Naegleria fowleri in a warming world: epidemiology, genotypic distribution, management challenges, and priorities for public health action

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***Naegleria fowleri* causes primary amoebic meningoencephalitis, a fatal infection with profound implications for global health burden and water safety. Rising global temperatures and ageing water infrastructure are expanding the ecological range of this pathogen, increasing exposure risk in both high-income and resource-constrained settings. The infection mainly occurs through contact with warm freshwater and brackish waters and inhalation of cyst-laden dust; however, surveillance systems remain fragmented and under-resourced, particularly in low-income and middle-income countries. Diagnosis is often delayed or made post-mortem, and survival is exceedingly rare. In this Review, we synthesised the current knowledge on global epidemiology, genotypic distribution, and environmental drivers of *N fowleri* emergence and identified key gaps in monitoring, water treatment, and governance. We propose that climate-resilient water safety strategies, integrated surveillance, and cross-sectoral collaboration are urgently needed to mitigate the risk of *N fowleri* exposure and to minimise future cases of primary amoebic meningoencephalitis. Strengthening global health systems to address this emerging threat will require coordinated investment, policy innovation, and research to close knowledge gaps and protect clinically vulnerable populations.**

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Introduction

Protozoan infections of the CNS are crucial health concerns, ranking second only to HIV in terms of CNS-related morbidity and mortality.[1] *Naegleria* is a genus of free-living amoebae comprising 47 species, commonly found in warm freshwaters and soils.[2] *Naegleria fowleri* is the only recognised human-pathogenic species within this genus, causing primary amoebic meningoencephalitis (PAM). Effective treatments against this infection are scarce, and the infection is often challenging to diagnose, with a mortality rate of more than 95%.[3] *N fowleri* infection has drawn increasing public health concerns and scientific interest recently, because of its rising prevalence and expanding distribution. First, increased prevalence of PAM is associated with the climate-driven expansion of *N fowleri* habitats, with elevated water temperature facilitating the proliferation of free-living amoebae.[3,4] Reported cases increased from 381 (1965–2018) to 431 in 2019, corresponding to a 13% rise between 2018 and 2019.[2,3] Second, contaminated water is typically brackish to saline in Pakistan, raising concerns about saline environments as a source of infection beyond freshwater. Studies suggest that *N fowleri* has moderate tolerance to salinity levels of approximately 1·5% for short periods.[5,6] These findings warn of the potential short-term survival of *N fowleri* in brackish water, which could, therefore, pose a transient exposure risk. Last, low diagnostic capabilities and insufficient clinical awareness result in the delay of effective treatment regimens, contributing to the high mortality rate.[7]

Advances in molecular diagnostic tools have substantially increased the detection and reporting of *N fowleri* infections, although diagnosis is often only performed post-mortem.[4] Consequently, estimates of prevalence and epidemiological trends remain uncertain. Moreover, integrated analyses that link genotypic diversity, evolutionary dynamics, and global epidemiological trends remain scarce. With the increasing incidence of PAM cases, its expanding geographical distribution, and the uneven distribution of clinically relevant genotypes, a clear synthesis of current evidence is needed. In this Review, we provide an updated overview of the global distribution of clinically relevant *N fowleri* genotypes, examine evolutionary relationships within the *Naegleria* genus, and integrate current epidemiological findings to better characterise emerging patterns of transmission and risk. The goal is to advance understanding of shifting epidemiological patterns, support risk assessments, and inform future research and public health strategies aimed at mitigating the impact of this increasingly recognised, free-living waterborne pathogen.

Complexity and diversity of *N fowleri* genotypes

N fowleri is a unicellular protozoan eukaryote classified within the *Heterolobosea* lineage and the *Excavata* supergroup.[8] The *N fowleri* genome comprises approximately 29 Mbp of nuclear chromosomal DNA, a 50 Kbp mitochondrial genome, and 16·5 Kbp of enclosed circular extrachromosomal ribosomal DNA elements (CEREs).[8] A single *N fowleri* cell is estimated to contain around 4000 copies of CEREs, contributing to approximately 47–56% of the total DNA mass.[8] Each CERE encodes a single copy of the 18S, 5.8S, and 28S rRNA genes (figure 1A).

N fowleri is classified into different genotypes, defined by variations in the number of repeat short sequences within the internal transcribed spacer 1 (ITS1) region and a single nucleotide polymorphism (31C→T) at position 31 of the 5.8S rRNA gene.[10] Zhou and colleagues[11] proposed a genotyping scheme with three short repeat sequences in the ITS1 region and classified *N fowleri* into six genotypes.

