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**Field Epidemiology & Disease Surveillance Division**  
**Focal Point for International Health Regulations**  
**National Institute of Health, Islamabad**  
**Ministry of National Health Services, Regulations & Coordination**  
Phone: (92-051) 9255117, Fax: (92-051) 9255099

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**WHO Collaborating Centre for Research & Training in Viral Diagnostics**

13/7 July 2016

**Subject: Advisory; Detection, Prevention and Control of Naegleria Fowleri Infection/  
Primary Amebic Meningoencephalitis (PAM)**

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In Pakistan since 2012, a total of 40 fatal documented cases were reported from different tertiary care hospitals of Karachi (12 fatal cases during 2015). One fatal at Karachi case reported 30<sup>th</sup> June 2016. As the disease is endemic in some areas of Karachi, so it is imperative to be vigilant in detection of any suspected case especially in persons having symptoms compatible with PAM. The objective of this advisory is to sensitize health care authorities to further strengthen and improve the level of preparedness in prevention and control of PAM Infection.

2. Primary Amebic Meningoencephalitis (PAM) is a rare disease with mortality rate of greater than 95%. The causative agent is *Naegleria Fowleri*, a thermophilic, free-living amoeba found in freshwater environments. Since detection of first case in Australia during 1965; more than 300 documented cases have so far been reported from 16 countries. Clinical features of PAM are similar to meningitis; include headache, fever, stiff neck, anorexia, vomiting, altered mental status, seizure, and coma. Death typically occurs in 3–7 days.

3. Most *Naegleria Fowleri* infections are associated with swimming in warm freshwater lakes. *Naegleria fowleri* can grow in public and private water tanks and pipes, especially with little or no disinfectant. This infection has also been reported when people rinse their sinuses through the nose, and cleanse their noses during religious practices using contaminated tap or faucet water.

4. **Prevention & Control:** Both trophozoites and cysts forms are sensitive to adequate levels of chlorination. The Municipal Public Health Authorities therefore, must ensure that adequate levels of chlorine are maintained in the supplied tap water along with strict monitoring arrangements. Awareness and education in the affected areas must also be undertaken to educate people on requisite preventive measures.

5. **Enhancing Disease Surveillance and Notification:** Strengthening Surveillance and notification of PAM infection should be enhanced with the dissemination of standard case definitions and diagnostics to areas of transmission and at-risk areas.

6. **Risk Communications:** Symptoms of *N. Fowleri* infection are clinically similar to meningitis, further lowering the index of suspicion. Making doctors more aware about the disease might improve case detection. Such information might enable earlier detection of infections, provide insight into the human or environmental determinants of infection, and allow improved assessment of treatment effectiveness.

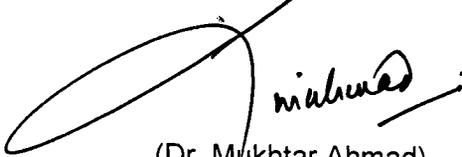
Rarely, infections have been associated with people submerging their heads or irrigating their sinuses (nose) by using heated and contaminated tap water. In such cases, the

monitoring and maintaining adequate levels of chlorine in the tap water at the consumer's end may yield desired results.

7. **Clinical care:** Any of the suspected cases should immediately be reported to health authorities for corrective measures. In 02 documented cases of survival, the combination of three drugs; **Amphotericin B** (IV+/Intrathecal), Rifampicin (Oral 10 mg/Kg/day) and **Fluconazole** (IV or oral 10 mg/kg/day) was used along with steroids. **Azithromycin** has both *in vitro* and *in vivo* efficacy against *Naegleria fowleri* and may be tried as an adjunct to AMB. Intensive supportive care is required.

8. The NIH has already communicated about PAM in the 34<sup>th</sup> and 35<sup>th</sup> issue of Seasonal Awareness and Alert Letter (SAAL) disseminated to the healthcare providers and management authorities. It is imperative that the concerned public sector departments be motivated to enhance the preventive efforts / steps. The situation may please be continuously monitored and updates along with the actions taken be kindly communicated to the NIH regularly on phone No. +92-51-9255237, Fax: +92-51-9255575, E-mail: eic.nih@gmail.com. The updated Guidelines for the prevention and control of Primary Amebic Meningoencephalitis (PAM) is also available on NIH website: [www.org.pk](http://www.org.pk) also annexed herewith for ready reference.

