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ADVANCEMENTS IN *NAEGLERIA* DETECTION: A COMPREHENSIVE REVIEW ON DIAGNOSIS AND PREVENTIVE STRATEGIES

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Abstract

Primary amoebic meningoencephalitis (PAM) is a rare but highly fatal central nervous system infection caused by the free-living amoeba Naegleria fowleri, commonly found in warm freshwater environments. This review aims to explore recent advancements in diagnostic approaches and preventive strategies associated with N. fowleri infection. The organism typically enters the human body through the nasal cavity during water-related activities, rapidly migrating to the brain via the olfactory nerves, leading to severe inflammation, cerebral edema, and death within a few days if untreated. Clinical presentation often mimics bacterial meningitis, including symptoms such as fever, headache, neck stiffness, and altered mental status, which frequently result in delayed or misdiagnosis.

Diagnostic methods include direct microscopic visualization of trophozoites in cerebrospinal fluid (CSF), culture techniques, immunohistochemistry, and advanced molecular approaches such as polymerase chain reaction (PCR) and next-generation sequencing (NGS). Among these, molecular diagnostics have shown higher sensitivity and specificity, enabling earlier and more accurate detection. However, limited availability of advanced diagnostic facilities in resource-constrained settings remains a significant challenge.

Preventive strategies primarily focus on minimizing exposure to contaminated water, maintaining proper chlorination in recreational water bodies, and promoting public awareness regarding safe water practices. Emerging interventions, including vaccine research and enhanced environmental monitoring, offer promising future directions. Overall, early diagnosis, improved surveillance, and strengthened public health interventions are critical to reducing the mortality associated with PAM. This review highlights the urgent need for integrating advanced diagnostic tools with effective preventive measures to improve patient outcomes and control the spread of this deadly infection.

INTRODUCTION

The central nervous system illness known as amoebic encephalitis is very rare but frequently fatal. There are two forms of amoebic encephalitis that can affect people: granulomatous amoebic encephalitis and primary amoebic meningoencephalitis (PAM). Free-living amoebae (FLA), which are naturally occurring microorganisms in freshwater environments like lakes and rivers, are the cause of amoebic encephalitis. Nevertheless, FLA can continue to exist without a particular host. Human disease transmission is attributed to four main genera of amoebae: *Naegleria*, *Acanthamoeba*, *Sappinia*, and *Balamuthia*.(1)

Worldwide, freshwater lakes, hot springs, inadequately chlorinated pools, and thermally contaminated water bodies are the primary habitats for *Naegleria fowleri*. Seawater has not been discovered to contain it. The first place

this organism was discovered was Australia; other reports of invasive human infections have come from New Zealand, Europe, Africa, Asia, and Latin America. It has largely been found in the southern states of the United States.It has also been isolated from thermally contaminated waters in the northern states, such as Minnesota and Connecticut, in more recent times. from 2008 and 2017, 34 instances were reported in the US, while from 1962 and 2017, the Centers for Disease Control (2) received reports of 143 illnesses.The majority of *N. fowleri* infections have resulted from swimming or diving in freshwater during leisure time.In Arizona, two kids contracted the infection while taking a bath at home. The organism was identified in a community well-water system that was not treated. It was believed that inhaling *Naegleria* cysts was the cause of another instance in Nigeria.(3)

Characteristics and Distribution

Protists of the class Heterolobosea, family Vahlkampfiidae, include *Naegleria* spp. Similar to every other protist in this class, it is a free-living organism that mostly consumes bacteria. Since *Naegleria* species are amoeboflagellates, they have the ability to change from amoebas to flagellates. For protection against harsh environments, the amoeba can potentially change into a cyst. Although *Naegleria* species have been recognized for more than a century, it wasn't until roughly 40 years ago that it was determined that one species is the cause of primary amoebic meningoecephalitis (PAM), an illness so severe that few persons who contract it survive. Mostly because of its virulence, this pathogenic species was awarded species status and given the name *Naegleria fowleri*. Since then, pathogenicity testing on experimental animals have been rendered unnecessary due to the development of genetic techniques that can quickly and precisely identify this pathogenic species.

Furthermore, it appears that *N. fowleri* isolation is still a challenge when the pathogen is only found in trace amounts in surface water, as opposed to high concentrations in water, like cooling waters used by enterprises. This disease might only be a tropical one if it weren't for the cooling waters of industry, where this infection develops in temperate climate zones.(4)

Life Cycle and Pathogenicity

Primary amebic meningoencephalitis (PAM) is a rapidly progressive and often fatal condition caused by the free-living ameba *Naegleria fowleri*. PAM occurs upon accidental introduction of *N. fowleri* into the nose, after which the ameba invades the central nervous system (CNS) through the cribriform plate and olfactory nerves. Invasion of the CNS results in cerebral edema, necrosis, herniation, and, in most cases, death. A presumptive diagnosis of *N. fowleri* infection can be made by microscopic examination of the cerebrospinal fluid (CSF) or brain tissue, and a definitive diagnosis can be made by immunohistochemistry (IHC), indirect immunofluorescence (IIF), polymerase chain reaction (PCR) , or next-generation sequencing (NGS).(5)

The most favorable environment for *N. fowleri* to produce PAM is warm water, hence swimmers and divers are particularly vulnerable to this infection.(6) Australia made the first report identifying PAM illnesses in 1965. Three deadly infections from 1965 and one from 1961 were found in the report. After being determined to be a novel species, the ameba responsible for the illnesses was given the name *Naegleria fowleri* in honor of M. Fowler, one of the report's original authors. The initial study in 1966 identified illnesses in the United States, first reported in Florida in 1962. Using tissue samples from autopsies that had been preserved, later studies discovered PAM infections that had happened in Virginia as early as 1937.(7)

It is concerning to note that despite advancements in antimicrobial chemotherapy and supportive care, the death rates associated with PAM are still quite high. Because it is a free-living amoeba, *N. fowleri* has the ability to change its phenotype in response to its surroundings. It displays a reproductively active trophozoite stage in favorable conditions. The infectious stage is recognized as the trophozoite stage. In the absence of nutrients but in the presence of water, trophozoites transition to a transitory flagellate stage that permits long-distance migration, frequently in search of food. *N. fowleri* neither reproduces nor forms cysts at this stage. In unfavorable or unfavorable environments, trophozoites transform into a dormant, metabolically inactive form called the cyst form. Similar to the flagellate phase, the

cysts do not feed or reproduce. Neisseria fowleri only produces cysts, reproduces,and/or can feed its trophozoites. Through the synthesis of PAM, the parasites enter hosts through the nasal pathway, passing through the olfactory neuroepithelia and entering the central nervous system.(8)

When infectious amoeba in the trophozoite phase enter a human being through the nose, they traverse the cribriform plate and make their way to the brain, where they severely damage the central nervous system (CNS). In cases that are misdiagnosed or receive inadequate treatment, brain injury results in cerebral hemorrhage and death within three to seven days. Even though the precise pathogenesis of *N. fowleri* is still unknown, the pathogen primarily invades the host central nervous system (CNS) through two main mechanisms: contact-independent (brain damage through different proteins) and contact-dependent (brain damage through food cup surface structures).(6)

