GOVERNMENT OF KERALA

Abstract

Health & Family Welfare Department - Action Plan to optimize the Prevention, Diagnosis, and Treatment of Amoebic Meningoencephalitis in Kerala - Revised - Orders issued.

HEALTH & FAMILY WELFARE (F) DEPARTMENT

G.O.(Rt)No.2617/2024/H&FWD Dated,Thiruvananthapuram, 02-11-2024

Read:- 1 . Technical Workshop on Amoebic Meningoencephalitis conducted on 27.08.2024.

2. E- mail message dated 09.09.2024 and 10.09.2024 from Dr. Aravind R, Member, State Medical Board.

3. E- mail message dated 21.10.24 from the Chairperson, State Medical Board.

ORDER

In the context of reporting an apparent increase in the number of cases of Amoebic Meningoencephalitis in Kerala, in order to ensure effective control and management of disease with focus on technical aspects of diagnosis, prevention and treatment, Government are pleased to issue the revised *"One Health based Multi-Pronged Action Plan to optimize the Prevention, Diagnosis and Treatment of Amoebic Meningoencephalitis in Kerala*", as annexed to this order.

(By order of the Governor)

Dr. Rajan Namdev Khobragade I A S

ADDITIONAL CHIEF SECRETARY

To:

The State Mission Director - National Health Mission,

Thiruvananthapuram.

The Director of Health Services, Thiruvananthapuram.

The Director of Medical Education, Thiruvananthapuram.

The Chairperson, State Medical Board.

All District Medical Officers (Health .

Principal Accountant General (A&E/Audit) Kerala.

Information & Public Relations (Web & New Media) Department.

Stock File/ Office Copy to file no.F2/270/2024-HEALTH.

Forwarded /By order

Section Officer

ONE HEALTH BASED MULTI-PRONGED ACTION PLAN TO OPTIMIZE THE PREVENTION, DIAGNOSIS AND TREATMENT OF AMOEBIC MENINGOENCEPHALITIS IN KERALA

Background

Amoebic encephalitis is a rare but lethal central nervous system infection caused by free-living amoebae found in freshwater, lakes, and rivers. There are two types of amoebic encephalitis, namely primary amoebic meningoencephalitis (PAM) and granulomatous amoebic encephalitis (GAE). Primary amebic meningoencephalitis (PAM) is a disease caused usually by infection with *Naegleria fowleri*, a microscopic amoeba commonly called a "brain-eating amoeba." This infection destroys brain tissue, causing severe brain swelling and death in most cases.

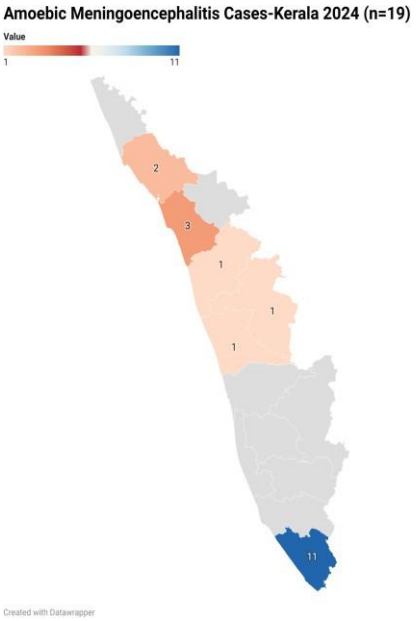
Naegleria fowleri is a free-living protozoan that resides in soil and freshwater. Human intranasal amoebae exposure through water or potentially dust particles can culminate in primary amoebic meningoencephalitis, which generally causes death. While many questions remain regarding pathogenesis, the microbial ecology of *N. fowleri* and the environmental abiotic and biotic factors that affect the distribution and abundance of *N. fowleri* is even less understood.Although the impacts of some abiotic factors remain poorly investigated or inconclusive, *N. fowleri* appears to have a wide pH range, low salinity tolerance and thermophilic preference. From what is known about biotic factors, the amoebae preferentially feed upon bacteria and are preyed upon by other free-living amoebae. Additional laboratory and environmental studies are needed to fill in knowledge gaps, which are crucial for surveillanceand management of *N. fowleri* in freshwaters. As surface water temperatures increase with climate change, it is likely that this amoeba will pose a greater threat to human health, suggestingthat identifying its abiotic and biotic preferences is critical to mitigating this risk.This underlines the importance of having a “One Health” approach to understand the threat potential and mitigation strategies to be adopted to prevent occurrence of PAM in Kerala.