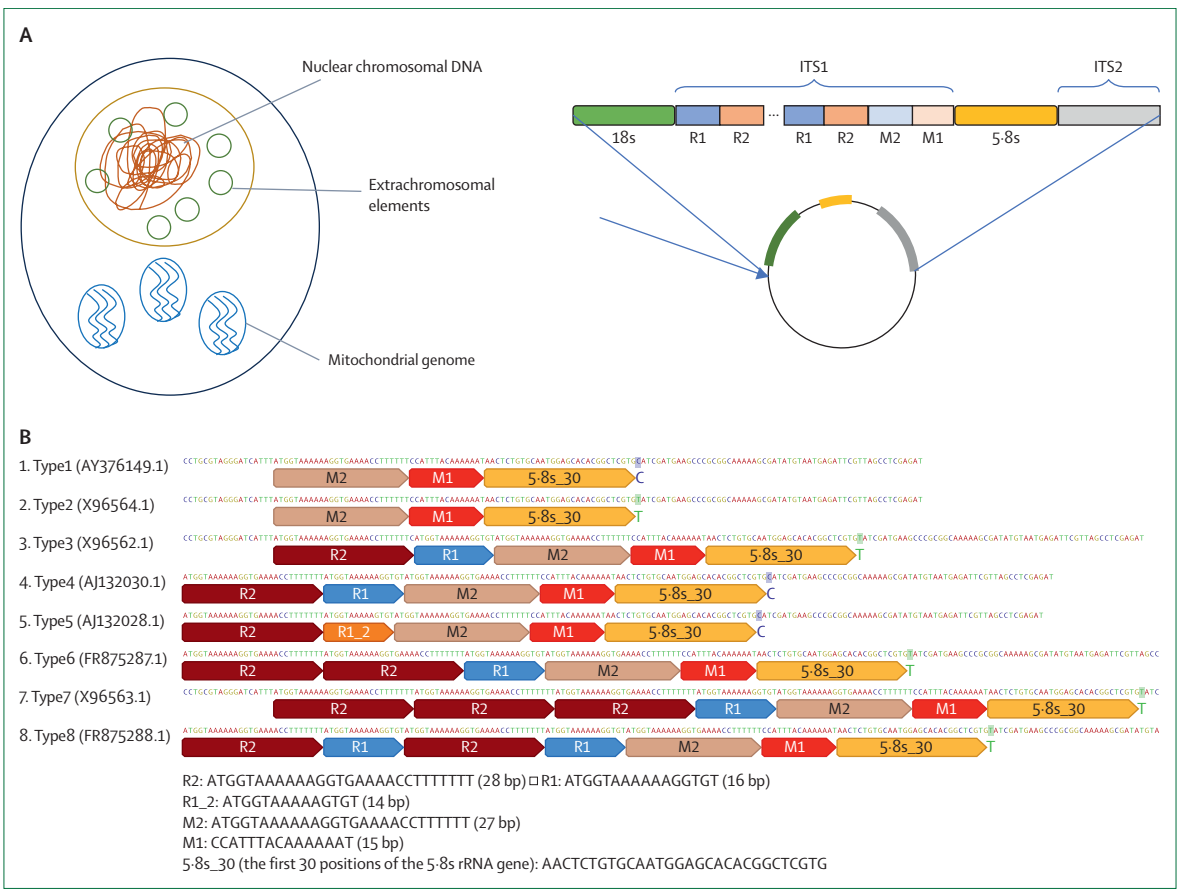


Figure 1: **Traditional ITS-based genotyping system of *Naegleria fowleri***
(A) Diagram illustrating the genome content of *Naegleria* species, including the closed circular extrachromosomal rDNA element. (B) Genetic variations among the eight *N fowleri* genotypes,[9] highlighting sequence differences in the ITS1 region and repeat motifs. ITS=internal transcribed spacer.

With the availability of additional sequence data, De Jonckheere[9] classified *N fowleri* into eight genotypes, which were used in this Review. Specifically, within the ITS1 region, the sequence motifs M1 and M2 are consistently present across all genotypes, whereas the repeat motifs R1 and R2 vary in copy number among genotypes (figure 1B). The motif patterns within the ITS1 region of *N fowleri* are distinct from those found in other *Naegleria* species, providing a key molecular signature for species-level discrimination (appendix p 2).[9] A cross-referencing comparison of the genotyping schemes is shown in the appendix (p 3).[9,11]

See Online for appendix

The eight genotypes present uneven global distribution.[9] Genotypes 1, 2, and 3 are reported in the Americas, whereas all genotypes, except Genotype 1, are present in Europe. In Asia, Genotypes 2 and 3 are predominant, whereas Oceania (Australia, Papua New Guinea, and New Zealand) has only reported Genotype 5.[9] Despite all genotypes being present in the environment, only Genotypes 1, 2, 3, and 5 appear to be clinically relevant.[12]

According to CERE sequence genetic content, 73 publicly available CERE sequences were successfully assigned to specific genotypes. Seven sequences could not be assigned a genotype, with 13 remaining unclassifiable (appendix pp 6–15), possibly owing to short sequence lengths or incorrect taxonomic annotations. Furthermore, the possibility of undiscovered genotypes cannot be excluded. Of the eight genotypes originating from 18 countries across five continents, ITS sequences reported worldwide include 22 from Asia, 41 from Europe, 21 from North America, six from Oceania, and two from South America (figure 2A), suggesting an expansion in genotype distribution range, with Genotype 5 now reported in Asia and Genotype 8 identified in the Americas. Oceania continues to be dominated by Genotype 5, whereas Europe harbours all genotypes except Genotype 1, aligning with findings of an earlier report (figure 2B).[9] Among the 92 sequences, 36 were derived from cerebrospinal fluid samples of infected individuals, all belonging to Genotypes 1, 2, 3, and 5, which have shown clinical relevance. Notably, a single sequence classified as Genotype 8 was reported from a cow's brain in Brazil.[13] The remaining 52 sequences originated from diverse environmental sources, including recreational baths, water reservoirs, treated drinking water, dam water, pool water, lake water, irrigation channels, nuclear power facilities, hot springs, and cooling water systems (appendix pp 6–15).