The life cycle of *Naegleria fowleri* consists of three stages: cysts (1), trophozoites (2), and flagellated forms (3) presented in Figure 1 and 2. Through promitosis, the trophozoites proliferate while maintaining an intact nuclear membrane (4).*Naegleria fowleri* can be found in soil, fresh water, geothermal wells, power plant thermal discharges, and tap and recreational water with low chlorine content. Trophozoites can briefly change into flagellated, non-feeding forms, but they typically return to the trophozoite stage. Trophozoites enter the nose mucosa of people or animals, usually through swimming or sinus irrigation (5), and go via the olfactory nerves to the brain (6), where they cause PAM.Cerebrospinal fluid (CSF) and tissue contain *Naegleria fowleri* trophozoites, with flagellated forms infrequently detected in CSF. Brain tissue doesn't have cysts.(2)

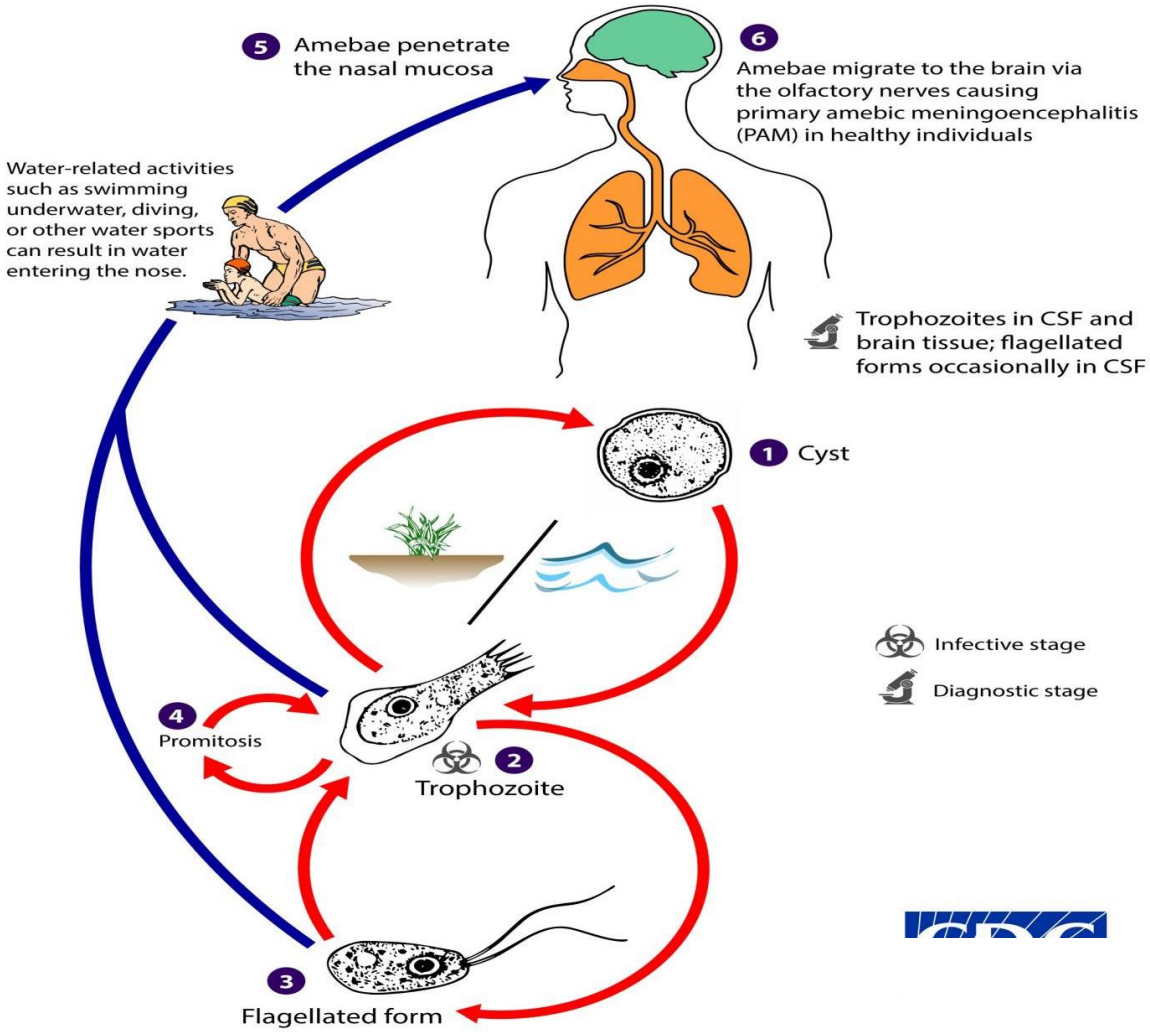

Figure 1 *Naegleria fowleri* life cycle in human body

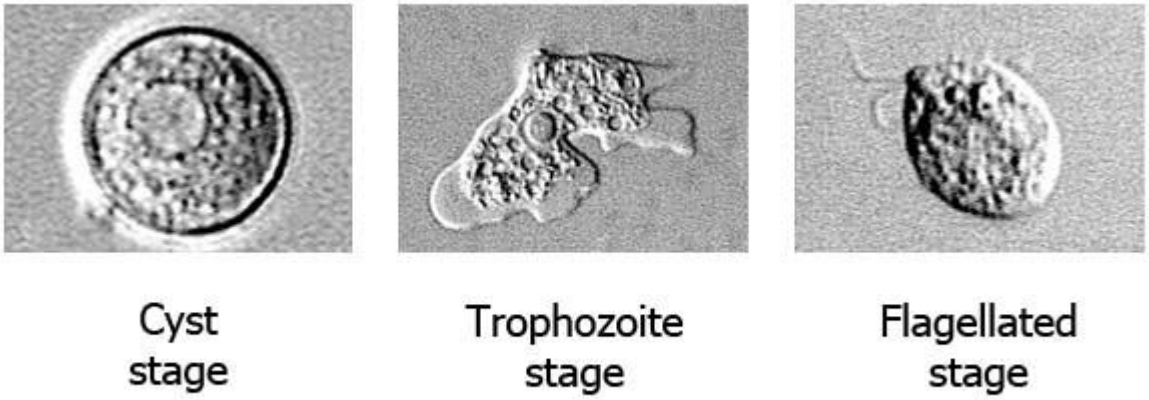

Figure 2 ***Naegleria fowleri*** **stages**

Clinical Presentation and Diagnosis

Most cases of *N. fowleri* infection appear two to eight days after infection, but some are documented as soon as 24 hours. Severe headache, fever, chills, positive signs of Brudzinski and Kernig, photophobia, disorientation, convulsions, and possibly even a coma are typical symptoms. In certain instances, myocardial necrosis and irregular cardiac rhythms have been noted.(9) Fever, frequent headaches, neck stiffness, nausea, vomiting, photosensitivity, unbalance, and sleepiness are some other symptoms. As soon as the body comes into contact with the pathogen, infection symptoms start to show. Every day that goes by, the symptoms get worse and become more noticeable.(10)

