seizures, coma, and death. The incubation period is short, and death usually occurs within one to two weeks of symptom onset. This aggressive course underscores the urgent need for early diagnosis and intervention, yet such outcomes remain rare due to nonspecific clinical presentations and limited diagnostic infrastructure.[3]Global epidemiological reports indicate that most cases have historically been concentrated in the United States, India, Pakistan, and several countries in Southeast Asia. However, recent case reports from Europe, South America, and non-endemic regions highlight the pathogen's expanding range.[4] The impact of climate change, particularly rising freshwater temperatures, is a significant driver of this shift. Furthermore, rapid urbanization, inadequate water treatment infrastructure, and increased recreational water use are amplifying human exposure risks.[5]From a molecular perspective, *N. fowleri* belongs to the family *Vahlkampfiidae* within the phylum Percolozoa, a group of amoeboflagellates characterized by their ability to transition between trophozoite, flagellate, and cyst stages. Advances in genomics and phylogenetics have begun to unravel its unique adaptations for neurotropism and immune evasion, offering potential targets for therapeutic development.[6] However, despite these insights, effective therapies remain elusive. Standard treatment regimens are inconsistent across regions, with most survivors reported only in cases of early diagnosis combined with aggressive multi-drug therapy including amphotericin B and miltefosine.The global health community faces a dual challenge: managing a pathogen with limited case numbers yet devastating outcomes, while simultaneously addressing its rising epidemiological footprint driven by ecological and climatic factors.[7] This review provides an integrated analysis of *N. fowleri*, with emphasis on global epidemiology, phylogenetic insights, clinical presentation, diagnostic advances, therapeutic challenges, and strategies for public health preparedness.

2. Biology and Pathophysiology of *Naegleria fowleri*

2.1 Taxonomy and Classification

Naegleria fowleri is a thermophilic, free-living amoeba that belongs to the family *Vahlkampfiidae*, order Schizopyrenida, within the phylum Percolozoa. Unlike classical amoebae of the phylum Amoebozoa, *N. fowleri* exhibits amoeboflagellate characteristics, enabling transitions between amoeboid and flagellated forms depending on environmental conditions. The genus Naegleria comprises more than 40 species, but only *N. fowleri* has been identified as pathogenic to humans, highlighting its unique neurotropism and virulence traits.[8, 9]

2.2 Life Cycle and Morphological Stages

The organism exhibits three distinct morphological stages:

Trophozoite (infective stage): The trophozoite is the active feeding and replicative form, measuring 10-25 µm, and is responsible for initiating human infection. It thrives in warm freshwater (25-46 °C), explaining its prevalence in hot springs, poorly maintained swimming pools, and warm lakes.[10]

Flagellate stage (transitional form): Under nutrient-deprived conditions, trophozoites can transform into a transient, non-replicating flagellated form with two flagella. This form aids dispersal in aquatic environments but does not contribute directly to infection.[11]

Cyst stage (resistant form): In unfavorable environments, *N. fowleri* encysts, forming a double-walled structure that ensures survival against

desiccation and nutrient deprivation. Unlike Acanthamoeba spp., cysts of *N. fowleri* are not known to be infective in humans.This morphological versatility contributes to its environmental persistence and pathogenic potential.[12]