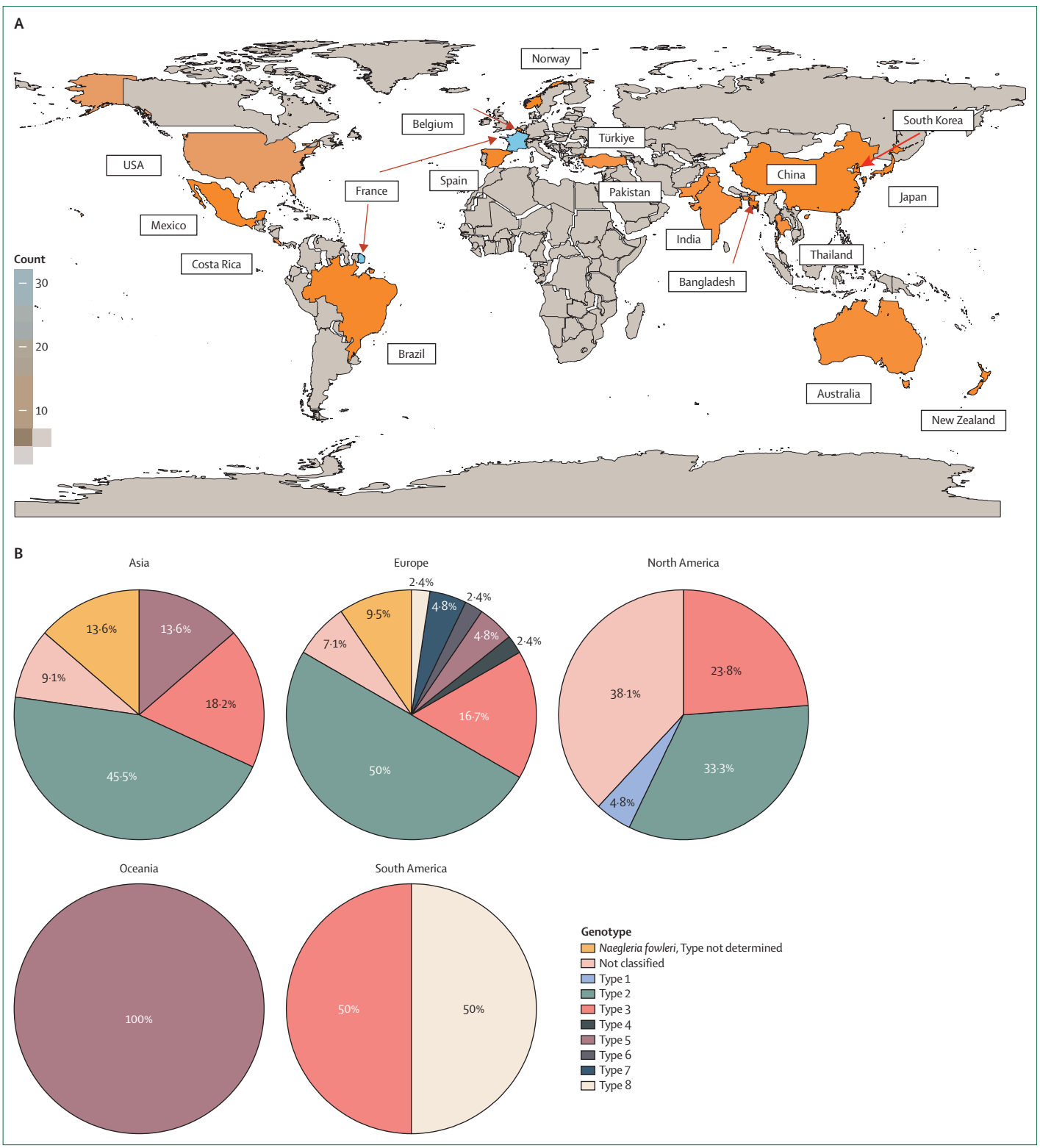


Figure 2: **Global distribution of *Naegleria fowleri* genotypes**
(A) The map shows the number of *N fowleri* ITS sequences reported worldwide, with colour intensity indicating prevalence. (B) The pie charts show the relative distribution of *N fowleri* genotypes across different continents or regions. ITS=internal transcribed spacer.

The reporting of Genotype 2 *N fowleri* in China and Genotypes 2 and 3 in Pakistan have been confirmed.[14,15] A study investigating four clinical *N fowleri* isolates from Thailand reported that three genotypes (2, 3, and 4) were associated with local PAM cases.[12] Notably, Genotype 4 had not been previously documented in clinical cases, raising possible misclassification. Further examination suggests that the Genotype 4 classification appears to be erroneous, because the sequence contained an 84-bp *ITS1* gene fragment characterised by two deletions in the R1 motif and a cytosine at position 31 of the 5.8S rRNA gene (appendix p 4). The sequence features are consistent with those seen in Genotype 5, supporting the reclassification of this Thailand case (MZ224444). Additionally, a sequence (MW033524) from an American individual with a thymine (T) at position 31 in the 5.8S rRNA gene was incorrectly classified as Genotype 1 by Harris and colleagues.[16] Based on the sequence characteristics, the correct classification should be Genotype 2 (figure 1B).

Evolution of *Naegleria* species

The direct origin of *N fowleri* is yet to be elucidated. A phylogenetic analysis of available *Naegleria* partial ITS1, 5.8S, and ITS2 rRNA sequences is shown in the appendix (p 5). The phylogenetic tree reveals that the thermophilic species *N fowleri* and *Naegleria lovaniensis* cluster together, supporting the hypothesis that *N fowleri* could have evolved from *N lovaniensis*.[8,9] We observed a monophyletic clade of *N fowleri* with Genotype 1 at its root, providing additional evidence for the hypothesis that Genotype 1 represents the earliest pathogenic lineage to have evolved from *N lovaniensis*.[9] Genotype 2, identified as an early variant, differs from Genotype 1 by a single base transition in the 5.8S rRNA, pointing to a sequential evolutionary pathway (figure 1B). All other genotypes are considered to have evolved sequentially from these two earlier genotypes.[9] Notably, Genotype 8 has longer phylogenetic branch lengths and has been found only in animal brain tissue, suggesting greater genetic divergence and the need for further evaluation of animal brain tissues for other possible genotypes.[9] Given no known human-to-human transmission, the primary host(s) driving the evolution of pathogenic *N fowleri* remain unknown.