The infection's symptoms line up with the immune system's reaction as well as the brain's affected regions. The majority of cases documented in the literature had constitutional symptoms, including fever, chills, and lethargy. These were caused by the generation of reactive oxygen species, which in turn activated the epidermal growth factor receptor pathway and induced the expression of pro-inflammatory cytokines, including IL-1β and IL-8, as well as MUC5AC (mucin).(11) In autopsies, PAM instances are diagnosed in about 75% of cases.The delay in diagnosis could be caused by the overlap of symptoms between PAM and bacterial meningitis.Getting a complete medical history can help reduce this potentially catastrophic delay.(2)

Since most deaths happen between days three and seven, prompt identification and treatment are essential. Finding out if the patient has recently been into touch with fresh water or has been using nasal irrigation is a crucial step in the diagnosing process. Although the majority of PAM instances in the US have happened in Southern states, questions concerning activities that raise the risk of PAM should always be asked during the health history. Inquire about allergies, rhinitis, and other upper respiratory tract conditions from the patient as well.(12)

Diagnostic modalities for ***Naegleria fowleri***

Diagnosing *Naegleria fowleri*, the amoeba responsible for the fatal Primary Amoebic Meningoencephalitis (PAM), can be challenging due to its rarity and the rapid progression of the disease. However, early and accurate diagnosis is crucial for prompt treatment and improving the chances of survival.

Table 1: Detection methods for *Naegleria fowleri* in clinical and environmental samples (13)

Detection method	Description
Direct visualization	The motile ameba can be observed under a microscope in a fresh sample of cerebrospinal fluid (CSF).
Antigen detection	Specific antibodies can be used in conjunction with immunohistochemistry (IHC) or indirect immunofluorescence (IIF) to directly stain amebic antigens in tissue.
Polymerase chain reaction (PCR)	Specific molecular tools can amplify DNA from the ameba in CSF or tissue.
Ameba culture	The ameba can be grown into culture, increasing the likelihood of detecting it by direct visualization or PCR.
Environmental detection	Water samples can be collected, concentrated, and put into culture to grow and select for *N. fowleri* .

Furthermore, elevated intracranial pressure and cerebral spinal fluid pressure have been linked to mortality; patients infected with *N. fowleri* have been reported to have CSF pressures of 600 mm H2O.(14) The patient's unrefrigerated cerebrospinal fluid (CSF) should be examined wet mount by the treating physician, ideally under a microscope equipped with phase-contrast optics to identify any trophozoites. As mentioned before, the ameboid trophozoites have a big nucleolus positioned in the center and range in size from 7 to 20 µm. The trophozoites most likely use eruptive pseudopodia to migrate quickly in a certain direction.(15)

Due to a substantial increase in red blood cells, CSF examination reveals color anomalies that vary in severity from gray in the early stages of the disease to red in the later stages.(16) Furthermore, hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), trichrome, Giemsa, or Wright-Giemsa stains can be used to identify *Naegleria* in CSF smears or cultures. Since the amebae may be damaged during heat fixation, a Gram stain should be avoided. Ameboid trophozoites with classic *Naegleria* morphology (i.e., a nucleus with a large, centrally placed, and densely staining nucleolus) will be visible in a stained CSF smear. The polymerase chain reaction (PCR) should be used to confirm the diagnosis of PAM if amebae are found in the CSF.(17)

Additionally, trophozoites with classic *Naegleria fowleri* form may be seen in microscopic inspection of hematoxylin and eosin, periodic acid-Schiff, trichrome, Giemsa, or Wright-Giemsa-stained smears of brain biopsy or autopsy tissues. The diameter of ameboid trophozoites is typically 10-15 µm when rounded, although they can measure 10-35 µm.

There are numerous vacuoles and granules in the cytoplasm. There is a big, thick karyosome inside the solitary, massive nucleus.In human tissues, *Naegleria fowleri* does not produce cysts.(2, 18)

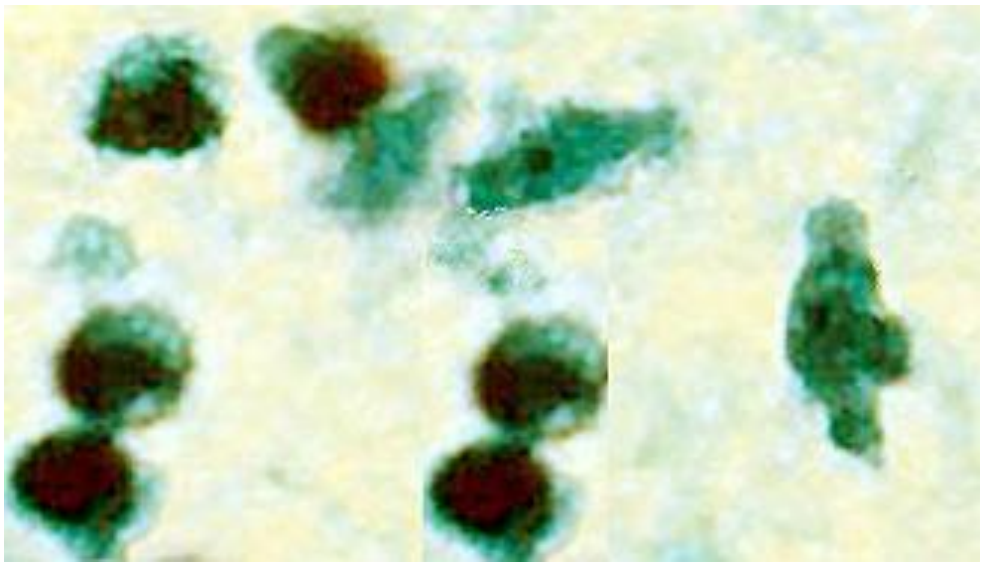

Figure 3 Trophozoite of *Naegleria fowleri* in CSF

Naegleria fowleri indirect immunofluorescence (IIF) experiment with 1000x oil magnification To identify *Naegleria fowleri* in formalin-fixed tissue, or CSF, immunohistochemical (IHC) staining and indirect immunofluorescent (IIF) staining employ antibodies specific for the bacterium. These techniques are then examined under a microscope.(2)