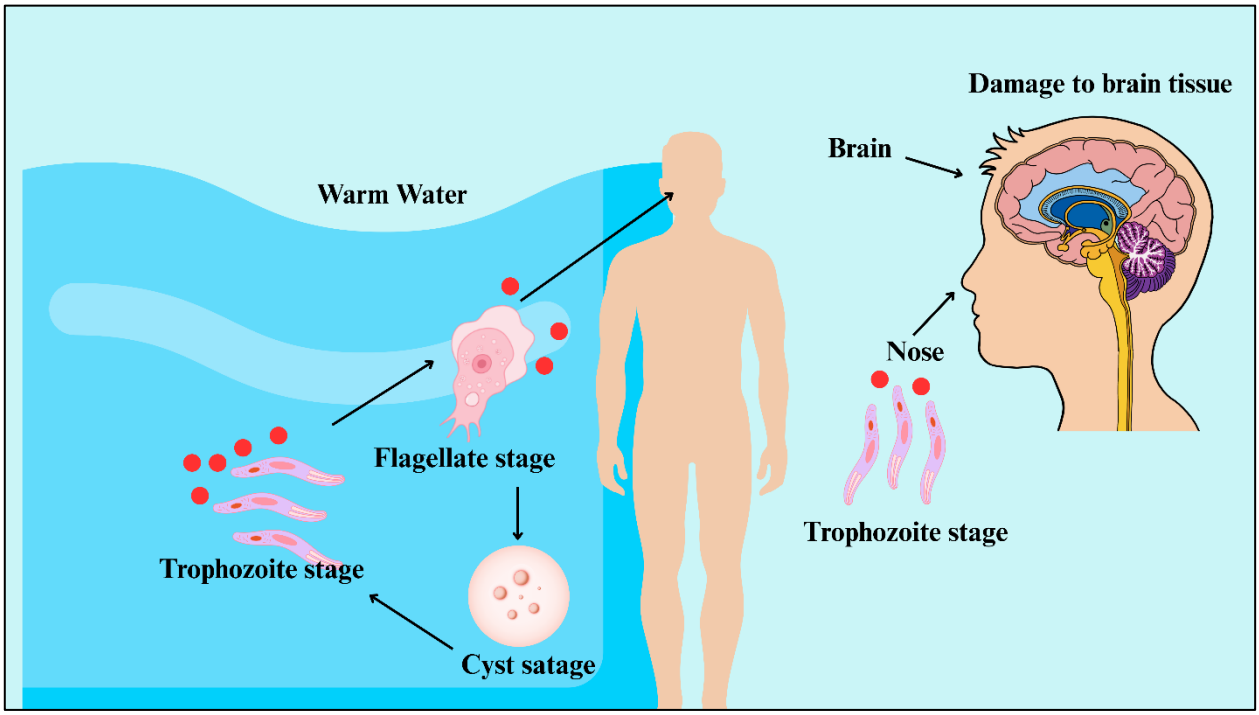


Figure 1: Life Cycle and Morphological Stages of*Naegleria fowleri*

2.3 Mechanisms of Pathogenesis

Human infection begins when contaminated water is forcibly introduced into the nasal cavity, typically during diving, swimming, or ablution practices. Trophozoites adhere to the olfactory epithelium, migrate along olfactory nerves, and penetrate the cribriform plate to invade the central nervous system (CNS).[13]

Key pathogenic mechanisms include:

Adhesion and invasion: Trophozoites express surface proteins such as integrins, adhesion molecules, and amoebastomes ("food cups") that facilitate tissue attachment and phagocytosis of host cells.[14]

Cytotoxicity: Secreted enzymes (proteases, phospholipases, neuraminidases) and pore-forming proteins degrade extracellular matrices and lyse host neural cells.[15]

Immune evasion:*N. fowleri* resists complement-mediated lysis and modulates host immune responses, including suppression of neutrophil and macrophage activity.[16]

Inflammation: The infection triggers intense neutrophilic infiltration and proinflammatory cytokine release, contributing to rapid CNS tissue destruction and cerebral edema.[17]

2.4 Host Immune Response and Disease Progression

The host immune system mounts both innate and adaptive responses against *N. fowleri*. Neutrophils, macrophages, and microglial cells form the first line of defense, releasing reactive oxygen species and antimicrobial peptides. However, the amoeba's resistance to oxidative stress and complement-mediated killing undermines these efforts.[18] Adaptive responses involving antibodies (IgA and IgG) have been demonstrated experimentally, yet their role in natural infections remains limited due to the rapid disease progression.Clinically, this results in a fulminant meningoencephalitis characterized by necrosis, hemorrhage, and inflammation of the brain tissue. Death typically occurs within 7-14 days after exposure, reflecting both the virulence of the pathogen and the insufficiency of host defenses.[19]

3. Global Epidemiology

3.1 Geographic Distribution and Hotspots

Since its first recognition in Australia in the mid-1960s, Naegleria fowleri infections have been reported across multiple continents, with the highest concentration of documented cases in the United States, India, Pakistan, Thailand, and several Latin American countries. In the U.S., most cases are associated with southern states such as Florida, Texas, and Arizona, reflecting the organism's thermophilic nature.[20] In South Asia, particularly in Pakistan's Sindh province, recurring clusters of infections have been reported, often linked to inadequately chlorinated municipal water supplies.Although previously considered a pathogen restricted to tropical and subtropical climates, cases in temperate regions of Europe (e.g., Czech Republic, Spain), North America (Minnesota), and South America (Argentina, Chile) indicate a geographical expansion that is increasingly concerning for global health authorities.[21]

3.2 Environmental Reservoirs and Transmission Dynamics

N. fowleri thrives in warm freshwater ecosystems, including lakes, rivers, geothermal hot springs, and poorly maintained swimming pools. It has also been detected in drinking water distribution systems, particularly in areas with low residual chlorine levels and high ambient temperatures.[22] The pathogen is not transmitted by ingestion of contaminated water but requires direct nasal exposure, usually during diving, swimming, or ritual ablution.Recent studies suggest that biofilm formation within water systems provides an additional niche for amoebic survival and dispersal. The presence of thermophilic bacteria in these environments may serve as food sources, sustaining *N. fowleri* populations in aquatic ecosystems. [23]

3.3 Incidence and Mortality Trends

Globally, fewer than 500 cases have been confirmed to date, though underreporting is highly likely due to diagnostic challenges. The case fatality rate exceeds 95%, with only a handful of documented survivors worldwide. Mortality trends remain consistent despite sporadic therapeutic successes, reflecting the limitations of current diagnostic and therapeutic modalities.In the United States, the Centers for Disease Control and Prevention (CDC) recorded.[24] approximately 0-8 cases annually over the past five decades, with the majority occurring during the summer months. In contrast, South Asia, particularly Pakistan and India, reports higher case numbers annually, suggesting environmental, infrastructural, and socio-behavioral factors significantly influence disease burden.[25]

3.4 Seasonal Variation and Demographic Profiles

Infections exhibit a marked seasonal trend, with most cases reported in summer and early autumn, coinciding with peak water temperatures. Children and young adults constitute the majority of cases, likely due to increased recreational water exposure and more vigorous aquatic activities that facilitate nasal water entry.Males are disproportionately affected, with a male-to-female ratio exceeding 2:1 in most case series, possibly reflecting gender-based differences in recreational exposure rather than biological susceptibility.[26]

3.5 Emerging Epidemiological Patterns and Risk Factors

Recent epidemiological shifts raise significant concerns:

3.5.1. Climate Change: Rising global temperatures caused by climate change are significantly influencing the ecology of freshwater systems. Warmer conditions are extending both the seasonal duration and the geographical distribution of thermophilic organisms such as *N. fowleri*, a pathogenic amoeba that thrives in warm freshwater environments.[27] Traditionally confined to subtropical and tropical regions, this microorganism is now being detected in more temperate areas as lakes, rivers, and reservoirs experience prolonged periods of elevated temperatures. These shifts increase the risk of human exposure through recreational water activities, underscoring the importance of monitoring freshwater ecosystems and developing adaptive public health strategies to mitigate emerging threats.[28]