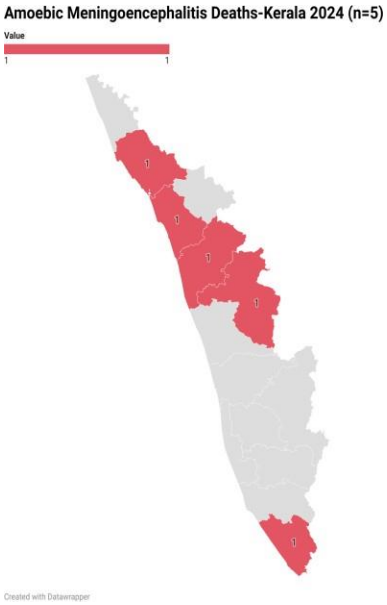
The apparent increase in number of cases of amoebic meningoencephalitis in Kerala needs to be studied in depth and hence a technical workshop was conducted on 27/8/2024 were all the technical aspects with regard to diagnosis, prevention and treatment of amoebic meningoencephalitis were discussed in detail. The biotic and abiotic factors which influence the growth of amoeba were discussed in detail and based on inputs from experts from State pollution control board and Department of Environmental sciences Kerala University,this action plan has been drafted.

The Kerala Scenario

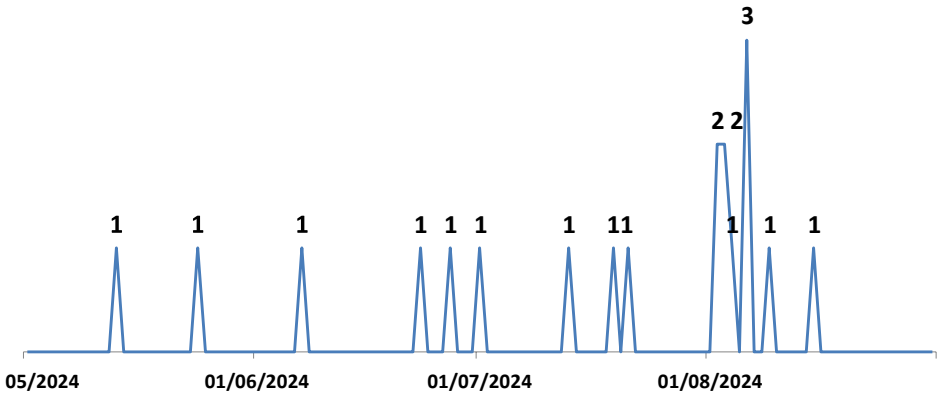
Sporadic cases of PAM have been reported from many parts of Kerala in the last decade. However in 2024, 19 cases of amoebic meningoencephalitis have been reported from Kerala in the last four months.

Year	No of Cases	No of Deaths	District	Diagnosis
2016	1	1	Alappuzha	Naeglaria Fowleri
2019	1	1	Malappuram	Naeglaria Fowleri
2020	2	2	Malapuram	Naeglaria Fowleri
2022	1	1	Thrishur	Naeglaria Fowleri
2023	2	2	Kasaragode	Acanthamoeba
			Alappuzha	Naeglaria Fowleri / ? Vermamoeba Vermicularis
2024	19	5	Malappuram - 1 Thrishur -1 Kannur - 2 Kozhikode - 3 Palakkad – 1 Trivandrum - 11	Naeglaria Fowleri Vermamoeba Acanthamoeba