Furthermore, the two thermophilic species (*N fowleri* and *N lovaniensis*) form a distinct evolutionary branch sister to the psychrotolerant *Naegleria* species from the Arctic and Antarctic regions (*Naegleria antarctica*, *Naegleria neopolaris*, *Naegleria neoantarctica*, *Naegleria polaris*, and *Naegleria arctica*), separate from other *Naegleria* species.[17] This divergence highlights the evolutionary adaptation of *N fowleri*, *N lovaniensis*, and their relatives to various extreme environments. Although eukaryotes were traditionally not considered extremophiles, a 2023 study identified phylogenetically diverse protists capable of surviving in extreme conditions.[18] *Naegleria* species have remarkable adaptability, potentially occupying even more extreme environments. However, because of the physical inaccessibility of many extreme habitats and limitations in available study materials, crucial knowledge gaps regarding their broad adaptation to multiple extreme conditions remain.

Epidemiology of PAM

543 PAM cases were identified in the literature across 39 countries (figure 3; appendix pp 16–17). The highest number of PAM cases have been reported from the USA, Pakistan, Mexico, India, and Australia.

The PAM cases in the Americas amount to 220 (41%) of the 543 global total reported. Of the 543 cases, 203 (37%) were reported in North America, 164 (30%) in the USA, 33 (6%) in Mexico, four (<1%) in Costa Rica, and two (<1%) in Cuba. In South America, 17 cases (3% of 543) were documented, with seven (<1%) in Venezuela (1%), followed by five (<1%) in Brazil, three (<1%) in Colombia (<1%), and two (<1%) in Peru. 236 (43%) of the 543 PAM cases were reported in Asia, making Asia the region with the highest number of reported PAM cases. Pakistan accounted for 159 (29%) of the 543 cases, followed by 32 (6%) in India, 17 (3%) in Thailand, and 16 (3%) in China. Additionally, Nepal reported four cases (<1%), and Iran, Viet Nam, and Japan each reported two cases (<1%). Bangladesh and South Korea each reported one case (<1%). Oceania accounted for 37 (7%) of the 543 cases, with Australia reporting 22 (4%), New Zealand nine (2%), and Papua New Guinea six (1%) cases. Europe accounted for 34 (6%) of the 543 cases, distributed across eight countries: Belgium, Czech Republic, France, Italy, Norway, Spain, Türkiye, and the UK. Meanwhile, Africa reported 16 (3%) of the 543 cases from nine countries.

Historically, most PAM cases were reported from Western countries; yet, Asia has emerged as a hotspot of late, possibly reflecting both increased pathogen prevalence and improved awareness. Pakistan has seen several *N fowleri* outbreaks nearly every year since the first reported case in 2008, presenting serious public health challenges.[19] Of note, within just one decade, Pakistan has emerged as the second most affected country worldwide, reporting nearly as many PAM cases as the USA has documented over the past 60 years.[20] South Korea reported its first PAM case in late 2022.[21] Epidemiologists speculate, on the basis of the individual's travel history, that the pathogen might have been introduced from Thailand. Surveillance of tap water in South Korea has revealed that *N fowleri* is present in 11% of the tested samples.[22] Kerala, India has reported a surge of free-living amoebic meningoencephalitis with no clear exposure and expanding regions since 2024.[23,24] This free-living amoebic meningoencephalitis outbreak is mainly caused by *N fowleri*, *Acanthamoeba* spp, and *Vermamoeba vermiformis* (incorrectly named *Vermamoeba vermicularis*).[24] Free-living amoebic meningoencephalitis has a lower mortality rate (approximately 20%) in Kerala, which underscores that early recognition, strengthened diagnostics, and prompt anti-protozoan therapy can improve outcomes.[25] However, of note, most cases were caused by