3.5.2. Urbanization and Infrastructure: In many developing countries, insufficient municipal water treatment combined with aging or poorly maintained infrastructure contributes to persistent vulnerability to waterborne pathogens. Suboptimal chlorination fails to achieve effective disinfection, allowing resilient microorganisms such as *N. fowleri* to survive within distribution systems.[29] Cracked pipelines, leaky joints, and intermittent water supply further exacerbate contamination risks by enabling infiltration of untreated surface water and sewage. These systemic deficiencies create favorable niches for pathogen persistence and proliferation, particularly in warm climates. Consequently, inadequate water quality management not only undermines public health but also amplifies the potential for outbreaks of preventable, yet life-threatening infections.[30]

3.5.3. Increased Global Travel: Increasing human mobility, driven by globalization, migration, and tourism, is reshaping the epidemiology of infectious diseases, including those caused by *N. fowleri*. Individuals traveling from endemic to non-endemic regions may inadvertently introduce cases, particularly when engaging in freshwater-based recreational activities. Changing leisure practices, such as the growing popularity of water parks, artificial lakes, and ecotourism, further amplify opportunities for exposure to warm freshwater habitats that support pathogen survival. These dynamics blur traditional geographic boundaries of disease occurrence, complicating surveillance and response. Consequently, public health systems must adapt to evolving behavioral and mobility patterns that increase the risk of sporadic outbreaks.[31,32]

3.5.4. Religious and Cultural Practices: In South Asia and the Middle East, reported infections of *N. fowleri* have frequently been associated with ritual nasal ablution practices performed with inadequately treated tap water, underscoring the influence of cultural behaviors on disease transmission. Such cases highlight how everyday religious or traditional activities can inadvertently increase exposure risk when safe water infrastructure is lacking. These epidemiological patterns challenge the conventional view of N. fowleri as a rare, geographically restricted pathogen. Instead, the organism must now be recognized as an emerging global health concern, warranting enhanced surveillance, culturally sensitive public health education, and internationally coordinated prevention and control strategies.[33,34]

4. Phylogenetic Insights

4.1 Molecular Classification and Genetic Diversity

Molecular studies have placed *N. fowleri* within the phylum Percolozoa, distinct from true amoebae in the phylum Amoebozoa. Phylogenetic analyses based on ribosomal RNA (18S rRNA) and internal transcribed spacer (ITS) sequences have revealed the presence of

several genotypes or subgroups of *N. fowleri*, with at least eight distinct genotypes (I-VIII) described globally.[35] These genotypes often correlate with specific geographic regions, suggesting evolutionary divergence shaped by environmental niches.Genotypic classification has provided valuable insights into the distribution and pathogenic potential of isolates. For example, genotype I strains predominate in the United States, whereas genotypes II and III are frequently reported from Asian and European regions. Despite genetic variation, all identified genotypes retain pathogenic potential, underscoring the uniform virulence of the species.[36]

4.2 Comparative Genomics with Other Free-Living Amoebae

Comparative genomic investigations between *N. fowleri* and its non-pathogenic relative *Naegleria gruberi* have provided valuable insights into the molecular basis of pathogenicity. The genome of *N. gruberi* functions as an evolutionary reference point, allowing researchers to distinguish genetic features that are specifically associated with the virulent phenotype of N. fowleri.[37] These include gene families encoding proteases, phospholipases, and pore-forming proteins, all of which contribute to host tissue degradation and immune evasion. In addition, N. fowleri possesses surface-associated proteins that enhance adhesion to epithelial cells, facilitating colonization of the nasal mucosa and subsequent invasion of the central nervous system. Genomic evidence also highlights genes involved in thermo-tolerance and stress adaptation, which enable survival under fluctuating environmental conditions and within the warm host environment.[38] Collectively, these findings indicate that *N. fowleri* has selectively retained or evolved a specialized genetic toolkit that supports its dual existence as a free-living amoeba and an opportunistic human pathogen.

4.3 Evolutionary Adaptations Driving Pathogenicity

The evolutionary success of *N. fowleri* is closely tied to its thermophilic nature. Its ability to thrive at temperatures up to 46 °C provides a selective advantage in heated aquatic habitats and partially explains its propensity to invade the human brain, an environment with elevated basal temperature.Additionally, phylogenetic studies suggest that the amoebastome ("food cup") structure and cytolytic effector molecules may have originally evolved for bacterial predation in environmental settings but were co-opted for tissue invasion in human infections. This highlights the concept of "accidental pathogenicity"-where environmental adaptations inadvertently enhance human virulence.[39]

4.4 Phylogeography and Global Strain Variability

Global phylogeographic mapping of *N. fowleri* has shown that strains from different continents often cluster together, indicating both regional evolution and intercontinental dispersal. Strain variability may influence disease epidemiology, with some studies suggesting differences in virulence, growth rate, and drug susceptibility between genotypes. However, the clinical significance of these differences remains poorly understood.Advances in whole-genome sequencing (WGS) and next-generation sequencing (NGS) technologies are expected to refine our understanding of strain-level diversity, enabling more precise epidemiological tracing and potentially informing targeted therapeutic strategies.[40]