2024Amoebic Meningoencephalitis – Timeline -Kerala

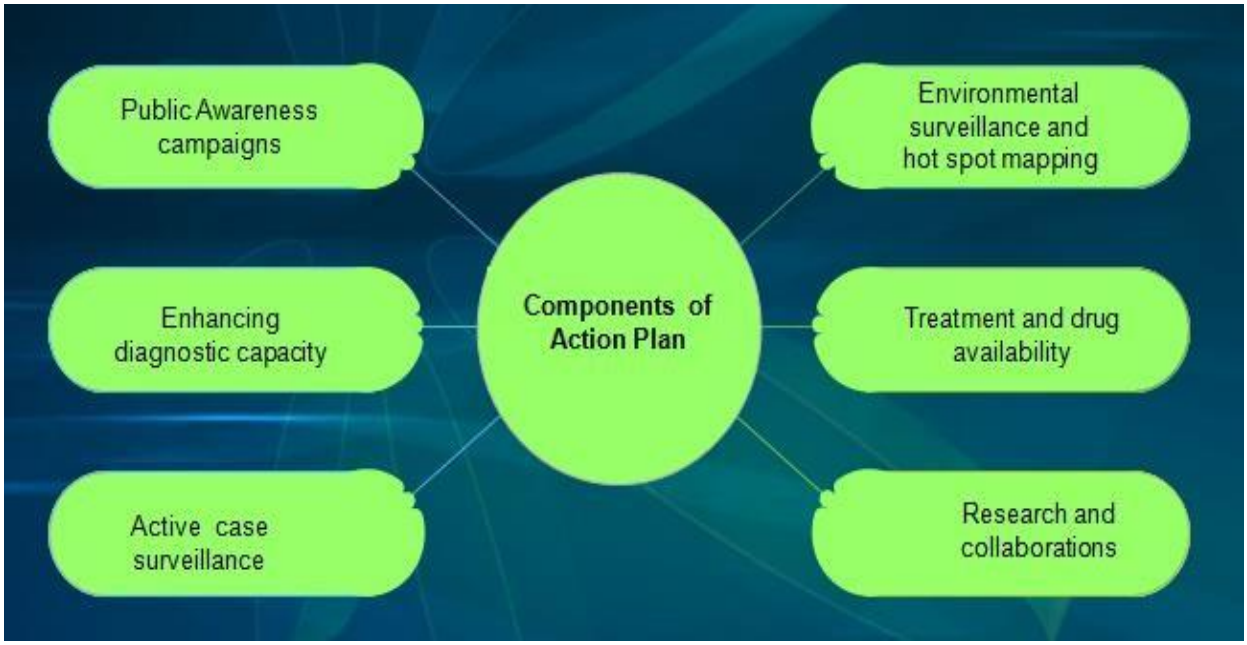


Impact of Global Warming

The free-living amoeba with the most evidence of the effects of climate change is *N. fowleri*. *N. fowleri* is thermophilic and one of its food sources is cyanobacteria, which flourishes in warmer waters. Climate change raising the water temperature and the heat driving more people to recreational water use are likely to increase encounters with this pathogen. Furthermore, warming temperatures may be expanding the geographical range of *Naegleria* species. Many reported cases of PAM occurred in the weeks after an increase in air temperature, indicating a potential relation to climate change.

Hence every year before the onset of summer [ie by February] public education campaigns must be launched focusing on preventive methods to be adopted to avoid getting infected with Naegleria.LSGDs must actively engage in IEC campaigns and through them aim must be to bring in behaviour change among people who routinely use freshwater bodies like ponds.

Components of Mullti-pronged Action Plan



1. PUBLIC AWARENESS CAMPAIGNS

- Main stakeholders Department of Health, LSGDs, Education department,department of irrigation, Water authority and Tourism.
- Timing of the campaign—year round campaign which should intensify from just before onset of summer [ie.January].
- Target group-Students, who are likely to come in contact with natural or artificial water sources for bathing, swimming, occupational or recreational activities.
- Materials-For IEC to be adopted and customized from Kerala State Technical guidelines on treatment and prevention of Amoebic meningoencephalitis.
- Each LSGD can identify the high risk groups ie people who regularly come into contact with fresh water bodies like ponds, and conduct focused IEC[Information, education,communication] sessions with them.
- Focussed IEC also should be given to those who work in water theme parks,swimming pools etc
- LSGDs should display billboards with IEC material should be displayed near waterbodies which have been epidemiologically linked to diagnosed cases of Naegleria fowleri infection or from where N.fowleri have been isolated during surveillance.
- All LSG institutions should ensure that the waterbodies in their jurisdiction are not contaminated by solid and liquid wastes.
- Cleaning of waterbodies where people swim, or bath should be a priority in pre-monsoon activities conducted by the local FHC and LSG institution
- The campaign should focus on children and people at occupational risk [like cattle rearers who may contact waterbodies to bath them, those who collect river sands,mussels etc)
- LSG institutions and FHCs should ensure the chlorination of swimming pools in resorts, hotels, schools and other recreational places like water theme parks.