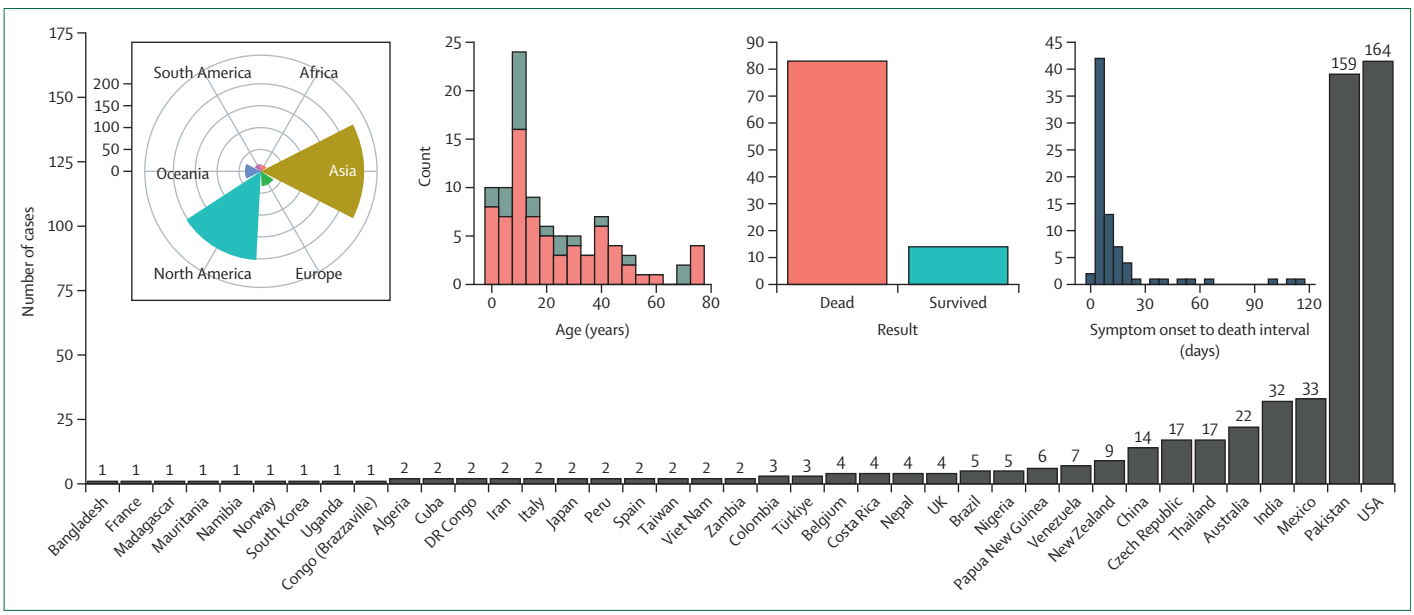


Figure 3: **Global distribution of PAM cases by continent, age, symptom onset to death interval, outcome, and country**
A horizontal bar plot of reported case counts by country, with case numbers labelled above each bar, is given in the bottom panel. The distribution of cases by continent is given in the top-left polar plot, wherein bar length represents the case count. A stacked bar plot illustrating age distribution by sex (red: female; blue: male) and a histogram depicting the symptom onset to death interval are given in the top-centre panels, with days on the x-axis and case count on the y-axis. The top-right bar plot displays case outcomes, with red indicating fatal cases and teal representing survivors. PAM=primary amoebic meningoencephalitis.

less virulent and more treatable amoebae, such as *Acanthamoeba* spp.[26] None the less, successful treatment of the PAM cases was still low, with only two survivors.[27,28] Public health actions, such as aggressive water treatment, enhanced diagnostic capacity, and improved awareness, were implemented in Kerala.[24,27] The lessons learnt from the India experience call for increased caution regarding the increasing risk of the threat these emergent free-living amoebae pose. Moreover, tropical nations such as Malaysia and the Philippines have not yet reported any PAM cases, despite studies showing a considerable presence of *Naegleria* spp in up to 70% of collected water samples.[29,30] These findings have prompted Malaysian health authorities to issue public advisories encouraging caution in recreational water activities.[30]

The sensitivity of diagnostic tools and clinical awareness among health-care personnel have facilitated the analysis of the PAM-related disease burden. However, the precise global prevalence of PAM remains uncertain because of potential misdiagnosis and possible under-reporting, especially in low-income countries.[19] For example, Africa with its tropical climate, documented 47 deaths per 100 000 population, caused by exposure to unsafe water.[31] To date, only 16 cases have been recorded, although high temperatures and low chlorination levels might have favoured *N fowleri* propagation.[32] Africa not only faces a high burden of various diseases but also has scarce resources, which result in under-reporting and misdiagnosis of PAM cases.[32]

Additionally, cases among animals have been reported. The first documented animal case involved a 6-year-old American tapir living in a zoo.[33] Brazil has reported two PAM cases in cows, and Algeria has documented cases in a pregnant cow and an ewe.[13,34] Seasonal outbreaks were also reported at a cattle ranch in San Bernardino, California, USA, where Genotype 2 was subsequently confirmed.[35] A case of a 20-year-old rhinoceros has been reported from central Texas, USA.[36] In addition, Genotype 8 was detected in a cow from Brazil,[13] and the genotypes associated with other reported animal cases remain under investigation.

We selected 98 (18%) of the 543 PAM cases described in literature for incidence analyses by group; the remaining cases were excluded because of insufficient information. Among the cases, 24 involved women and 74 involved men (figure 3). Consistent with findings of previous studies, the proportion of men (76%) exceeded that of women (24%).[2] The cases spanned a wide age range, from 11 days to 75 years (median age 13·5 years). Cases reported in Pakistan predominantly affected adults aged 26–45 years, in contrast to that in the USA, where infections mainly occurred in teenagers.[14] This discrepancy indicated potential cryptic *N fowleri* strain(s), which require further characterisation.[20] Furthermore, ten cases involved infants younger than two years, including two newborns, among whom only one survived. Six of the infants were from rural areas with documented exposure to a private well or stagnant water, suggesting the risk associated with the use of untreated water for infant bathing.

Since the first reported PAM cases, which were in South Australia in 1965, timely and effective countermeasures continue to be a want.[37] The main factor that continues to undermine the therapeutic success of PAM is delayed

diagnosis. The early symptoms of PAM mimic bacterial meningitis, and the trophozoites of *N fowleri* are morphologically similar to macrophages, making microscopic identification challenging.[38] Despite multiple cerebrospinal fluid examinations, the amoebae frequently go undetected because of poor clinical expertise and a low index of suspicion.[38] Moreover, the disease appears to be indolent, with waxing and waning symptoms and an absence of classic meningeal signs.[39] Additionally, there is currently no consistently effective treatment for PAM. The most common treatment is intravenous amphotericin B, oral rifampicin, and intravenous fluconazole. Although the antifungal drug amphotericin B is the drug of choice for treating PAM, its success rate (albeit possibly reduced owing to late diagnosis) remains very low at approximately 2·7%. Additionally, the likelihood of successful treatment is not associated with patient age, rather with early intervention.[7] Although more men have been successfully treated than women, this occurrence could simply be attributed to the higher number of infections among men. To our knowledge, 17 survivors have been recorded worldwide (table).