5. Clinical Spectrum of Primary Amoebic Meningoencephalitis (PAM)

5.1 Early Clinical Manifestations

Primary amoebic meningoencephalitis (PAM), a fulminant and often fatal infection caused by *N. fowleri*, generally manifests after an incubation period ranging from two to seven days following exposure to contaminated freshwater sources such as lakes, rivers, or inadequately treated tap water.[41] The initial clinical presentation is typically nonspecific, resembling common viral or bacterial infections, which complicates timely recognition. Early symptoms include severe frontal headache, intermittent or persistent fever, nausea, vomiting, fatigue, and a general sense of malaise. Because these manifestations closely mimic viral meningitis or bacterial meningoencephalitis, clinicians frequently encounter diagnostic challenges, and treatment is often delayed.[42] Such delays are critical, as the disease progresses rapidly, with the amoeba invading the central nervous system via the olfactory nerve, leading to devastating neurological damage. The overlap in symptomatology underscores the need for heightened clinical suspicion, especially in patients with recent freshwater exposure, to facilitate early diagnosis and improve survival outcomes.[43]

5.2 Progression of Neurological Symptoms

The clinical course of primary amoebic meningoencephalitis (PAM) is alarmingly rapid, with symptom progression occurring within 24 to 72 hours after the onset of early flu-like manifestations. As the infection advances, neurological involvement becomes pronounced, reflecting extensive cerebral inflammation, edema, and necrosis caused by the aggressive invasion of *N. fowleri*.[44] Patients typically develop classic meningeal signs such as stiff neck and photophobia, accompanied by progressive neuropsychiatric disturbances including confusion, disorientation, hallucinations, and marked personality changes. Seizure activity and cranial nerve palsies frequently emerge as the disease worsens, further impairing neurological function. A progressive decline in consciousness is often observed, culminating in deep coma and, in most cases, death.[45] Autopsy studies consistently demonstrate hemorrhagic necrotizing meningoencephalitis, with the most severe damage localized in the frontal lobes, olfactory bulbs, and brainstem regions directly invaded via the olfactory nerve pathway. This devastating pathology underscores the urgent need for early recognition and intervention in suspected cases.[46]

5.3 Prognosis and Mortality

PAM is associated with an exceptionally poor prognosis, with a case fatality rate exceeding 95%. Most patients succumb to the disease within 7-14 days of symptom

onset despite aggressive treatment. Only a small number of survivors have been reported worldwide, and in nearly all cases, early recognition and immediate initiation of combination therapy were critical factors in survival.Long-term neurological sequelae in survivors may include cognitive impairment, motor dysfunction, and seizure disorders, although detailed follow-up data remain limited.[47]

5.4 Differential Diagnosis

PAM poses a significant diagnostic challenge because its early manifestations overlap with those of more common central nervous system infections. As a result, it is frequently misdiagnosed as bacterial, viral, or fungal meningitis, delaying the initiation of appropriate treatment. The differential diagnosis includes bacterial meningitis caused by organisms such as *Neisseria meningitidisor Streptococcus pneumoniae*, both of which present with acute onset of fever, headache, and meningeal irritation.[48] Viral encephalitides, including those due to enteroviruses or herpes simplex virus, can also mimic PAM with similar nonspecific neurological features. Fungal infections, particularly *Cryptococcus neoformans*, represent another diagnostic consideration, especially in immunocompromised hosts. Additionally, infections caused by other free-living amoebae, such as Acanthamoeba species and *Balamuthia mandrillaris*, must be distinguished from PAM. However, unlike the fulminant and rapidly fatal course of PAM, these pathogens more commonly cause granulomatous amoebic encephalitis (GAE), which progresses in a chronic or subacute manner.[49]

5.5 Clinical Red Flags

Clinicians should suspect PAM in patients who present with acute meningitis-like symptoms following recent freshwater exposure, particularly in warm climates or during the summer season. Awareness of this association is crucial to prompt diagnosis and life-saving treatment.[50]

6. Diagnostic Approaches

6.1 Conventional Diagnostic Methods

Historically, the diagnosis of PAM has depended largely on microscopic examination of cerebrospinal fluid (CSF), a method that remains challenging due to the rapid course of the disease and the nonspecific overlap with other central nervous system infections. Wet mount microscopy may reveal motile trophozoites, typically measuring 10-25 µm, which exhibit a distinctive eruptive, directional movement that differentiates them from white blood cells. Staining techniques such as Giemsa, trichrome, or Wright stain can provide additional clarity by highlighting nuclear morphology, particularly the presence of a prominent central karyosome.[51] CSF analysis often shows turbid fluid, increased opening pressure, neutrophilic pleocytosis, elevated protein concentrations, and decreased glucose levels, features that strongly resemble bacterial meningitis and contribute to frequent misdiagnosis. Although culture on non-nutrient agar overlaid with Escherichia coli can confirm N. fowleri infection, the process requires several days, rendering it impractical for urgent clinical decision-making in this rapidly progressive, often fatal disease.[52]

6.2 Molecular Diagnostics

Advancements in molecular diagnostics have greatly enhanced the accuracy and timeliness of detecting *N. fowleri* in suspected cases of primary amoebic meningoencephalitis (PAM), overcoming many limitations of conventional microscopy and culture-based methods. Polymerase chain reaction (PCR)-based assays, particularly real-time PCR (qPCR), have become the cornerstone of modern diagnostics due to their ability to provide rapid, sensitive, and highly specific detection.[53] These assays often target conserved genetic regions such as the 18S rRNA and internal transcribed spacer (ITS) sequences, allowing reliable identification of the pathogen even in low-concentration samples. Moreover, multiplex PCR platforms have been developed to simultaneously detect and differentiate Naegleria from other free-living amoebae of clinical significance, including *Balamuthia mandrillaris*.[54] In addition, next-generation sequencing (NGS) applied to cerebrospinal fluid has emerged as a powerful, unbiased approach for identifying elusive pathogens in undiagnosed encephalitis cases, though widespread clinical use remains limited by high costs, infrastructure requirements, and longer turnaround times.[55]