2. ENHANCING DIAGNOSTIC CAPACITY

- IAV,Thonnakkal will be the designated apex centre in the state for molecular and genomic diagnosis of Amoebic meningoencephalitis.For this purpose IAV will get equipped to perform real-time PCR to diagnose free living amoeba infections of consequence to humans ie Naegleria, acanthamoeba, balamuthia, sappinia and vermamoeba vermiformis.Genomic sequencing of the diagnosed isolates also will be carried out at IAV.
- A division of Medical Parasitology will be established in the Departments of Microbiology at GMC Thiruvananthapuram and GMC Kozhikode, Microbiology departments at these two institutes will be equipped with state of art equipments needed for the microscopic and molecular diagnosis of amoebic meningoencephalitis.
- Molecular diagnostic facility to diagnose free living amoeba infections will be established at State public health laboratory also.
- A short primer video on the morphological and molecular diagnosis of amoebic meningoencephalitis will be made and through the State training site, it will be disseminated to all Microbiologists in Kerala.

3. ACTIVE CASE SURVEILLANCE

The key to optimizing the treatment outcomes in PAM and GAE is in making an early diagnosis and initiating the treatment protocol at the earliest.For this the following points has to be meticulously followed

- A history of nasal exposure to fresh water in the 14 days before symptom onset should be asked of any patient who presents with symptoms of acute meningitis.
- For patients with meningitis who have a history of recent nasal exposure to fresh water, the CSF specimen should undergo rapid testing for *N. fowleri/FLA*.Microbiologist should be immediately alerted about the clinical suspicion prior to sending CSF sample.
- In patients with clinical and CSF picture suggestive of bacterial meningitis who are not responding to antibiotics or are rapidly deteriorating consider PAM even in the absence of exposure to fresh water.
- CSF wet mount examination to be done on all turbid/opalescent CSF samples.
- All cases diagnosed as PAM through CSF microscopy should be immediately initiated on the recommended multi-drug regimen and supportive therapy aimed at lowering intracranial pressure.
- All cases diagnosed by CSF microscopy should be immediately informed to DSO and the CSF sample should be sent for PCR and genomic sequencing [if needed].
- A history of a definite exposure to fresh water could not be ascertained in certain cases of PAM/GAE and hence it will be better to request for a CSF wet mount examination for all cases of suspected meningitis/meningoencephalitis especially if an alternate diagnosis could not be made. And hence in all suspected cases of meningitis/meningoencephalitis, CSF Wet mount should be sought and performed.

4. ENVIRONMENTAL SURVEILLANCE AND HOT SPOT MAPPING

Action Plan on Environmental factors affecting distribution and abundance of free-living amoebas (FLAs) has been prepared by Kerala State Pollution Control Board and Department of Environmental Sciences, University of Kerala

Objectives are to

• Regularly monitor water quality (pH, temperature, TDS, turbidity, DO, BOD, salinity, ammoniacal nitrogen, nitrate, phosphate, iron, total coliform, faecal coliform, faecal streptococci and abundance of free-living amoeba (FLAs) in stagnant water bodies of affected zones in Kerala.

• Establish the relation between land use/ temperature/ eutrophication/organic

matter/coliform/ nutrient contamination with abundance of FLAs.

• Assess the concentration of iron and correlate with the presence of FLAs in polluted surface water

• Examine the presence of Naegleria fowleri and Acanthamoeba sp. in the surface waterbodies and ground water of affected areas by culture dependent methods and molecular tools.

• Develop standards for FLAs in surface water

• Land use of the affected areas

• Identification of source of pollution in the affected areas by conducting sanitation survey using mobile application namely Envisan and development of action plan for abatement of pollution

• Suggestion on the mechanism to be adopted for cleaning of different type of waterbodies

Work plan

Surveillance of surface water bodies of affected area and sanitation survey of affected areas using mobile app for arriving at action plan for abatement of pollution

5. OPTIMIZING TREATMENT AND ENSURING DRUG AVAILABILITY

- Cornerstone of combination treatment of PAM and GAE is Miltefosine. Steps have been made to ensure continous availability of Miltefosine--KMSCL.
- An online training module on treatment of Amoebic meningoencephalitis will be made available on State Training site.

6. RESEARCH AND COLLABORATIONS

- Collaborations will be established with Division of Medical Parasitology, PGIMER Chandigarh and AVM Institute Pondicherry.
- Abiotic and biotic factors influencing growth of free living amoeba will be studied in collaboration with State pollution control board and department of environmental engineering, Kerala University.
- Possibilities for Collaboration with Indian Institute of science/other agencies will be explored for Genomic sequencing of amoeba isolates.
- A case control study will be undertaken with help of NIE-ICMR to identify the risk factors responsible for acquisition of amoebic meningoencephalitis.