9. This communication may please also be distributed to the districts health officials for information.



(Dr. Mukhtar Ahmad)  
Executive Director

Distribution list Attached

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- vii. PS to the Secretary, Ministry of National Health Services, Regulations & Coordination, GoP, Islamabad
- viii. Focal Person FELTP, FDSRU, NIH Islamabad



## Guidelines and Technical Information for the Prevention and Control of Primary Amebic Meningoencephalitis (PAM) Caused by *Naegleria fowleri*

Updated: July 2016

### **Background:**

The Primary Amebic Meningoencephalitis (PAM), a rarely occurring disease is caused by a parasite, *Naegleria fowleri*; highly fatal disease having CFR.99%. First detected in Australia during 1965; since then about 300 cases have so far been reported from 16 countries. In Pakistan cases have been reported from different tertiary care hospitals of Karachi since 3<sup>rd</sup> July 2012 and to date a total of 45 fatal cases (01 during 2016) have been reported.

The *Naegleria fowleri* is naturally found in warm freshwater, transmitted primarily through inhalation of infested water during swimming or putting contaminated water in nostrils during ablution. Disease however, does not occur by drinking contaminated water or by swimming in the sea. Symptoms usually start from 1-7 days after exposure, while death may occur in 1-12 days of illness. Because of rapid progression, the diagnosis is usually made after death.

The municipality & public health authorities must ensure adequate levels of chlorine are maintained in the supplied tap water along with strict monitoring arrangements as the parasite is sensitive to chlorine. Awareness and education in the affected areas must also be undertaken to educate people on requisite preventive measures.

### **1. The Agent:**

*Naegleria fowleri* (commonly referred to as the "brain-eating amoeba"), is a single-celled free-living microscopic and heat-loving amoeba found around the world. The organism grows best at higher temperatures up to 46°C and is naturally found in warm freshwater environments such as lakes, rivers, canals, hot springs, warm water discharge from industrial or power plants, poorly maintained or minimally chlorinated swimming pools, water heaters and soil, where it feeds on bacteria and other microbes.

### **2. Transmission:**

The infection usually occurs by entry of the contaminated water through the nose i.e. swimming or diving in freshwater places or putting contaminated water to the nose during ablution with inadequately chlorinated pool or contaminated tap water. The amoeba migrates to the brain along the olfactory nerve causing serious disease. Infections however do not occur by drinking contaminated water.

### **3. The Illness:**

PAM is a rare but almost invariably fatal disease of the central nervous system. In US, only 01 out of 123 cases reported during 1962 to 2011 could survive whereas the other documented survival occurred in Mexico. From subsequent laboratory experiments performed on the California survivor's strain, it appears that the lesser virulence of the infecting strain might have contributed to his recovery.

### **3.1 Signs and Symptoms:**

Clinical similarity to bacterial meningitis increases chances of misdiagnosing PAM. Symptoms usually start 1-7 days after exposure with death in 1-12 days of illness.

Because of rapid progression, the diagnosis is usually made after death.

**Stage 1** is characterized by severe frontal headache, fever, nausea and vomiting. Usual presenting symptoms include severe headache, meningeal signs, fever, vomiting, seizures, and focal neurologic deficits. Other symptoms such as photophobia, mental status abnormalities, lethargy, dizziness, ataxia, cranial nerve palsy, hallucinations, and delirium have also been reported. Moreover in some cases, abnormalities in taste or smell, nasal obstruction, and nasal discharge have been observed.

**Stage 2:** Stiff neck, Seizures, Altered mental status, Hallucinations and coma rapidly progress to death. Autopsy findings include hemorrhagic necrosis of the olfactory bulbs and cerebral cortex.

### 3.2 Diagnosis:

Cerebrospinal fluid (CSF) studies of PAM patients typically demonstrate a pattern similar to bacterial meningitis with an elevated opening pressure, a polymorphonuclear pleocytosis, normal or low glucose, and elevated protein. However the observations of blood in the CSF and/or motile ameba serve as clues for potential diagnosis of PAM.

#### Diagnostic Options:

##### Direct Visualization in CSF:

The diagnosis can be made most quickly by microscopic examination of fresh, unfrozen, unrefrigerated cerebrospinal fluid (CSF). Frozen or refrigerated samples are not appropriate because cold temperatures kill the amebae.