6.3 Rapid and Point-of-Care Diagnostics

Given the rapidly progressive and often fatal course of PAM, there is increasing emphasis on developing point-of-care diagnostic tools capable of providing results within hours, enabling timely clinical intervention. Loop-mediated isothermal amplification (LAMP) assays have shown considerable promise for rapid, sensitive detection of *N. fowleri* in field or resource-limited settings, circumventing the need for complex laboratory infrastructure. Immunofluorescence assays employing monoclonal antibodies allow direct identification of amoebic antigens in cerebrospinal fluid or tissue samples, enhancing diagnostic specificity. Additionally, innovative biosensor technologies and nanoparticle-based platforms are under investigation for bedside application, offering potential for real-time detection and streamlined integration into clinical workflows.[56]

6.4 Neuroimaging and Ancillary Investigations

Neuroimaging findings in PAM are nonspecific but may include diffuse cerebral edema, hydrocephalus, and hemorrhagic lesions, often localized to the frontal lobes. Magnetic resonance imaging (MRI) may help identify early olfactory bulb involvement but is not diagnostic.[57]

6.5 Challenges in Early and Accurate Diagnosis

Despite significant advances in molecular and immunological diagnostics, substantial challenges remain in the timely identification of PAM. Low clinical suspicion often delays consideration of the disease until advanced neurological symptoms appear, while overlapping cerebrospinal fluid profiles with bacterial meningitis contribute to frequent misdiagnosis. Furthermore, resource constraints limit access to

advanced molecular assays, which are predominantly available in specialized reference laboratories in high-income settings. Given the fulminant progression of PAM, even delays of 24-48 hours can prove fatal. Enhancing clinician awareness, establishing systematic surveillance, and developing cost-effective, rapid point-of-care diagnostic tools are essential strategies to reduce diagnostic delays and improve patient survival.[58]

7. Therapeutic Interventions and Challenges

7.1 Current Treatment Protocols

Treatment of PAM remains highly challenging, primarily due to the pathogen's rapid CNS invasion and the difficulty of achieving therapeutic drug concentrations in brain tissue. Current treatment regimens are largely based on case reports and small case series, as no randomized clinical trials exist.[59]

Amphotericin B (AmB): The first-line therapy, typically administered intravenously, sometimes in combination with intrathecal delivery. AmB is a polyene macrolide antifungal and anti-amoebic agent that exerts its activity primarily by targeting sterol components of the plasma membrane. At the molecular level, AmB has high affinity for ergosterol and ergosterol-like sterols present in *N. fowleri*, where it binds and self-assembles into trans-membrane aggregates that form pores or ion channels. These pores allow uncontrolled leakage of intracellular cations such as K^+ and Mg^{2+}, together with proton influx, leading to disruption of membrane potential, ionic gradients, and overall cellular homeostasis.[60] In addition, AmB can induce oxidative stress by generating reactive oxygen species that damage lipids, proteins, and nucleic acids, and it may also destabilize membranes through sterol sequestration by extracting sterols from the bi-layer. At the cellular level, these effects culminate in profound membrane disruption, collapse of mitochondrial potential, and impairment of ATP synthesis, enzyme activity, and signaling pathways.[61] The resulting osmotic imbalance drives cell swelling, vacuolization, and necrotic lysis of trophozoites, though in some protozoa, apoptosis-like processes such as DNA fragmentation and caspase-like activity have also been observed. AmB's therapeutic action is partly selective because mammalian cell membranes contain cholesterol rather than ergosterol, for which the drug has lower affinity; however, at higher concentrations AmB can also interact with cholesterol, causing toxicity in host cells, most notably renal tubular cells, leading to dose-limiting nephrotoxicity. Thus, AmB's mechanism integrates sterol binding, pore formation, ionic imbalance, and oxidative stress to achieve amoebicidal effects, while its clinical utility is constrained by host toxicity.[62]

Miltefosine: Originally an antileishmanial drug, miltefosine has shown *in-vitro* amoebicidal activity and contributed to several survivor cases when used in combination therapy. Miltefosine, an alkylphosphocholine originally developed as an antitumor agent, exerts its anti-*N.fowleri* activity through a multifaceted mechanism involving both membrane and mitochondrial targets. The drug integrates into phospholipid bi-layers, altering membrane composition, fluidity, and lipid raft organization, which disrupts sterol- and sphingolipid-rich domains essential for parasite survival.[63] It further interferes with Phosphatidyl choline and phosphor inositide metabolism, impairing lipid-dependent signaling cascades that regulate proliferation, motility, and stress responses. Within mitochondria, miltefosine disrupts membrane potential, causing energy collapse and initiating apoptosis-like signaling, while simultaneously generating excess reactive oxygen species that damage DNA, proteins, and lipids. These molecular events manifest at the cellular level as membrane destabilization, increased permeability, mitochondrial dysfunction, ATP depletion, and oxidative injury, culminating in either apoptosis-like death characterized by chromatin condensation and DNA fragmentation or necrotic lysis under severe stress.[64] Additionally, membrane disruption and antigen release may synergize with host immune responses to enhance parasite clearance. Miltefosine displays relative selectivity for protozoan membranes due to their unique lipid composition, though it is not without host toxicity, as prolonged use can cause gastrointestinal, hepatic, and renal side effects, and resistance may develop through altered drug uptake or lipid remodeling. Altogether, miltefosine's combined effects on membranes, signaling pathways, and mitochondria underpin its therapeutic utility against *N. fowleri*, making it one of the few orally available agents with clinical relevance in this otherwise highly lethal infection.[65]