- Epidemiologic profile and Natural history of all the reported cases of Amoebic meningoencephalitis in Kerala in 2024 should be published as a research article.

Annexure1

PREVENTION

Measures to prevent amebic meningoencephalitis include the following:

- Avoidance of diving and jumping into stagnant freshwater.
- Consider using nose plugs for unavoidable exposures or pinching your nose shut when diving or swimming in freshwater.
- Keep your head above water when swimming in freshwater, hot springs, and other untreated thermal bodies of water.
- When participating in water-related activities, avoid digging, or stirring up, the sediment.
- Use boiled, filtered, or sterile water for nasal or sinus irrigation, not tap water.
- Wading pools should be emptied each day
- Swimming pools/water theme parks and spas should be kept clean,chlorinated and maintained correctly
- Keep sprinklers and hoses away from noses.
- Flush still water from hoses before letting children play with them
- Nasal irrigation should only be carried out with water that is sterile, distilled, filtered (using a filter with an absolute pore size of ≤1 μm) or previously boiled and left to cool. Neti pots or other devices that are used in the practice of nasal irrigation should be rinsed after each use using sterile, distilled, filtered, or boiled water or put in the dishwasher if dishwasher safe.
- Ensure that natural waterbodies are free of organic pollutants like sewage [coliform count may be used as a surrogate marker]

If you are using unchlorinated water:

- Don’t let water to go up your nose when showering or washing your face
- Watch children playing with hoses or sprinklers
- Teach children not to squirt water up their nos

Public water supplies are chlorinated with levels high enough to protect against N. fowleri. Nevertheless, N. fowleri has been periodically identified in some water supply systems where chlorine levels have fallen below the required level.Therefore, there is a very small risk that if domestic water is allowed to warm or stagnate and chlorine levels fall (ie. a hose left in the sun or overland pipes) it can become a breeding ground for N. fowleri. If this water is then used in swimming pools or for washing out the nose, it carries with it the potential for infection.

Annexure 2

Protocol for drinking water supply systems.

The Australian Drinking Water Guidelines, published by the National Health and Medical Research Council as "Australian Drinking Water Guidelines 2011 - Version 3.5 Updated August 2018", provides an authoritative reference on what defines safe, good quality water, how it can be achieved and how it can be assured.

The Guidelines state: "Two groups of free-living amoebae, Naegleria and Acanthamoeba, have been responsible for human infections in Australia. Infection is opportunistic and generally results from contact during recreational bathing, or domestic uses of water other than drinking. Public water supplies can contaminate swimming pools. The occurrence of these organisms is unrelated to faecal contamination and their ecology in aquatic environments is more complex than that of enteric protozoa."

"Cerebral infection by Naegleria fowleri is strictly waterborne and although rare is usually fatal. Since these amoebae are able to colonise piped water supplies, disinfection at the water source may not adequately control them unless the disinfectant pervades the whole distribution system."

"Acanthamoeba species cause both cerebral and corneal disease. An environmental source of infection has rarely been identified with certainty."

As free-living environmental organisms, Naegleria are not associated with faecal contamination of water and can be detected in the absence of Escherichia coli. Whilst only Naegleria fowleri has caused amoebic meningitis, other species of thermophilic Naegleria may indicate the potential presence of Naegleria fowleri. Detection of any thermophilic Naegleria (also known as Naegleria tolerant to 42º C) in drinking water should therefore initiate corrective actions while speciation is undertaken to determine if Naegleria fowleri is present. A detection of thermophilicNaegleria in treated water may indicate that preventive measures and barriers have failed.Naegleria are most likely to enter a water supply system at the source or at breaks in the

distribution system, such as open reservoirs and tanks. Under favourable conditions, such as warm air and water temperatures, low water usage, stagnant water in pipes, inadequate chlorination and ample food source,they can proliferate in pipework and tanks. Under unfavourable conditions, Naegleria can encyst and when in this state are more resistant todisinfection, readily surviving in tank sediments and pipe biofilm. Unless chlorine residual is continuous and adequate, decystation to the active trophozoite form will remain a threat.