- i. A wet mount of freshly-centrifuged CSF sediment might demonstrate actively moving trophozoites in a generally linear forward direction.
- ii. Additionally, *Naegleria* can be identified in CSF smears or cultures using hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), trichrome, Giemsa, or Wright-Giemsa stains. A stained smear will show ameboid trophozoites with morphology typical of *Naegleria* i.e. Nucleus with a centrally located, densely staining large nucleolus.

##### Note:

- a. Gram stain must be avoided as the amebae gets destroyed during heat fixation.
- b. If amebae are identified in the CSF, the diagnosis should be subsequently confirmed with culture, PCR, or immunohistochemical (IHC) tests.

##### Direct Visualization in Tissue:

The diagnosis can be made from microscopic examination of stained smears of brain biopsy or autopsy specimens demonstrating trophozoites with typical morphology

##### Immunohistochemical staining:

Techniques, such as indirect immunofluorescent antibody (IFA) staining and immune alkaline phosphatase staining (IHC), use an antibody specific for *Naegleria fowleri* followed by microscopy to identify *Naegleria fowleri* in tissue, culture, or CSF.

##### Culture:

Culture is a routine procedure used to identify free-living amebae in clinical and environmental specimens. It involves inoculating mammalian cell cultures and monitoring for cytopathogenicity, or inoculating plates of *E. coli* lawns and monitoring for tracks where the amebae have moved through the lawn eating the bacteria. A negative culture result however, does not rule out the presence of free-living amebae.

### 3.3 Treatment:

In 02 documented cases of survival, the combination of three drugs; **Amphotericin B** (IV+/Intrathecal), **Rifampicin** (Oral 10 mg/Kg/day) and **Fluconazole** (IV or oral 10 mg/kg/day) was used along with steroids. **Azithromycin** has both *in vitro* and *in vivo* efficacy against *Naegleria fowleri* and may be tried as an adjunct to AMB.

### 4. Epidemiological Highlights and Risk Factors:

- Since reporting of first cases in Australia during 1965, the infections have now been reported from across the world.
- 80% reported cases in US are <18 years of age; more than 75% being males. The reason is unclear but may reflect the types of water related activities.
- Available data is not sufficient to accurately estimate the PAM risk however; infections are mostly linked to nasal exposure to contaminated drinking water
- The ameba has never been shown to have spread from one person to another. It remains unknown why certain persons become infected out of millions exposed to similar water activities.
- Both trophozoites and cysts forms are sensitive to adequate levels of chlorination.
- Drying makes trophozoites nonviable instantaneously and cysts in <5 minutes.
- *Naegleria fowleri* does not survive in sea water because of salinity.
- Because of environmentally tough cysts, no means are yet known to control the natural *Naegleria fowleri* levels in lakes and rivers.

### 5. Prevention & Control:

- Both trophozoites and cysts forms are sensitive to adequate levels of chlorination. The Municipal Public Health Authorities therefore, must ensure that adequate levels of chlorine are maintained in the supplied tap water along with strict monitoring arrangements. Awareness and education in the affected areas must also be undertaken to educate people on requisite preventive measures.
- Rarely, infections have been associated with people submerging their heads or irrigating their sinuses (nose) by using heated and contaminated tap water. In such cases, the monitoring and maintaining adequate levels of chlorine in the tap water at the consumer's end may yield desired results.
- The Health education and personal actions should focus on:
  - a. Limiting the amount of water going up the nose
  - b. Lowering the chances that *Naegleria fowleri* may be in the water.
- People should accordingly avoid getting water too deep into their nostrils and ensure adequate treatment of their water supply besides avoiding swimming in infected water.
- Patients with suspected symptoms should seek medical help immediately.
- **Swimming pools/water disinfection:**
  - DO keep your swimming pool adequately disinfected before and during use. Adequate disinfection means:
  - Pools: free chlorine at 1–3 parts per million (ppm) and pH 7.2–7.8

- Hot tubs/spas: free chlorine 2–4 parts per million (ppm) or free bromine 4–6 ppm and pH 7.2–7.8
- If you need to top off the water in your swimming pool with tap water, **DO** place the hose directly into the skimmer box and ensure that the filter is running.
- **DO NOT** top off by placing the hose in the body of the pool.

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