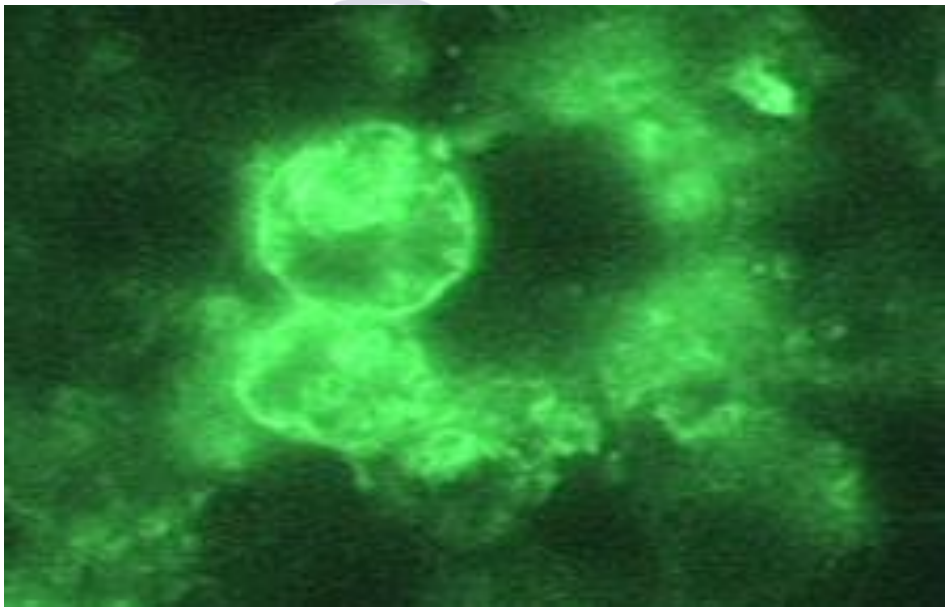

Figure 4 Indirect Immunofluorescence (IIF) assay for *Naegleria fowleri*

Since most patients with PAM pass away before an immune response is generated, serologic testing for *Naegleria fowleri* utilizing indirect immunofluorescent antibody (IFA) to evaluate serum antibody titers in patient sera has minimal diagnostic utility. A California PAM survivor did, nevertheless, show signs of a serologic reaction. To specifically determine whether amebae are present in tissue or CSF, several molecular techniques can amplify DNA

from the amebae. Successful amplification of DNA has been achieved from CSF and unfixed tissue samples. Isolate typing is possible, but the wild populations are not well understood enough to place this results in a biological context.(19)

Conventional and real-time PCR are two of the several PCR-based methods that have been reported for the detection and identification of free-living amebic infections in clinical specimens; nevertheless, these methods are restricted to use in a few reference diagnostic laboratories. For the purpose of qualitatively evaluating *Naegleria fowleri*, Acanthamoeba spp., and Balamuthia mandrillaris in clinical samples, the CDC developed a real-time PCR. In this assay, all three free-living amebae are simultaneously identified using different primers and TaqMan probes.It is possible to examine strains or subtypes of *Naegleria fowleri*, but interpreting the results is challenging because little is known about the wild populations in the environment. Subtyping, however, can assist in determining the *Naegleria fowleri* environmental exposure in PAM patients. Next-generation sequencing has also been utilized recently to diagnose PAM.(2, 20, 21)

Clinical and environmental materials containing free-living amebae can be identified using the culture technique. Mammalian cell cultures are inoculated, and the proliferation of E. coli on lawns or cytopathogenicity is observed. The sample is placed on a growth plate coated in bacteria that can act as a food source for *Naegleria fowleri* in order for it to develop on an E. coli lawn.To perform the first screening, the plate is incubated at a temperature of 108°F/42°C, which is high enough to kill most free-living amebae and select for thermophilic amebae, like *Naegleria fowleri* or other amebae. The tracks left behind by an ameba as it travels over the plate consuming the bacteria are visible on the first screen. *Naegleria fowleri* is going to grow at a higher temperature if the plate lacks of amebae. Since other thermophilic free-living amebae might also be present, if thermophilic amebae are found on the plate grown at the higher temperature, these amebae are subjected to additional, specialized testing to ascertain whether *Naegleria fowleri* is present.(22)

Hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), trichrome, Giemsa, or Wright-Giemsa stains can all be used to uniquely identify *Naegleria* in cultures from clinical specimens. As previously documented, stained cultures may show trophozoites with the characteristic appearance of *Naegleria fowleri*. Trophozoites can grow to a size of more than 40 µm in culture. Further testing should be done because a negative culture result does not rule out the presence of free-living amebae.(2)

Diagnostic Techniques for PAM

More focused symptoms and indicators are produced by different brain regions that are impacted. Therefore, identifying the focal area implicated may be aided by neuroimaging approaches.(23) There are also noticeable increases in the concentrations of polymorphonuclear cells and trophozoites in the CSF. The brain's magnetic resonance imaging frequently reveals anomalies in different areas.(24)

Protozoan bacteria, *N. fowleri* , are known to cause PAM, a rare condition marked by their presence in the CSF (cerebrospinal fluid). For a laboratory diagnosis, *N. fowleri* in the CSF must normally be grown and confirmed. This is followed by an ELISA test, a flagellation test (FT), or another diagnostic method such as PCR or restriction fragment length polymorphism (RFLP). While RFLP is based on the restriction profiles of the genus *Naegleria*, ELISA provides postmortem, retrospective, and late diagnosis. The 1990s saw the introduction of DNA probe-based detection techniques as an alternative to microscopy and culture.(6)

These techniques do, however, have several drawbacks, including lack of data, time, expense, and retroactive or delayed diagnosis. More effective PCR diagnostic methods, like LAMP, can be used to environmental and clinical specimens. LAMP has the advantages of being easy to use, precise amplification products may be produced without expensive equipment, and amplification response outcomes can be visually assessed. It is also sensitive, fast, and reproducible. Thus, molecular methods such as PCR and LAMP provide useful substitutes for PAM diagnosis, especially in developing nations with limited resources.(13)

OBJECTIVE

To determine the advancements in *Naegleria* detection considering a comprehensive Review on Diagnosis and Preventive Strategies.

RESEARCH QUESTIONS

- What are the latest advancements in *Naegleria* detection methods?
- What is the diagnostic accuracy of these methods?
- What is the potential impact of these advancements on patient outcomes?

METHODOLOGY

To develop a systematic review on the advancements in *Naegleria* detection, the following methodology was employed:

Inclusion Criteria

The following inclusion criteria was applied to select studies for the systematic review:

- Research focusing on the methods and techniques for detecting *Naegleria*, including but not limited to microscopy, molecular methods, and neuroimaging techniques.(25)
- Studies evaluating the accuracy of diagnostic tests for *Naegleria*, such as sensitivity, specificity, and predictive values.(25)
- Research on preventive measures, including but not limited to water treatment, public health interventions, and clinical management strategies.(25, 26)
- Case studies, clinical trials, cross-sectional studies, cohort studies, and diagnostic accuracy studies were considered to provide a comprehensive understanding of *Naegleria* detection and prevention.(26)
- Studies from any time period, published in any language, and involving any population were included to ensure a broad representation of the available literature.(26)

Exclusion Criteria

The following exclusion criteria was applied to the selection of studies:

- Research that does not directly address *Naegleria* detection methods, diagnostic accuracy, or preventive strategies were excluded to maintain the focus of the review.(25)
- Research with incomplete or insufficient data that hinders the assessment of the relevance and quality of the study.(25)
- Studies conducted on non-human subjects or models were excluded, as the review focuses on human-relevant research.(26)

Search Strategy

A comprehensive search was conducted using electronic databases such as PubMed, Google Scholar, Scopus, and Web of Science. Relevant keywords and Medical Subject Headings (MeSH) terms were used to ensure a thorough search such as *Naegleria* ,*Naegleria fowleri*, *Naegleria* Infections , Diagnostic Techniques Microbiological, Molecular Diagnostic Techniques , Neuroimaging, Water Microbiology, Water Purification and Preventive Health Services.