Source of infections

PAM cases are often associated with accidental or forced introduction of the amoeba into the nasal cavity. Sources of infection include exposure to various artificial or natural freshwater environments such as surf venues, splash pads, rivers, ponds, hot springs, tap water, irrigation canals, private wells, and stagnant water bodies. Common activities associated with infection include swimming, rafting, bathing, wakeboarding, surfing, and nasal irrigation. Although most PAM cases have been linked to freshwater recreational activities, an increasing number of infections have been associated with forceful nasal entry of municipal drinking water.[57] For example, *N fowleri* has been detected in 10% of groundwater supply wells in Arizona, USA and 27% in potable water systems in Louisiana, USA.[58] This finding highlights the importance of surveillance of *N fowleri* in water systems to ensure the safe management of drinking and recreational water sources. Maintaining a chlorine residual and periodic monitoring is particularly valuable for water supplies that seasonally exceed a temperature of 30°C or consistently remain at a temperature of more than 25°C.[59] Additionally, several cases have been associated with religious practices, specifically during ritual ablutions that involve drawing inadequately chlorinated water into the nostrils.[39] As *N fowleri* is ubiquitous in soil, humans could inhale amoebal cysts. The first documented case of dust-related infection dates to 1977 in Nigeria, where an 8-month-old baby girl, with no history of contact with water, got infected. *N fowleri* was cultured from the child's nose, supporting the dust-harboured infection.[60] Another instance occurred in Türkiye; a 68-year-old woman with no contact with water was diagnosed with PAM. The amoeba was detected in the soil used for planting orchids, further underscoring the potential risk from soil contact.[61]

Most confirmed *N fowleri* infections are associated with freshwater exposure because the amoeba is not typically expected to survive in salty water, but it can persist (presumably as cysts) at salinities of approximately 1·6% for short durations.[6] However, an exceptional case in China was associated with exposure to seawater, possibly via the more resistant cyst form.[62] Additionally, in Karachi, where surface waters are predominantly brackish to saline, *N fowleri* continues to pose a substantial PAM health problem. These observations suggest the potential of saline environments as the source of *N fowleri* infection or that more diverse genotypes have evolved that are not yet completely understood, or both.[20]

Practical monitoring and regulation strategies

N fowleri is listed as a high-risk pathogenic microorganism in both drinking water and recreational water, as suggested by WHO, the Australian Government, and the US Environmental Protection Agency.[59,63,64] Although routine monitoring of *N fowleri* is not conducted by most water utilities,[57] periodic prospective studies are recommended for any water supply with temperatures exceeding 25°C continuously or 30°C seasonally.[59,64] Despite these recommendations, most water utilities pay little attention to *N fowleri*, largely because of the scarcity of validated detection methods and the shortage of trained personnel. To translate these risk-based recommendations into routine practice, appropriate and accessible detection methods are essential. Molecular diagnostic methods using quantitative PCR are commonly used to detect *N fowleri* at present. These methods typically target regions within the ribosomal operon, including the 18S rRNA gene and ITS-5.8S rRNA regions or the *Mp2Cl5* gene, which are considered specific to *N fowleri*.[65,66] The membrane protein *Mp2Cl5* gene is present as a single-copy gene per cell, yielding an assay with high specificity but low sensitivity (limit of detection being five intact *N fowleri* amoebae). The 18S rRNA assay, with multiple copies per cell, shows higher performance, with detection limits ranging from 0·8 to 0·05 amoebae, but its sensitivity varies significantly across *N fowleri* genotypes.[66] Although the 18S rRNA qPCR assay has high accuracy and specificity,[66] our group found that the primers (NaeglF192-NaeglR344) can also amplify *N lovaniensis* and yield false-positive results (Y Wang, unpublished data). Upon this finding and further literature review (aside from a recent study[67] in January, 2025), this study is one of the first instances in which these specific 18S rRNA gene primers (NaeglF192-NaeglR344) have been shown to detect several closely related *Naegleria* species (*N lovaniensis, Naegleria jamiesoni,* and *Naegleria australiensis*). Therefore, to accurately detect pathogens, qPCR results should be interpreted with caution, and these assays are recommended to be complemented with Sanger sequencing or an equivalent. Given the rise of public health concerns and outbreaks in countries such as Pakistan and India, coordinated efforts between government agencies and industry stakeholders are urgently needed to consider