Azoles (e.g., fluconazole, ketoconazole, voriconazole): Azole compounds such as fluconazole, ketoconazole, and voriconazole act against Naegleria fowleri primarily by disrupting sterol biosynthesis, a process critical for maintaining the structural integrity and function of the plasma membrane. At the molecular level, azoles inhibit the cytochrome P450-dependent enzyme lanosterol 14α-demethylase, which catalyzes the conversion of lanosterol to ergosterol or ergosterol-like sterols in protozoan membranes.[66] Inhibition of this enzyme leads to depletion of functional sterols and accumulation of toxic 14-methylated sterol intermediates that alter membrane fluidity, permeability, and protein function. These disruptions impair membrane-bound enzymes, ion channels, and signaling pathways essential for trophozoite survival. At the cellular level, loss of membrane integrity compromises nutrient uptake, ion homeostasis, and stress responses, while impaired sterol composition in mitochondrial membranes disrupts electron transport and ATP generation.[67] The cumulative effects include mitochondrial dysfunction, oxidative stress, inhibition of growth, and eventual parasite death. Although azoles demonstrate activity against Naegleria in vitro and some clinical contexts, their efficacy may be limited by variable sterol metabolism in amoebae, suboptimal penetration into the central nervous system, and emerging resistance mechanisms such as efflux pump over-expression or altered drug binding to the target enzyme. Thus, azoles contribute to therapeutic regimens against *N. fowleri* by targeting sterol biosynthesis, but their effectiveness is context-dependent and often

enhanced when used in combination with other agents such as amphotericin B.[68]

Rifampin and azithromycin: Occasionally, adjunctive agents are incorporated into treatment protocols for *N. fowleri* infections because of their potential synergistic antimicrobial effects when combined with established anti-amoebic drugs. Evidence from reported cases indicates that the most favorable therapeutic outcomes have been achieved through aggressive multi-drug regimens rather than monotherapy. These regimens are often complemented by comprehensive supportive care measures, including meticulous management of intracranial pressure to counteract cerebral edema and prevent herniation.[69] In select cases, therapeutic hypothermia has also been employed to reduce metabolic demand and limit neuronal damage. This multimodal approach highlights the necessity of integrating pharmacological and supportive strategies to improve survival.[70]

7.2 Experimental and Adjunctive Therapies

Novel approaches under investigation include:

Nanoparticle-based drug delivery: Nanoparticle-based drug delivery systems represent a promising strategy to overcome the challenges of treating N. fowleri infections, particularly the difficulty of achieving therapeutic concentrations of anti-amoebic agents within the central nervous system due to the restrictive nature of the blood-brain barrier (BBB). By encapsulating drugs within biocompatible nanoparticles, including liposomes, polymeric nanoparticles, or solid lipid carriers, these formulations can enhance drug stability, prolong circulation time, and facilitate targeted transport across the BBB.[71] For example, amphotericin B, the mainstay of PAM treatment, has demonstrated improved CNS bioavailability and reduced systemic toxicity when delivered in liposomal or nanoparticulate forms. Similarly, azole antifungals like fluconazole and miltefosine have been explored in nanoformulations to increase penetration into brain tissue while minimizing adverse effects. These approaches not only enhance therapeutic efficacy against *N. fowleri* but also offer a platform for combination therapy, potentially improving survival outcomes in this otherwise rapidly fatal disease.[72]

Immunotherapy: Immunotherapeutic strategies targeting *N. fowleri* are emerging as adjuncts to conventional anti-amoebic treatment by enhancing host immune defenses. Monoclonal antibodies can be designed to recognize surface antigens of the amoeba, promoting opsonization and complement-mediated lysis, while cytokine modulation aims to stimulate protective innate and adaptive immune responses, such as increased production of interferon-gamma or interleukin-12. Integration of these immunomodulatory agents into nanoparticle-based delivery systems has been explored to improve stability, targeted CNS delivery, and controlled release. For instance, liposomal encapsulation of cytokines or antibody fragments can facilitate blood-brain barrier penetration, offering a promising approach to augment host immunity against this rapidly fatal pathogen.[73]

Repurposed drugs: High-throughput screening of FDA-approved drugs has revealed several compounds with potent amoebicidal activity against *N. fowleri*, offering the potential for rapid repurposing in the treatment of primary amoebic meningoencephalitis. Notably, statins, such as simvastatin, and certain anti-cancer agents, including miltefosine analogs, have demonstrated significant inhibition of trophozoite viability *in vitro*. Incorporating these drugs into nanoparticle-based formulations, such as liposomes or polymeric nanoparticles, can enhance blood-brain barrier penetration, improve bioavailability, and reduce systemic toxicity. Such nano-formulated repurposed therapeutics hold promise for accelerating clinical translation and improving survival outcomes in this otherwise rapidly fatal infection.[74]

Combination therapy strategies: Combining amphotericin B with agents such as miltefosine, azole antifungals, or rifampin has demonstrated promising synergistic activity against *N. fowleriin vitro*, suggesting enhanced amoebicidal efficacy compared to single-drug exposure. Amphotericin B remains the cornerstone therapy due to its membrane-disrupting action, while miltefosine and azoles contribute additional mechanisms, including interference with lipid metabolism and ergosterol biosynthesis. Rifampin may further augment activity through inhibition of RNA synthesis. Despite these encouraging laboratory findings, robust clinical evidence remains scarce, as most reported cases rely on limited patient cohorts or anecdotal outcomes. Rigorous clinical validation through systematic trials is therefore essential before such combinations can be formally integrated into treatment guidelines.[75]