Additionally, conference proceedings and grey literature was included in the search to identify potentially relevant studies that may not be indexed in traditional databases.The search results provided relevant studies on the prevalence of *Naegleria* spp. and the diagnosis of *Naegleria fowleri*. These studies, including systematic reviews, meta-analyses, and CDC guidelines, contributed a valuable information to the systematic review on *Naegleria* detection and diagnosis.(2, 25)

Data Collection

For the narrative review on *Naegleria* detection methods, diagnostic accuracy, and preventive strategies, the data collection process were involved the extraction of relevant information from each included study. The data collected encompass various aspects of the studies, including but not limited to the year of publication, country, number of cases, patient demographics, diagnostic results, and clinical outcomes. Additionally, information on the methods and techniques for *Naegleria* detection, diagnostic accuracy measures, and preventive strategies were systematically extracted from the selected studies.(26, 27).

Ethical Considerations

In the context of conducting a systematic review, ethical considerations primarily revolve around the responsible and transparent handling of data and the adherence to ethical guidelines in research (28). As such, the following ethical considerations were upheld throughout the review process. The integrity of the data extracted from the included studies wias maintained, and any potential sources of bias or conflict of interest was carefully addressed. The review was conducted in a

transparent manner, with clear documentation of the data collection and analysis processes to facilitate the reproducibility of the findings. The review was adhere to established ethical guidelines for research, ensuring that the data collection and analysis procedures align with ethical standards and best practices in the field.(29)

LITERATURE REVIEW

Diagnosis:

This study provided new information on brain-eating amoebae and brought attention to the uncommon instance of *N. fowleri* infection, which has resulted in more case reports in the past ten years. An overview of *Naegleria fowleri* infection cases from different nations is given in the table 2 below, together with information on related symptoms, onset time, complete blood count, CSF result and PCR result.

Table 2: PAM cases' clinical, laboratory and epidemiological aspects examined.

No	Citation	Symptoms	Time to Onset	Full Blood Count	CSF Result	PCR Result
1	Chen et al., 2019 (30)	Fever; myalgia; fatigue; persistent occipital headache (for 2 days)	5 days	Leucocytosis; CRP	Turbid; high pressure; glucose low; leucocyte high; protein high; Pandy's test positive; trophozoites of *N. fowleri* with Wright–Giemsa stain	*N. fowleri* positive
2	Celik and Arslankoylu 2021 (31)	Fever; inability to suck; irritability; convulsion (for 2 days)	4 days	Leucocytosis; CRP	Leucocytosis; protein high; glucose low; negative for microbial growth, TORCH, acid-resistant bacilli, Ziehl–Neelsen staining	*N. fowleri* positive
3	Chomba et al., 2017 (32)	Fever; convulsion	2 days	No data	No bacterial or fungal pathogens were detected in CSF day 1 and day 3; numerous highly motile amoebic trophozoites and cysts day 8	*N. fowleri* positive
4	Stowe et al., 2017 (33)	(1) Fever; convulsion; headache, vomiting; difficulty ambulating; altered mental status; (2) generalised muscle weakness; tactile fever; vomiting;	Both 8 days	No data	Grossly abnormal; (1) free-swimming amoebae	*N. fowleri* positive

No	Citation	Symptoms	Time to Onset	Full Blood Count	CSF Result	PCR Result
		confusion; convulsion				
5	McLaughlin and O'Gorman 2019 (34)	Headache; photophobia; nausea; vomiting; neck stiffness	No data	No data	Turbid; glucose low; protein high; leucocytosis; free-living amoebae	*N. fowleri* positive
6	Cope et al., 2018 (35)	Fever; headache; lethargy	14 days	No data	High pressure; leucocytosis; glucose low; protein high; wet mount of the CSF revealed possible motile trophozoites	*N. fowleri* positive, with concomitant detection of Balamuthia mandrillaris and Acant hamoeba spp.
7	Sazzad et al., 2020 (36)	Fever; generalised headache; vomiting; weakness; neck stiffness; unconscious	No data	No data	Protein high; glucose low; leucocytosis; negative Ziehl–Neelsen stain and acid-fast bacilli	*N. fowleri* positive
8	Baral and Vaidya 2018 (37)	Fever; global headache; feature of anomic aphasia but no vomiting, seizure, or neurological deficit at presentation; altered sensorium and agitation; gradual weakness of bilateral lower limb and trunks	No data	No data	Leucocytosis; protein high; glucose low; negative for microbial growth, TB, AFB, HSV	Not performed
9	Huang et al., 2021 (38)	Fever; headache; vomiting; altered consciousnes	3 days	Leucocytosis; CRP	Leucocytosis; glucose low; protein high; pale, pink, thick necrotic fluid drawn out from the syringe	*N. fowleri* positive

No	Citation	Symptoms	Time to Onset	Full Blood Count	CSF Result	PCR Result
		s; convulsion				
10	Mushtaq et al., 2020 (39)	Fever; worsening headache; generalised weakness	No data	Leucocytosis;	Leucocytosis; negative Gram stain, India ink, cryptococcal antigen; positive wet prep for *Naegleria*	*N. fowleri* positive
11	Mittal et al., 2019 (40)	Fever; chills; rigors; abnormal body movement; vomiting; generalised tonic–clonic seizures; decreased oral intake; decrease urine output	No data	Microcytic hypochromic anaemia; raised CRP	High pressure; protein high; glucose low; leucocytosis; wet mount positive for moving trophozoites of amoeba; negative for India ink, Gram stain	*N. fowleri* positive
12	Anjum et al., 2021 (41)	Headache; fever; intractable emesis; poor oral intake	3 days	Leucocytosis; raised CRP	Pressure high; turbid; glucose low; protein high; leucocytosis; EVD protein high; Wright–Giemsa stain showed amoebic trophozoites	*N. fowleri* positive
13	Heggie and Küpper 2017 (42)	Fever; vomiting; headache; difficulty waking up from sleep; difficulty holding head up; unable to open eyes; hallucination	Less than 1 week	No data	*N. fowleri*	Not performed

Additionally, the PCR results consistently confirm the presence of *Naegleria fowleri* in the CSF of the affected patients, supporting the definitive diagnosis of PAM.The table 2 also alludes to the use of advanced diagnostic techniques such as loop-mediated isothermal amplification (LAMP) for the rapid and visual detection of *Naegleria fowleri* in clinical and environmental samples. This highlights the ongoing efforts to improve diagnostic capabilities, particularly in regions with limited resources.

