

REVIEW

PRIMARY AMEBIC MENINGOENCEPHALITIS AND *NAEGLERIA FOWLERI*: AN UPDATE

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Abstract. Free living amebae, *Naegleria* spp, are found in natural and recreation water worldwide. Pathogenic *N. fowleri* causes primary amebic meningoencephalitis (PAM), which leads to lethal infection of the central nervous system in humans and animals. PAM and bacterial meningitis have similar clinical manifestations, such as high grade fever with flu-like symptoms, nausea, vomiting and neurological changes, thereby leading to possible misdiagnosis. As PAM progresses rapidly, diagnosis is usually made after death. This review describes recent incidents of PAM in particularly those in Thailand due to water irrigation, pathogenesis and detection of *N. fowleri* in Thailand.

Keywords: *Naegleria fowleri*, distribution, PAM incidence, primary amebic meningoencephalitis (PAM), Thailand

INTRODUCTION

Being a free-living amoeba in the environment, *Naegleria fowleri* trophozoites feed on bacteria using pseudopodia (Siddiqui *et al*, 2016). In addition, trophozoites use their pseudopodia for actively progressive and directional movement. Trophozoite switches to a flagellate stage for feeding more food as this allows long distance movement. When the environment becomes unfavorable, trophozoite switches into a dormant form (cyst),

which is non-feeding and non-reproducing (Siddiqui *et al*, 2016).

N. fowleri is a causative agent of fulminant brain infection, also known as primary amebic meningoencephalitis (PAM). In 1962, the first case was found by brain autopsy samples in Florida, USA (Butt, 1966). However, cases of PAM have been reported in Virginia, USA since 1937 (Gustavo, 1970). There were several cases of PAM found in Australia (1965), Europe (1973), Asia (1978) and Africa (1980) (De Jonckheere, 2011). The root of infection of PAM is commonly through activity in *N. fowleri* infected water; however two fatal cases of PAM without a history of swimming were reported from South India (Shenoy *et al*, 2002). In Thailand, PAM was first reported in Si Sa Ket Province

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in 1983 (Jariya *et al*, 1983). Sporadic cases have since been reported in many provinces of the country: Nakhon Pathom, Samut Prakan and Suphan Buri (central region), Trat (eastern), and Si Sa Ket and Surin (north eastern) (Wiwanikit, 2004; *ibid*, 2016).

Previous studies have documented intracellular multiplication of *Legionella* enclosed in vacuoles within mitochondria of *N. fowleri* trophozoite (Newsome *et al*, 1985). Under these conditions, amebae provide both nutrition for proliferation and intracellular environment conducive to multiplication of *L. pneumophila* (Newsome *et al*, 1985). The coexistence of *L. pneumophila* is abetted by biosynthesis of a mucopolysaccharide matrix, enabling them to colonize free-living amebae, leading to a risk of *Legionella* infection in humans (Żbikowska *et al*, 2014).

Herein, we review cytopathogenesis of PAM, *N. fowleri* identification, distribution of *N. fowleri* and incidences in Thailand, and chemotherapy.

CYTOPATHOGENESIS

Naegleria trophozoite mainly invades the central nervous system (CNS) through passage of the nostrils (Visvesvara, 2010). Once in the brain, trophozoites secrete a variety pathogenic enzymes, which damage red blood and white blood cells, and brain tissue leading to hemorrhagic-necrotizing meningoencephalitis (Siddiqui *et al*, 2016). The inflammation of brain occurs during the infection by trophozoites activating the innate immune system, neutrophils and macrophages (Marciano-Cabral and Cabral, 2007; Tiewcharoen *et al*, 2017, in press).

Cytopathogenesis of *N. fowleri* towards mammalian cells has been studied through analysis of cellular interaction

(Grace *et al*, 2015). Human neuroblastoma SK-N-MC cells provide the best model of neuropathogenesis in PAM patients (Tiewcharoen *et al*, 2008a). The process of *N. fowleri* infection in SK-N-MC cells demonstrated engulfment of the ameba at the cell surface of a food-cup structure, followed by feeding on the host cell nutrients (Tiewcharoen *et al*, 2008b). During infection, the ameba up-regulates acetyl Co-A synthetase, isocitrate dehydrogenase, pore B precursor pyruvate kinase, and 18S rRNA transcript for destruction of host cell. The ameba also releases soluble factors to regulate proteins involved in host cell signal transduction (Tiewcharoen *et al*, 2012). In addition, the infected host cells secrete soluble proteins involved in angiogenesis, fatty acid metabolism, signal transduction, translation, stress response, signal pathways, and respiration of neighboring cells, leading to inflammation and severe damage to host cells and finally death (Tiewcharoen *et al*, 2013).

IDENTIFICATION OF *N. FOWLERI*

N. fowleri can be identified in the environment through morphological examination and, in the laboratory, by molecular techniques (CDC, 2017). Using *N. fowleri* cultured in non-nutrient agar supplemented with heat-killed *Escherichia coli*, it is possible to obtain all three forms of *N. fowleri*, namely, trophozoite (Fig 1a), flagellate (Fig 1b) and cyst (Fig 1c). All stage of *N. fowleri* is characterized by a large nucleolus and halo in the nucleus.