7.3 Barriers to Successful Treatment

The effectiveness of current therapeutic interventions for PAM remains severely constrained by multiple factors. Foremost is delayed diagnosis, as the disease is typically recognized only after extensive central nervous system involvement, when treatment options are less effective. In addition, many conventional and repurposed drugs demonstrate poor penetration across the blood–brain barrier, resulting in sub-therapeutic concentrations within brain tissue. The infection itself progresses with alarming rapidity, often leading to death within 7-14 days of symptom onset, thereby leaving clinicians with a very limited therapeutic window. Furthermore, the extreme rarity of PAM restricts opportunities for large-scale clinical trials, hindering the development of evidence-based, standardized treatment protocols. These combined challenges underscore the urgent need for improved diagnostics, innovative drug delivery systems, and international collaboration to optimize therapeutic strategies.[76]

7.4 Recommendations for Clinical Management

Effective clinical management requires a proactive and multidisciplinary approach. Maintaining high clinical suspicion in individuals with recent freshwater

exposure is crucial, particularly in endemic or high-risk regions, to enable timely recognition of the disease. Early initiation of combination therapy, most notably regimens incorporating amphotericin B with miltefosine or other synergistic agents, offers the best chance of survival. Equally important is aggressive supportive care aimed at controlling cerebral edema, preventing seizures, and stabilizing vital functions. Incorporating rapid molecular diagnostic tools into routine practice can significantly reduce diagnostic delays, facilitating earlier intervention and improving clinical outcomes.[77]

8. Global Public Health Threats and Challenges

8.1 Underreporting and Limited Surveillance

Despite its extremely high mortality, remains significantly underreported worldwide, particularly in low- and middle-income countries. Contributing factors include limited access to advanced laboratory facilities capable of molecular diagnosis, frequent misdiagnosis as more common conditions such as bacterial or viral meningitis, and the absence of centralized reporting systems to capture confirmed cases. This widespread underreporting obscures the actual global burden of *N. fowleri* infections, making it difficult for public health authorities to accurately assess risks, prioritize surveillance, and allocate resources effectively, thereby hindering the development of comprehensive strategies for prevention and control.[78]

8.2 Climate Change and Expanding Habitat Range

Global warming has expanded the range of warm freshwater habitats, allowing *N. fowleri* to thrive in previously non-endemic regions. Extended summer seasons and increased frequency of heatwaves are correlated with higher incidence rates in temperate zones. Additionally, extreme weather events such as flooding and droughts may concentrate thermophilic amoebae in small water bodies, heightening human exposure risk.[79]

8.3 Socioeconomic and Healthcare Infrastructure Gaps

A significant number of PAM cases are reported from regions with inadequate water treatment infrastructure, where poorly chlorinated municipal supplies, contaminated recreational water facilities, and reliance on untreated freshwater for daily activities create persistent exposure risks. These challenges are further compounded by socioeconomic disparities, which not only increase the likelihood of contact with unsafe water sources but also limit timely access to advanced diagnostics and appropriate medical care. Together, these factors contribute to the consistently high mortality associated with *N. fowleri* infections and highlight the urgent need for targeted public health interventions.[80]

8.4 Public Awareness and Misconceptions[81]

Public perception of *N. fowleri* is often influenced by sensationalized media coverage, yet awareness of preventive measures remains low. Key gaps include:

- ✓ Lack of knowledge about safe swimming practices (e.g., avoiding diving or submerging the head in warm freshwater)
- ✓ Insufficient guidance regarding nasal protection during ablution or recreational activities
- ✓ Minimal emphasis on water treatment policies in educational campaigns

Strengthening community-level education and health communication is critical to reducing exposure risk.

8.5 Emerging Global Health Threat Considerations

With its exceptionally high fatality rate, expanding geographical distribution, and ability to persist in diverse aquatic environments, *N. fowleri* has emerged as a growing global health concern. The combined effects of climate change, rapid urbanization, and increasing recreational use of freshwater sources are amplifying exposure risks across both endemic and non-endemic regions. Addressing this challenge requires coordinated surveillance systems, standardized case reporting, and strengthened international collaboration to enable early detection, improve data sharing, and guide evidence-based prevention strategies aimed at reducing the likelihood of future outbreaks and mitigating the public health impact of this deadly pathogen.[82]

9. Strategies to Combat *Naegleria fowleri* Infections

9.1 Preventive Measures

Prevention remains the most effective strategy for controlling Naegleria fowleri infections, given the extremely high mortality and limited therapeutic options. Ensuring proper water treatment through consistent chlorination and routine monitoring of municipal supplies, swimming pools, hot springs, and other public water sources is critical to reducing amoebic proliferation. Equally important are safe recreational practices, such as avoiding head submersion or diving in warm freshwater, using protective nose clips, and steering clear of stagnant or poorly maintained water bodies. Public education and awareness campaigns, particularly in endemic areas and during peak summer months, play a vital role in reducing exposure risks.[83]

9.2 Policy and Regulatory Frameworks

To effectively reduce the risk of *N. fowleri* infections, countries must implement comprehensive national guidelines that regulate water quality, enforce proper chlorination, and promote safe recreational water practices. Establishing robust surveillance networks with centralized reporting systems is equally important for the rapid detection of outbreaks and accurate epidemiological monitoring. At the global level, international collaboration is essential, with the sharing of genomic, clinical, and epidemiological data playing a key role in strengthening early-warning systems, advancing research, and coordinating public health responses aimed at mitigating the impact of this emerging pathogen.[84]

9.3 Research Priorities

Ongoing research is essential for advancing effective interventions against N. fowleri and reducing the mortality of primary amoebic meningoencephalitis. Promising avenues include experimental vaccine development aimed at amoebic surface proteins and cytolytic enzymes, as well as novel drug discovery efforts that focus on repurposed compounds, nanoparticle-based delivery systems, and combination therapies to enhance central nervous system penetration and therapeutic efficacy. Parallel to treatment advances, innovation in diagnostics, such as rapid point-of-care assays, portable PCR platforms, and biosensor technologies offers the potential for early detection and timely intervention. Additionally, genomic surveillance through whole-genome sequencing provides critical insights into strain variation, virulence determinants, and the global spread of this deadly pathogen.[85]