Prevention:

The World Health Organization (WHO) states that immunization is the greatest and most efficient method of disease prevention. As a result, a team of researchers created a DNA vaccine that produces the Nfa1 gene of *N.*

fowleri using a lentiviral vector (43). An in vivo study on mice was conducted to assess the impact of the vaccine on the amoeba. The findings demonstrated that the vaccinated animals had increased IgG levels as well as enhanced IL-4 and IFN-γ production, suggesting a mixed-type Th1/Th2 immune response. Furthermore, the mice who received the vaccination showed a greater survival rate (90%) than the mice in the control group, which all perished after two weeks post-inoculation. Despite the fact that these findings suggest that vaccination may be a preventive measure against *N. fowleri* , more research is necessary to fully appreciate its potential (44).

As an alternative, one can employ additional preventive measures to ward off *N. fowleri* infection. The majority of PAM cases happen when people engage in certain activities in amoeba-infested waterways, like swimming pools or natural bodies of water. Pools and water parks should be chlorinated to prevent PAM because *N. fowleri* is vulnerable to chlorine and is destroyed at one part per million. To find out if *N. fowleri* is present in such waters, the South Australian High Commission's ameba-monitoring program regularly measures total coliform counts and residual chlorine levels. Global implementation of comparable initiatives could stop new cases of *N. fowleri* infection. Since *N. fowleri* infection has also been linked to household water sources, medical equipment, and hygiene products, chlorine should also be used to sanitize these items. Moreover, when flushing, washing, or irrigating nasal passages, always use boiling water (6, 45).

Recreational activities like swimming, water skiing, and diving should be avoided in lakes, rivers, and ponds since they cannot be chlorinated, especially in the summer. If not, it is advised to use nose plugs to prevent the amoeba from entering the nasal canal. Additionally, it is best to avoid digging in or agitating the silt that is present in these bodies of water's shallow end. Furthermore, the prevalence of *N. fowleri* in high-risk locations should be continuously monitored by local public authorities (46). In the case that the amoeba is found, signs advising people not to enter the water should be put up. Implementing awareness campaigns in schools, colleges, religious organizations, local government buildings, hospitals, and recreational areas is essential for the success of these preventive efforts. Implementing awareness campaigns in schools, colleges, religious organizations, local government buildings, hospitals, and recreational areas is essential for the success of these preventive efforts. Preventive measures are the best method to avoid PAM because there is now no therapy for this fatal illness (6).

Moreover as *Naegleria fowleri*, a deadly amoeba, can cause brain infection when water enters the body through the nose.To prevent infection, avoid swimming or diving in warm freshwater places, using sterile water when irrigating nasal passages, and holding your nose shut during water-related activities. Disinfect swimming pools and avoid activities where water is difficult to prevent.(47, 48) To prevent infection, following prevented measures should be taken as presented in table 3.

Table 3:Preventive Strategies *N. fowleri* infection

Preventive Measure	Description
Avoid swimming in warm freshwater	Refrain from water-related activities in warm, untreated, or poorly treated water, especially during periods of high water temperature and low levels.
Minimize water entry into the nose	Hold your nose shut, use nose clips, or keep your head above water when taking part in water-related activities to prevent water from entering the nose.
Avoid tap water for nasal hygiene	Avoid using tap water when irrigating, flushing, or rinsing nasal passages. Use sterile, distilled water for these purposes.
Maintain swimming pool	Keep swimming pools adequately disinfected before and during use.

hygiene	Maintain proper chlorine and pH levels.
Minimize sediment disturbance	Avoid digging in or stirring up sediment surrounding warm, fresh water when engaged in water-related activities.

The sudden, deadly illness Given that the thermophilic amoeba that causes PAM multiplies in less chlorinated water during the warmer months, PAM is linked to water. Since all PAM instances are associated with inadequately chlorinated water consumption, maintaining chlorinated water levels is imperative. Public awareness campaigns should be conducted in schools, universities, mosques, and public areas. This is especially important in developing nations like Pakistan (6).

DISCUSSION

Due to difficulties in detecting the illness, such as its initial ambiguous presentation and ability to mimic bacterial meningitis, PAM infection may be underestimated in clinical settings. The infrequent occurrence of PAM can result in additional delays. Some centers may not have access to diagnostic equipment that can identify the culpable organism, such as *N. fowleri* , because they rely on the operator. FLA cultures take longer to complete and require certain media; negative test results can also need more correlational investigation (26).

The study makes important recommendations for physicians who treat patients with comparable PAM presentations. Usually accompanied by a fever, headache, and vomiting, the illness needs to be tested right away to be diagnosed. While there might not be any noticeable changes in baseline blood values, general infectious indicators such as CRP and ESR will always be increased (46). Accurate diagnosis requires careful and timely interpretation of preliminary data. For the early diagnosis, medical professionals need to ask about travel history and considerable exposure to warm freshwater. A high clinical index of suspicion is also necessary (49).

The thermophilic amoeba *N. fowleri* can be found in a variety of settings, such as water parks, thermal waters, ponds, rivers, freshwater lakes, drinking water distribution systems, untreated swimming pools, and fountains. It can advance quickly and cause death in a matter of days (50). PAM enters the brain directly, which causes its onset to occur quickly. *N. fowleri* enters the nasal cavity after engaging in water contact activities, adheres to the nasal mucosa, burrows into it, passes through the cribriform plate, and then continues along the olfactory nerves to the olfactory bulb. Brain edema, cerebral artery herniation, and finally death are the consequences of brain penetration (6).

The degree of symptoms affecting the central nervous system is correlated with both the pathogenicity of *N. fowleri* and the strength of the host immune response. To clarify the pathophysiology of PAM, two hypothesized models contact dependent mechanisms and contact-dependent mechanisms have been created. The infection's symptoms match the immune system's reaction as well as the brain's affected regions. Nearly all cases documented in the literature had constitutional symptoms, including fever, chills, and lethargy (51). These were caused by reactive oxygen species, which then activated the epidermal growth factor receptor pathway and induced the expression of pro-inflammatory cytokines and MUC5AC (mucin). It may be possible to identify the focal area involved by using neuroimaging techniques, as different portions of the brain that are afflicted produce more specific indications and symptoms (23).

Furthermore the results of the CSF sample may be able to rule out a viral cause for involvement of the central nervous system. Viral causes are ruled out by low CSF glucose levels and concurrently high CSF protein levels. The US CDC recommends utilizing molecular methods to diagnose PAM when *N. fowleri* is found in a brain tissue sample or CSF sample (52). A precise diagnosis is essential because it allows for the quick start of an intensive treatment plan that will guarantee survival.

However, there needs to be a strong public health response since *N. fowleri* infections have a high death rate. First, a persistent health awareness campaign must inform the public

about this ailment. Reminding people attending freshwater contact events of the importance of protective practices should be a regular part of health promotion campaigns. Broad distribution of health education messages is also necessary.