	Year (month)	Age; sex	Infection source	Diagnosis	Clinical treatment	Reference
Punjab, India	NA	73 years; male	Unknown	Cerebrospinal fluid wet mount revealed trophozoites	Amphotericin B (1 mg/kg per day); rifampicin (600 mg once daily)	40
Bristol, UK	1969	6 years; male	Muddy puddle	Amoeba isolate from the cerebrospinal fluid	Amphotericin (0·25–0·75 mg/kg per day)	41
Queensland, Australia	1971 (5)	14 years; male	Unknown	Unknown	Amphotericin B (dose not reported)	42
California, USA	1978 (6)	9 years; male	Swimming in hot springs	Cerebrospinal fluid wet mount revealed amoebae	Amphotericin B (1·5 mg/kg per day); rifampicin (10 mg/kg per day); sulfisoxazole (4 g/day); miconazole (350 mg/m^2 per day)	43
Pennsylvania, USA	1990 (8)	32 years; male	Waterskiing in a small, stagnant, freshwater lake	Cerebrospinal fluid wet mount revealed amoebae	Amphotericin B (75 mg/day); rifampicin (600 mg/12 h)	44
Chandigarh, India	2002	26 years; female	Air	Cerebrospinal fluid wet mount revealed amoebae	Rifampicin (450 mg/day); amphotericin B (1 mg/kg per day); ornidazole (1500 mg/day)	45
Sonora, Mexico	2003 (4)	10 years; male	Swimming in an irrigation canal	Cerebrospinal fluid wet mount revealed amoebae	Amphotericin B (0·25 mg/kg per day); dexamethasone (0·6 mg/kg per day); ceftriaxone (100 mg/kg per day); rifampicin (10 mg/kg per day); fluconazole (10 mg/kg per day)	46
Qom, Iran	2011	5 months; male	No record about contact with water source	*Naegleria fowleri* was observed in cerebrospinal fluid wet mount	Rifampicin (10 mg/kg); amphotericin B (1 mg/kg per day)	47
Uttar Pradesh, India	2013	25 days; male	Bath water from nearby shallow well	Cerebrospinal fluid wet mount revealed amoebae	Amphotericin B; rifampicin; fluconazole (dose not reported)	48
Arkansas, USA	2013 (7)	12 years; female	Swimming in a lake-based water park	The Wright-Giemsa staining of the cerebrospinal fluid revealed amoebae consistent with *N fowleri*	Amphotericin B (1·5 mg/kg per day); fluconazole (10 mg/kg per day); rifampicin (10 mg/kg per day); azithromycin (10 mg/kg per day)	49
Texas, USA	2013 (8)	8 years; male	Surface water	Wright staining was performed, revealing amoebic trophozoites	Amphotericin B (1 mg/kg per day); rifampicin (12 mg/kg per day); fluconazole (12 mg/kg per day loading dose, then 9 mg/kg per day); azithromycin (10 mg/kg per day); miltefosine (150 mg/day)	50
Himachal Pradesh, India	2014	6 years; female	Cement tank, natural freshwater	Cerebrospinal fluid wet mount revealed amoebae	Amphotericin B (1 mg/kg per day); fluconazole (8 mg/kg per day); rifampicin (10 mg/kg per day)	51
Karachi, Pakistan	2015	25 years; male	Unknown	Unknown	Amphotericin B (1·5 mg/kg per 12 h); azithromycin (10 mg/kg per day); fluconazole (800 mg/day); rifampicin (600 mg/day); chlorpromazine (2 g/day); miltefosine (150 mg/day)	52
Tamil Nadu, India	2019	47 years; male	Bathing in a river	Flagellated trophozoites shown from the cerebrospinal fluid sample	Amphotericin B 150 mg; azithromycin (dose not reported)	53
Cartago, Costa Rica	2020	5 years; female	Hot spring	No trophozoites in cerebrospinal fluid, confirmed with qPCR	Amphotericin B (dose not reported)	54
Lima, Peru	2020	8 years; male	Swimming in a public pool	Cerebrospinal fluid wet mount showed forms suggestive of trophozoites	Amphotericin B deoxycholate (36 mg/day); voriconazole (220 mg/12 h); miltefosine (25 mg/8 h); rifampicin (360 mg/day)	55
Karachi, Pakistan	2023 (6)	22 years; male	Unknown	Cerebrospinal fluid wet mount showed trophozoite forms of amoebae	Miltefosine (50 mg/6 h); amphotericin B (75 mg immediately, then 50 mg/24 h); rifampicin (400 mg/12 h); fluconazole (400 mg/12 h); azithromycin (500 mg/24 h); sodium valproate (500 mg/8 h) 20% mannitol (200 mL/8 h)	56

NA=not available. PAM=primary amoebic meningoencephalitis. qPCR=quantitative PCR.

Table: **Surviving cases of PAM**

implementing monitoring of *N fowleri* in drinking water systems. At the same time, standardised, precise, and easy-to-implement detection methods need to be developed to support routine monitoring for delivery of safe drinking water.

Australia is the only country that has imposed a *N fowleri* threshold in drinking water distribution systems (DWDSs), recommending a density of two cells per litre (or detection in a 500-mL sample) triggering remedial action. The presence of any *Naegleria* species should prompt immediate intervention without waiting for species-level identification. Any thermophilic *Naegleria* species detected in drinking water serves as a proxy indicator for *N fowleri,* suggesting the failure of preventive measures and barriers.[59] For recreational water, *N fowleri* is listed as a contaminant, but no detection threshold has been assigned. The previous infection site at the geothermal bathing area in Guadeloupe had posted a sign indicating that the level of 100 cells/L was considered safe to swim and was permitted by law.[68] However, after a reported case of PAM in the region, a researcher regarded that labelling this concentration as safe was criminal and potentially negligent.[68]

Current response strategies are largely informed by practices in Australia. Of note, an undetectable disinfectant residual and warm water temperature favour *N fowleri* colonisation.[69] Thus, the recommended protocol for *N fowleri* management is to maintain a free chlorine or chloramine residual concentration of more than 0·5 mg/L throughout all points of the DWDS. If thermophilic *Naegleria* species

are detected, an immediate increase to at least 1 mg/L is required until any identified source of *Naegleria* or reasons for the positive result is identified or managed.[69] However, reduced effectiveness of chlorine disinfection against *N fowleri* associated with biofilms has been reported, as biofilms can protect amoebal cells and exert chlorine demand.[70] Biofilms are best controlled by a chlorine residual of more than 1 mg/L to inactivate any trophozoite or flagellate forms released from *N fowleri* cysts. Other disinfection approaches, including combined chlorination and UV treatment,[71] salinity,[72] and chlorine dioxide gas[72] have also shown effectiveness in managing *N fowleri*. However, these methods have been evaluated primarily under controlled laboratory conditions, with low validation under real-world field conditions. Consequently, their operational performance, robustness, and practical applicability remain uncertain and require further research. Additionally, the public health sector has suggested that maintaining a free chlorine level of 2 mg/L in all storage tanks for at least 60 days could be necessary to suppress the growth of *N fowleri*.[73] As for the public aquatic facilities, the Australian Government recommends that free chlorine should be maintained at a level of more than 1 mg/L, with a common target of 2 mg/L.[74] No standardised protocol exists as of now for managing *N fowleri* in open recreational water sources such as lakes or rivers. However, the use of a nose clip is recommended, especially during water activities in inadequately disinfected or untreated water environments.[68]

Conclusion and outlook

Cases of *N fowleri* infection are possibly substantially underestimated, with a growing body of literature indicating increasing prevalence and potential climate-driven expansion, contributing to its recognition as an emerging public health concern.[6] Given its high mortality and expanding geographical distribution, *N fowleri* remains an important public health concern. Climate warming could further influence the ecology and infection dynamics of *N fowleri*. Enhanced research, surveillance, proactive management, and public and clinical awareness are warranted.