9.4 One Health and Multisectoral Collaboration

A holistic, One Health approach that integrates human, animal, and environmental health is critical for mitigating the impact of *N. fowleri* infections. This includes systematic monitoring of freshwater sources for pathogenic strains, fostering collaboration between water management authorities, public health agencies, and research institutions, and actively engaging communities to implement preventive practices effectively. By combining environmental surveillance, inter-sectoral coordination, and public education, such strategies aim to minimize exposure risk, facilitate early diagnosis, and enhance treatment outcomes, ultimately reducing the global public health burden of primary amoebic meningoencephalitis and improving overall population health resilience.[86]

10. Future Perspectives

10.1 Predictive Modeling of Outbreaks

Recent advances in epidemiological modeling and climate analytics provide a powerful framework for predicting outbreaks of PAM. By integrating environmental parameters such as water temperature, pH, and seasonal rainfall patterns with historical incidence data, these models can identify regions and periods of heightened risk. Such predictive insights enable targeted preventive interventions, optimized resource allocation, and strategic public health planning, particularly in areas where *N. fowleri* is emerging or previously unrecognized. Ultimately, this approach enhances preparedness, reduces exposure risk, and supports timely responses to minimize the burden of this rapidly fatal infection.[87]

10.2 Integration of Genomics, AI, and Epidemiology

Integrating whole-genome sequencing (WGS) with artificial intelligence (AI) offers a transformative approach to enhancing surveillance and outbreak prediction for *N. fowleri*. AI-driven algorithms can analyze genomic data from environmental samples to detect novel pathogenic strains, predict virulence determinants, and identify potential drug resistance profiles. Additionally, these tools can inform and optimize the allocation of resources for water safety interventions and public health measures. By combining high-resolution genomic insights with predictive analytics, this integrative framework enables data-driven early warning systems and supports rapid, targeted responses to emerging PAM cases, ultimately strengthening global preparedness and mitigation efforts.[88]

10.3 Strengthening Global Health Preparedness

In response to the expanding environmental presence of *N. fowleri*, global health preparedness must prioritize strengthening diagnostic and treatment capacity, particularly in resource-limited regions where early detection and intervention are most challenging. Standardizing clinical management protocols including prompt initiation of combination therapy and comprehensive supportive care can improve patient outcomes and reduce mortality. Equally important is fostering international collaboration to facilitate knowledge sharing, professional training, and coordinated research efforts, enabling the global health community to respond more effectively to emerging cases and to implement evidence-based strategies for prevention, surveillance, and management of this highly lethal pathogen.[89]

10.4 Potential Breakthroughs in Therapy and Prevention

Future strategies to combat *N. fowleri* infections should focus on the development of highly effective pharmacologic agents capable of penetrating the central nervous system, alongside exploration of immunotherapeutic approaches or vaccines targeting key amoebic virulence factors. Equally critical is the deployment of rapid point-of-care diagnostic tools in endemic regions to enable early detection and timely initiation of treatment. By integrating technological innovation, molecular insights, and targeted public health interventions, these approaches collectively offer the potential to significantly reduce the global burden of this rapidly fatal pathogen and improve survival outcomes in affected populations.[90]

11. Conclusion

N. fowleri, often referred to as the "brain-eating amoeba," is a rare but highly lethal pathogen responsible for PAM, a disease with a mortality rate exceeding 95%. Its pathogenicity stems from thermophilic and neurotropic adaptations that enable survival in warm freshwater environments and invasion of the central nervous system. Historically confined to tropical and subtropical regions, reports from temperate zones indicate an expanding geographical and seasonal range driven by climate change, urbanization, and increased recreational water exposure. Early diagnosis is challenging because initial symptoms mimic bacterial or viral meningitis, and conventional laboratory methods are often too slow to guide timely therapy. Molecular diagnostics, including PCR and next-generation sequencing, improve detection but remain limited in resource-poor settings. Treatment is primarily based on

amphotericin B and miltefosine, while novel drug delivery systems, combination therapies, and experimental immunotherapies show promise but require further validation. Public health mitigation necessitates a multi-pronged strategy encompassing community education, water safety regulation, rapid diagnostics, therapeutic innovation, and international collaboration within a One Health framework. Ultimately, reducing the global burden of PAM depends on early detection, prompt treatment, and preventive measures informed by genomics, epidemiology, and environmental monitoring, with the integration of technological advances, surveillance, and preparedness offering the best prospects for improved survival.

List of abbreviations:

18S rRNA-18S Ribosomal Ribonucleic Acid

AI-Artificial Intelligence

AmB-Amphotericin B

ATP -Adenosine Triphosphate

BBB-Blood-Brain Barrier

CDC-Centers for Disease Control and Prevention

CNS-Central Nervous System

CSF-Cerebrospinal Fluid

DNA-Deoxyribonucleic Acid

FDA-Food and Drug Administration

FLA-Free-Living Amoeba

GAE-Granulomatous Amoebic Encephalitis

IgA-Immunoglobulin A

IgG-Immunoglobulin G

ITS-Internal Transcribed Spacer

LAMP-Loop-Mediated Isothermal Amplification

MRI-Magnetic Resonance Imaging

NGS-Next-Generation Sequencing

PCR-Polymerase Chain Reaction

qPCR-Quantitative Polymerase Chain Reaction (Real-Time PCR)

RNA-Ribonucleic Acid

WGS-Whole Genome Sequencing

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