Limitations and Recommendations:

Diagnosing *Naegleria fowleri* infection remains highly challenging due to its rarity and the rapid progression of primary amoebic meningoencephalitis (PAM). Although the organism can be identified microscopically in fresh cerebrospinal fluid (CSF) samples, this requires specialized laboratory equipment and experienced personnel, which may not be readily available in all clinical settings. Environmental detection in water sources is also complex, involving processes such as sample collection, concentration, and culturing, which are time-consuming and may not always produce definitive results. Furthermore, the clinical presentation of PAM often overlaps with bacterial meningitis, leading to potential misdiagnosis or delays in appropriate treatment. In such cases, obtaining a thorough medical history, particularly regarding recent freshwater exposure, becomes essential for accurate diagnosis.

To address these challenges, continued research and investment in advanced diagnostic methods, including polymerase chain reaction (PCR) and next-generation sequencing, are recommended to enhance both the speed and accuracy of detection. Public health initiatives should prioritize increasing awareness about *N. fowleri* infection, emphasizing preventive strategies such as avoiding the دخول of freshwater into the nasal passages and using protective measures like nose clips during water activities. Additionally, improving water treatment protocols and implementing routine monitoring in recreational water bodies can help minimize the presence of the amoeba. Finally, training healthcare professionals to recognize early symptoms of PAM and to consider environmental exposure in their diagnostic approach can significantly improve early detection and patient outcomes.

CONCLUSION

N.fowleri infection is rare but leads to PAM. Its occurrence is worldwide, with a significant risk of fatality. This study consolidated the most recent publications from the last decade featuring both epidemiology and clinical presentation. Understanding the significance of *N. fowleri* and PAM is essential for public health and safety. Early diagnosis and prompt treatment are crucial for improving the chances of survival. Preventive measures, such as avoiding contaminated water sources and following safety guidelines, are essential in minimizing the risk of PAM.

Key Findings:

- **High Mortality:** *Naegleria fowleri* infection leads to PAM, which progresses rapidly and is associated with an extremely high fatality rate, often within 3–7 days of symptom onset.
- **Diagnostic Delay:** The similarity of PAM symptoms to bacterial meningitis frequently results in misdiagnosis or delayed diagnosis, significantly reducing survival chances.
- **Molecular Methods:** Advanced diagnostic techniques such as PCR and next-generation sequencing (NGS) demonstrate higher sensitivity and specificity compared to conventional microscopy and culture methods.
- **CSF Findings:** Common laboratory findings include elevated protein, low glucose, high leukocyte count, and the presence of motile trophozoites in cerebrospinal fluid, supporting diagnosis.

Prevention: Due to limited effective treatment options, preventive strategies such as proper chlorination, avoiding contaminated water exposure, and public awareness are the most critical measures to reduce disease burden.

REFERENCES

1. Ong TYY, Khan NA, Siddiqui R. Brain-eating amoebae: predilection sites in the brain and disease outcome. Journal of clinical microbiology. 2017;55(7):1989-97.
2. CDC. Centers for Disease Control and Prevention National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Centre for Disease Control and Prevention, United States Department of ...; 2021.

3. Pervin N, Sundareshan V. *Naegleria*. 2018.
4. De Jonckheere JF. Origin and evolution of the worldwide distributed pathogenic amoeboflagellate *Naegleria fowleri*. Infection, Genetics and Evolution. 2011;11(7):1520-8.
5. Gharpure R, Bliton J, Goodman A, Ali IKM, Yoder J, Cope JR. Epidemiology and clinical characteristics of primary amebic meningoencephalitis caused by *Naegleria fowleri*: a global review. Clinical Infectious Diseases. 2021;73(1):e19-e27.
6. Jahangeer M, Mahmood Z, Munir N, Waraich UeA, Tahir IM, Akram M, et al. *Naegleria fowleri*: Sources of infection, pathophysiology, diagnosis, and management; a review. Clinical and Experimental Pharmacology and Physiology. 2020;47(2):199-212.
7. Hall AD, Kumar JE, Golba CE, Luckett KM, Bryant WK. Primary amebic meningoencephalitis: a review of *Naegleria fowleri* and analysis of successfully treated cases. Parasitology Research. 2024;123(1):84.
8. Siddiqui R, Ali IKM, Cope JR, Khan NA. Biology and pathogenesis of *Naegleria fowleri*. Acta tropica. 2016;164:375-94.
9. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, *Naegleria fowleri*, and Sappinia diploidea. FEMS Immunology & Medical Microbiology. 2007;50(1):1-26.
10. Naqvi AA, Yazdani N, Ahmad R, Zehra F, Ahmad N. Epidemiology of primary amoebic meningoencephalitis-related deaths due to *Naegleria fowleri* infections from freshwater in Pakistan: An analysis of 8-year dataset. Archives of Pharmacy Practice. 2016;7(4).
11. Cervantes-Sandoval I, Serrano-Luna JdJs, Meza-Cervantez P, Arroyo R, Tsutsumi V, Shibayama M. *Naegleria fowleri* induces MUC5AC and pro-inflammatory cytokines in human epithelial cells via ROS production and EGFR activation. Microbiology. 2009;155(11):3739-47.
12. Martínez-Castillo M, Cárdenas-Zúñiga R, Coronado-Velázquez D, Debnath A, Serrano-Luna J, Shibayama M. *Naegleria fowleri* after 50 years: is it a neglected pathogen? Journal of medical microbiology. 2016;65(9):885.
13. Nadeem A, Malik IA, Afridi EK, Shariq F. *Naegleria fowleri* outbreak in Pakistan: unveiling the crisis and path to recovery. Frontiers in Public Health. 2023;11.
14. Visvesvara GS. Pathogenic and opportunistic free-living amebae. Manual of clinical microbiology. 2015:2387-98.
15. Aldape K, Huizinga H, Bouvier J, McKerrow J. *Naegleria fowleri*: characterization of a secreted histolytic cysteine protease. Experimental parasitology. 1994;78(2):230-41.
16. Control CfD, Prevention. Primary amebic meningoencephalitis--Arizona, Florida, and Texas, 2007. MMWR Morbidity and mortality weekly report. 2008;57(21):573-7.
17. Nichols M, Gollarza L, Sockett D, Aulik N, Patton E, Francois Watkins LK, et al. Outbreak of multidrug-resistant Salmonella Heidelberg infections linked to dairy calf exposure, United States, 2015–2018. Foodborne pathogens and disease. 2022;19(3):199-208.
18. Duignan P. ParasiticDiseasesof MajorTaxa. Pathology and Epidemiology of Aquatic Animal Diseases for Practitioners. 2023:214.
19. Haselkorn TS, DiSalvo S, Miller JW, Bashir U, Brock DA, Queller DC, et al. The specificity of Burkholderia symbionts in the social amoeba farming symbiosis: prevalence, species, genetic and phenotypic diversity. Molecular Ecology. 2019;28(4):847-62.
20. Ali IKM, Roy S. A real-time PCR assay for simultaneous detection and differentiation of four common Entamoeba species that infect humans. Journal of Clinical Microbiology. 2020;59(1):10.1128/jcm. 01986-20.