Protocol for drinking water providers

Naegleria entering a distribution system during a barrier breach colonise the distribution system and may re-appear in the system when adequate residual is not maintained. Biofilms that form on the internal surfaces of water supply distribution system provides a bacterial food source and protection from environmental stresses, including chlorination. Biofilms can slough off ormigrate into the drinking water from these biofilms reservoirs and make their way into the reticulation system. Managing and monitoring bores and other raw water sources and maintaining adequate, permanent disinfection throughout the treatment, storage and reticulation system are thus critical to managing thermophilic Naegleria risk in drinking water supply systems. **Maintaining a free chlorine or chloramine residual at 0.5 milligrams per litre or higher will usually control Naegleria fowleri, provided the disinfectant residual persists throughout the water supply system at all times, the turbidity is low and biofilms are well managed**. In some circumstances 0.4 milligrams per litre of free chlorine residual is acceptablein the distribution system without being in breach of requirements, provided control of thermophilic Naegleria is maintained at all times **. Investigations carried out in Western Australia have shown that while 0.4 to 0.5 milligrams per litre of free chlorine was able to control the occurrence of Naegleria fowleri in the water stream in most circumstances, it was insufficient to eradicate the protozoön from biofilms coating the interior surface of some drinking water pipes. The research established that, for these circumstances, a constant free chlorine concentration greater than 1.0 milligram per litre is able to deactivate Naegleria fowleri from the bulk water and biofilm**. **Following a thermophilic Naegleria detection, drinking water providers must aim for a free chlorine residual of at least 1 milligram per litre to re-establish control throughout the distribution system.** Drinking water providers are reminded that in most circumstances and water temperatures , disinfection of drinking water supplies by ozonation or ultra violet irradiation only (that is, without a later step involving chlorination or chloramination) is unable to reliably control amoeba or biofilms throughout larger distribution systems, but may be effective in well-designed small distribution systems with cooler water.

Monitoring requirements

Routine monitoring of drinking water supplies for thermophilic amoebae (the most significant of which is Naegleria fowleri) is required during the months of the year when water temperatures within the distribution system are likely to exceed 20º Celsius.

Drinking water providers must establish and monitor temperature profiles of water within the distribution system to determine the months of the year when the water temperature is likely to exceed 20º Celsius. While amoebae may be detected below 20º Celsius, they are expected to

encyst and to reduce to low numbers. Cysts are not believed to be infective, as they have not been recovered from brain tissue or cerebrospinal fluid. Sampling of water below 20º Celsius is optional. Samples should be collected from the distribution system for thermophilic amoeba analysis at the same frequency and place as bacteriological samples, upstream of any end-of-line fitting in place that either filters or chills the water, or makes ice. This is because samples obtained from point-of-use filtered or chilled locations may underestimate the true risk, as they are not representative of the bulk distributed water received at fixtures such as shower heads or garden hoses where water may enter the nasal passage. Drinking water providers who manage significant building plumbing or circulation systems that involve chilling water should ensure that their water chiller management protocols are compatible with the amoeba monitoring requirements**. Unlike samples for bacteriological analysis, samples for amoeba analysis should not be chilled when sent to the laboratory.**

A water sample of 250 millilitre volume is considered acceptable, although some techniques utilise a 500millilitre sample volume. Samples must be transported at ambient temperature (do not include ice blocks) to the analysing laboratory within 24 hours (recommended) or within 96 hours (acceptable). Samplers are reminded to ensure that water temperature and chlorine residualare also recorded with each sample, as well as clear labelling of site codes and text descriptors of the location being sampled, as well as any other relevant Chain of Custody requirements.

Testing of freeliving amoeba in water samples must be performed by an accredited laboratory. It is only necessary to confirm the species as either N. fowleri or not fowleri, as per the Polymerase Chain Reaction (PCR) method, which gives the quickest result. Other methods that involve speciation are acceptable, but are disadvantaged by being slower. The PCR method examines water for free-living thermophilic amoebae by culturing on a seeded non-nutrient agar plate, followed by microscopic identification.