Genotyping plays a crucial role in epidemiological investigations, aiding in tracking pathogen evolution infection, or contaminant source.[9,16] ITS-based genotyping has been used to establish epidemiological links between clinical and environmental samples, whereas its discriminatory power is low. Isolates assigned to the same genotype do not consistently form a monophyletic clade (appendix p 5), thus reducing the reliability of linking infection sources across distinct exposures. In addition, genotypic variation could influence phenotypic traits such as growth and treatment response, highlighting the need for higher-resolution molecular tools.[14,75]

The complexity of eukaryotic genomes presents an important challenge to elucidating the molecular mechanisms underlying pathogenesis.[76] Nevertheless, such understanding is urgently needed to advance diagnostic and therapeutic strategies, particularly given that four (Genotypes 1, 2, 3, and 5) of eight *N fowleri* genotypes are clinically relevant. Accordingly, whole-genome DNA sequencing can provide crucial insights into cellular biology, the potential roles of endosymbionts, and the overall genetic architecture of *N fowleri*. However, generating genome-wide data at scale remains challenging because of the large and complex nature of *Naegleria* genomes, compounded by the difficulty of isolating this species from environmental samples, where it typically occurs at low concentrations. As *N fowleri* is the only known pathogenic species within the *Naegleria* genus, the reason why it causes fatal disease and its evolution remain incompletely characterised. Going forward, a comparative assessment of genomic content across diverse *Naegleria* species would be worthwhile to explore the pathogenicity of *N fowleri* and offer new opportunities for developing effective therapeutics.

DWDSs are not completely sealed and could have dust intrusion into storage reservoirs, in addition to ingress of contaminated water during main breaks or repairs and pressure fluctuations.[69] Once introduced, *N fowleri* can proliferate within warm drinking water biofilms, wherein trophozoites, flagellates, and cysts are protected from low chlorine residuals.[77] Importantly, free-living amoebae could serve as a host for a wide range of bacterial pathogens, including *Legionella pneumophila*, one of the most important water-related opportunistic pathogens globally. Therefore, the frequent co-occurrence of free-living amoebae and bacterial pathogens within engineered and natural water systems represents a crucial ecological nexus that could facilitate pathogen persistence, amplification, stress tolerance, and potentially enhance human transmission.[78] Many bacteria are capable of adapting to resist amoebal digestion, surviving, and replicating intracellularly within vacuoles, thereby gaining protection from environmental stresses, ultimately increasing public health risks.[79] Furthermore, interactions between free-living amoebae, including *N fowleri*, and drinking water biofilms can reduce the effectiveness of chlorine disinfection within DWDS biofilms.[70] Evidence also indicates that free-living amoebae could enhance pathogen virulence and facilitate the spread of antibiotic resistance genes.[80] Consequently, further investigation of the ecological interactions between *N fowleri*, biofilms, and internalised bacteria is warranted, together with the development and evaluation of more effective and ecologically informed disinfection and control strategies.

The poor understanding of the environmental ecology of *N fowleri* continues to constrain effective risk management and control strategies. Therefore, an improved understanding of the biotic and abiotic drivers influencing *N fowleri* genotype distribution, abundance, survival, and niche occupancy is crucial. These drivers include factors such as temperature; nutrient, food or prey availability; disinfectant residuals; biofilm interactions; hydrodynamic conditions; and general microbial community competition.

Search strategy and selection criteria

To inform this Review, we searched PubMed, Embase, and Web of Science to explore the global burden of primary amoebic meningoencephalitis from Jan 1, 1965, until August 31, 2025, using the following terms: (("primary amoebic meningoencephalitis" OR "primary amoebic meningoencephalitis" OR PAM) AND ("Naegleria fowleri" OR "N. fowleri") AND (case OR report OR outbreak)). No language restrictions were applied. Articles without detailed phylogenetic analyses were excluded from the analysis of the global distribution of cases by continent, age, symptom onset to death interval, outcome, and country. The internal transcribed spacer sequences in the National Center for Biotechnology Information were derived by searching ("Naegleria fowleri"[Organism] AND "internal transcribed spacer 1") in the nucleotide database.

Notably, pathogenic free-living amoebae are accidental human pathogens and are not known to be transmitted from person to person; however, they have virulence factors that might enhance their ability to prey on bacteria and possibly evade predation by other free-living amoebae[6] (such as the known predators *Balamuthia mandrillaris* and *Willaertia magna*) and amoebophagous fungi.[81] Although awareness regarding the role of extracellular vesicles and cytopathogenicity of *N fowleri* associated with human infections is growing,[82] the potential role of endosymbionts (and by genotypes) is unknown. In other free-living amoebae, however, endosymbionts are known to contribute to virulence factor expression and prey digestion modulation.[83,84]

Enhancing awareness and education regarding *N fowleri* among health-care professionals, water authorities, and the general public is equally important.[20] Improved awareness could help to reduce exposure risks, increase clinical suspicion, and facilitate earlier diagnosis and intervention in infected individuals. In parallel, the development of dedicated molecular surveillance tools capable of affordable, sensitive detection and high-resolution genotyping of *N fowleri* would substantially strengthen environmental and public health surveillance capacity. Such tools could improve risk assessment, accelerate clinical diagnosis, and support source tracking and the identification of transmission pathways and epidemiological linkages.

Together, these efforts offer the hope of reducing the global health burden of *N fowleri* infection and ultimately, transform this overwhelmingly fatal disease into a preventable and more effectively managed public health threat.

Contributors

YW, JG, and SH developed the initial outline of the manuscript. YW conducted the literature review and drafted the manuscript. JG and NJA provided critical feedback. LS reviewed the manuscript and provided comments. All authors contributed to the revision and fine-tuning of the manuscript and approved the final version.

Declaration of interests

We declare no competing interests.

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