21. Régoudis E, Pélandakis M. Detection of the free living amoeba *Naegleria fowleri* by using conventional and real-time PCR based on a single copy DNA sequence. Experimental Parasitology. 2016;161:35-9.
22. Amebae can be common in water systems, including thermophilic amebae other than *Naegleria fowleri*, however none of these other amebae induce PAM.
23. Kim J-H, Sohn H-J, Yoo J-K, Kang H, Seong G-S, Chwae Y-J, et al. NLRP3 inflammasome activation in THP-1 target cells triggered by pathogenic *Naegleria fowleri*. Infection and Immunity. 2016;84(9):2422-8.
24. Martinez AJ. Free-living amebas: natural history, prevention, diagnosis, pathology, and treatment of disease: Crc Press; 2019.
25. Saberi R, Seifi Z, Dodangeh S, Najafi A, Abdollah Hosseini S, Anvari D, et al. A systematic literature review and meta-analysis on the global prevalence of *Naegleria* spp. in water sources. Transboundary and Emerging Diseases. 2020;67(6):2389-402.
26. Ahmad Zamzuri MAI, Abd Majid FN, Mihat M, Ibrahim SS, Ismail M, Abd Aziz S, et al. Systematic review of brain-eating amoeba: a decade update. International Journal of Environmental Research and Public Health. 2023;20(4):3021.
27. Streby A, Mull BJ, Levy K, Hill VR. Comparison of real-time PCR methods for the detection of *Naegleria fowleri* in surface water and sediment. Parasitology research. 2015;114:1739-46.
28. Bell E, Bryman A. The ethics of management research: an exploratory content analysis. British journal of management. 2007;18(1):63-77.
29. Suri H. Ethical considerations of conducting systematic reviews in educational research. Systematic reviews in educational research: Methodology, perspectives and application. 2020:41-54.
30. Chen M, Ruan W, Zhang L, Hu B, Yang X. Primary amebic meningoencephalitis: a case report. The Korean journal of parasitology. 2019;57(3):291.
31. Celik Y, Arslankoylu AE. A newborn with brain-eating ameba infection. Journal of Tropical Pediatrics. 2021;67(1):fmaa100.
32. Chomba M, Mucheleng'anga LA, Fwoloshi S, Ngulube J, Mutengo MM. A case report: primary amoebic meningoencephalitis in a young Zambian adult. BMC Infectious Diseases. 2017;17(1):1-5.
33. Stowe RC, Pehlivan D, Friederich KE, Lopez MA, DiCarlo SM, Boerwinkle VL. Primary amebic meningoencephalitis in children: a report of two fatal cases and review of the literature. Pediatric Neurology. 2017;70:75-9.
34. McLaughlin A, O'Gorman T. A local case of fulminant primary amoebic meningoencephalitis due to *Naegleria fowleri*. Rural and Remote Health. 2019;19(2):1-3.
35. Cope JR, Murphy J, Kahler A, Gorbett DG, Ali I, Taylor B, et al. Primary amebic meningoencephalitis associated with rafting on an artificial whitewater river: case report and environmental investigation. Clinical Infectious Diseases. 2018;66(4):548-53.
36. Sazzad HM, Luby SP, Sejvar J, Rahman M, Gurley ES, Hill V, et al. A case of primary amebic meningoencephalitis caused by *Naegleria fowleri* in Bangladesh. Parasitology research. 2020;119:339-44.
37. Baral R, Vaidya B. Fatal case of amoebic encephalitis masquerading as herpes. Oxford Medical Case Reports. 2018;2018(5):omy010.
38. Huang S, Liang Xa, Han Y, Zhang Y, Li X, Yang Z. A pediatric case of primary amoebic meningoencephalitis due to *Naegleria fowleri* diagnosed by next-generation sequencing of cerebrospinal fluid and blood samples. BMC Infectious Diseases. 2021;21:1-5.

39. Mushtaq MZ, Mahmood SBZ, Aziz A. A fatal case of primary amoebic meningoencephalitis (PAM) complicated with diabetes insipidus (DI): a case report and review of the literature. Case Reports in Infectious Diseases. 2020;2020.
40. Mittal N, Mahajan L, Hussain Z, Gupta P, Khurana S. Primary amoebic meningoencephalitis in an infant. Indian journal of medical microbiology. 2019;37(1):120-2.
41. Anjum SK, Mangrola K, Fitzpatrick G, Stockdale K, Matthias L, Ali IKM, et al. A case report of primary amebic meningoencephalitis in North Florida. IDCases. 2021;25:e01208.
42. Heggie TW, Küpper T. Surviving *Naegleria fowleri* infections: a successful case report and novel therapeutic approach. Travel Medicine and Infectious Disease. 2017;16:49-51.
43. Zuo F, Abolhassani H, Du L, Piralla A, Bertoglio F, de Campos-Mata L, et al. Heterologous immunization with inactivated vaccine followed by mRNA-booster elicits strong immunity against SARS-CoV-2 Omicron variant. Nature communications. 2022;13(1):2670.
44. Kim J-H, Sohn H-J, Lee J, Yang H-J, Chwae Y-J, Kim K, et al. Vaccination with lentiviral vector expressing the nfa1 gene confers a protective immune response to mice infected with *Naegleria fowleri*. Clinical and Vaccine Immunology. 2013;20(7):1055-60.
45. Visvesvara GS. Infections with free-living amebae. Handbook of clinical neurology. 2013;114:153-68.
46. Güémez A, García E. Primary amoebic meningoencephalitis by *Naegleria fowleri*: pathogenesis and treatments. Biomolecules. 2021;11(9):1320.
47. Grace E, Asbill S, Virga K. *Naegleria fowleri*: pathogenesis, diagnosis, and treatment options. Antimicrobial agents and chemotherapy. 2015;59(11):6677-81.
48. Younus S, Fatima H, Rangwala BS, Munir A, Ahsan SM, Naeem W, et al. Knowledge, Attitude, and Practices related to *Naegleria fowleri* Among General Population of Karachi, Pakistan: A Cross-Sectional Study. Annals of Neurosciences. 2023:09727531231196996.
49. Pugh JJ, Levy RA. *Naegleria fowleri*: Diagnosis, pathophysiology of brain inflammation, and antimicrobial treatments. ACS Publications; 2016. p. 1178-9.
50. Maciver SK, Piñero JE, Lorenzo-Morales J. Is *Naegleria fowleri* an emerging parasite? Trends in parasitology. 2020;36(1):19-28.
51. Sohn HJ, Song KJ, Kang H, Ham AJ, Lee JH, Chwae YJ, et al. Cellular characterization of actin gene concerned with contact-dependent mechanisms in *Naegleria fowleri*. Parasite Immunology. 2019;41(8):e12631.
52. Stubhaug TT, Reiakvam OM, Stensvold CR, Hermansen NO, Holberg-Petersen M, Antal E-A, et al. Fatal primary amoebic meningoencephalitis in a Norwegian tourist returning from Thailand. JMM case reports. 2016;3(3).