Amoebae are identified by the presence of cysts and sometimes trophozoites. Genus identification is possible at the cyst stage by their characteristic size and shape. The result will be reported as:

Thermophilic amoebae	**Not Detected or Detected / 250 mL**
Thermophilic *Naegleria*	**Not Detected or Detected / 250 mL**

If thermophilic *Naegleria* has been detected, then the report will state:

Naegleria fowleri	**Pending**

Identification of *Naegleria fowleri* is performed by the Molecular Diagnostics laboratory using Real-time PCR. This result will be available 1-3 days after thermophilic *Naegleria* has been identified by microscopy. The result will be reported as:

Naegleria fowleri	**Not Detected or Detected**

Please note that remediation is required upon detection of thermophilic *Naegleria*. Hence, the drinking water supplier (or client requesting the analysis) should ensure that arrangements have been made for the analysing laboratory to advise the drinking water supplier of this result as soon as possible, both by email and telephone communication.

Action by the drinking water supplier should be initiated upon confirmation of thermophilic *Naegleria*, not be delayed until the presence of *N. fowleri* is established.

ADVICE FOR RECURRENT DETECTIONS

With respect to *Naegleria* species, the requirement for drinking water supplies is that:

 No sample of drinking water should contain *Naegleria fowleri*;

 No more than one water sample collected in any 12 month period in each locality supplied by the water supplier shall contain *Naegleria* species tolerant to 42° Celsius or above; and

 If *Naegleria* species tolerant to 42° Celsius or above (i.e. thermophilic *Naegleria)* are detected, immediate corrective action must be taken.

More than one thermophilic *Naegleria* event in a drinking water supply in a 12 month period per locality is thus considered to be a recurrent problem.
Drinking water providers must follow a risk management protocol which, depending on the characteristics of the system, should include the following actions to prevent further detections or continue the response to an ongoing problem:
 Review operational limits to ensure a chlorine residual of at least 0.5 milligrams per litre persists in all parts of the reticulation at all times;

 Install chlorine boosters to ensure continuous residual above 0.5 milligrams per litre (where drinking water is piped over long distances);

- Increase cleaning frequency to minimise sediment build-up in storage tanks or reservoirs;

- Introduce flushing and scouring programs to control biofilm and sediment build-up in reticulation;

- Increase tank turnover rates and investigate potential for dead legs or stagnant areas, to reduce water age in the system.

- Maintain complete records of any incident or detection, including any short term and long term responses, and ensure that system operational plans remain up-to-date.

- Keep the public informed of any relevant update in circumstances, especially if the circumstances necessitate a change in consumer behaviour from the status quo.

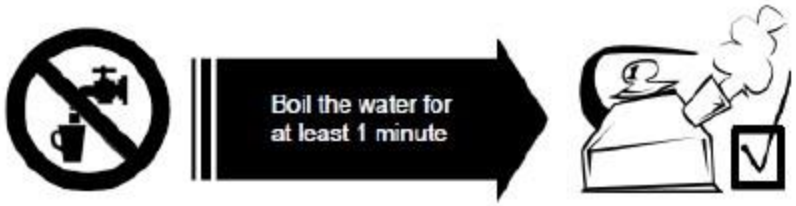
- Assess raw water sources and other drinking water supplies systems managed by that drinking water provider to determine if they are similarly susceptible to contamination.

Drinking water providers are also reminded that it is possible that a thermophilic *Naegleria* detection may coincide with an *E. coli* detection or some other adverse event in the same supply system. The responses to these detections need to run simultaneously.
Drinking water providers must also ensure that all *Naegleria* related incidents in a drinking water supply are included in the quarterly drinking water quality reports reported to the Department of Health, as well as any published annual water quality report.

Annexure-3

Public Health Messaging Examples

Water quality alert: BOIL ALL WATER



After boiling the water allow it to cool

Then the boiled water can be used for drinking, bathing, washing dishes, brushing teeth and food preparation.

CAUTION: Cool boiled water before use and keep children away from boiling water.

Water quality alert: playing with water

Also, be careful when playing with water or in the bath. Anyone playing in or with water needs to avoid getting water up their nose.

Water quality alert: NO PLAY

Water quality alert: playing with water

Be careful when playing with water, showering or in the bath. Anyone playing in or with water needs to avoid getting water up their nose.

References

1.Naegleria Response protocol for drinking water: Government of Western Australia, Department of Health.

2.Technical guidelines on prevention,diagnosis and treatment of amoebic meningoencephalitis in Kerala .

3. Stahl LM, Olson JB. Environmental abiotic and biotic factors affecting the distribution and abundance of Naegleria fowleri. FEMS Microbiol Ecol. 2020 Dec 30;97(1):fiaa238. doi: 10.1093/femsec/fiaa238. PMID: 33242082; PMCID: PMC8068756.