One useful molecular tool for rapid species differentiation and genotyping is by analysis of PCR-amplified *N. fowleri* internal transcribed spacer (ITS) region of 5.8S ribosomal (r)DNA gene (450 bp), NF gene (310 bp), and *nfa1* (360 bp) (Jeong *et al*, 2004, Tiewcharoen *et al*, 2007). In addition,

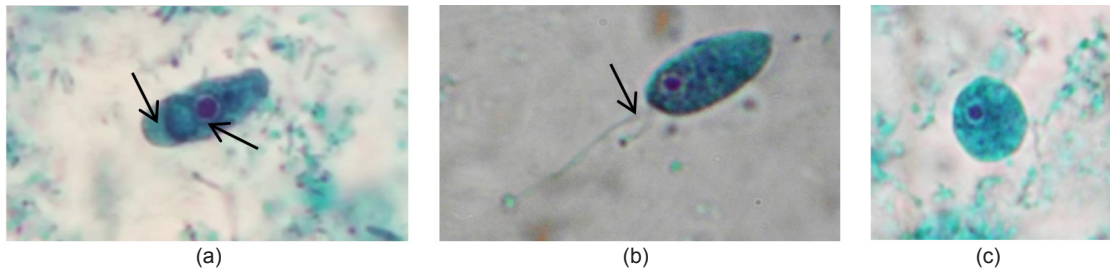


Fig 1—Photomicrograph of *Naegleria fowleri*. (a) Trophozoite (stained with Gormori's trichrome) showing semispherical pseudopodia (left arrow) and large nucleolus with halo (right arrow) in nucleus. (b) Pear-shaped flagellate form with two flagellae (arrow). (c) Spherical cyst. Magnification, 1,000x.

sequencing [5.8S ribosomal (r)DNA gene] of *N. fowleri* isolated from brain autopsies of three patients at Chachoengsao, Ramathibodi, and Siriraj hospitals, and one environmental sample from Khon Kaen Province, Thailand allowed their identification as Cattenom Japanese J16(1) 42 E strain (Tiewcharoen *et al*, 2007).

DISTRIBUTION OF *N. FOWLERI* AND INCIDENCES OF INFECTION

The geographical range of *N. fowleri* has been expanding worldwide over the past few years (Kemble *et al*, 2012; Capewell *et al*, 2015). A survey of *N. fowleri* was carried out in the central region of Thailand (Lopburi, Pathum Thani, and Samut Prakan Provinces) (Jariya *et al*, 1997). During 2001-2003, pathogenic *Naegleria* spp were identified in Bangkok (Taling Chan District), and in Chumphon, Nakhon Nayok, Nakhon Sawan, Prachuap Khiri Khan, Saraburi, Sukhothai and Surat Thani Provinces (Tiewcharoen *et al*, 2004). *N. fowleri* was also found in fresh water samples from Wang Lang and Wat Rakhang piers of Chao Phraya river, Bangkok employing PCR amplification of *N. fowleri nfa1* (Tiewcharoen *et al*, 2018 in press). Using the same technique (we

also identified *N. fowleri* in four tourism locations, namely, Khun Dan Prakan Chon Dam and Nang Rong Waterfall (Nakhon Nayok Province), Bo Khlueng Hot Spring (Ratchaburi Province) and Pha Wang Palace (Nakhon Pathom) (unpublished data).

In Thailand, the first case of PAM was reported in 1983 from Si Sa Ket Province in the northeastern region (Jariya *et al*, 1983). Since then, sporadic cases of PAM were reported in the central (5 cases) (Charoenlarp *et al*, 1988; Sirinavin *et al*, 1989; Wattanaweeradej *et al*, 1996; Viriyavejakul *et al*, 1997; Sithinamsuwan *et al*, 2001), northern (Petchsuwan and Jariya, 1997), northeastern (4 cases) (Poungvarin *et al*, 1991; Chotmongkol *et al*, 1993; Jariya *et al*, 1997; Bunjongpak, 2000), and eastern (2 cases) (Somboonyosdej and Pinkaew, 1987) regions of the country.

During 2007-2016, a number of cases had a history of recreational or tap water irrigation (3 cases) and tap water backyard 'slip-n-slide' contact (1 case) (Shenoy *et al*, 2002; De Jonckheere, 2004; CDC, 2017). PAM might be more common among young boys due to their greater propensity for activities exposing them to such risks (Capewell *et al*, 2014). Owing to the difficulty in diagnosing PAM, 75% of cases

were identified only after autopsy (da Rocha-Azevedo *et al*, 2009; Visvesvara, 2010). In Thailand, two fatal PAM cases were reported involving recreational or tap water irrigation. In one case, a 12-year old Thai boy from Udon Thani Province with a history of exposure to a school swimming pool water rapidly developed acute severe meningoencephalitis within a week and ultimately died (Pudthasa *et al*, 2016). In the other case, a 71-year old female Norwegian tourist became infected with *Naegleria* by nasal irrigation through contaminated tap water at an apartment at Pathaya. Subsequent brain autopsy revealed an accumulation of *Naegleria* trophozoites around the intra-parenchymal vessels with more heavy inflammatory infiltrates (Stubhaug *et al*, 2016).

CHEMOTHERAPY

The high death rate of PAM is still problematic. *N. fowleri* causes fatal CNS infection and there are no clinical trials accessing the efficacy of one treatment regimen over another. Anti-fungal amphotericin B has been the drug of choice for treating PAM (Vargas-Zepeda *et al*, 2005); however, the administration of amphotericin B is limited due its high toxicity to renal function (Laniado-Laborín and Cabrales-Vargas, 2009). A number of *in vitro* and *in vivo* amphotericin B combination therapies (Goswick and Brenner, 2003; Soltow and Brenner 2007; Tiewcharoen *et al*, 2009) as well as multidrug treatment with amphotericin B (Linam *et al*, 2015) against *N. fowleri* have been reported with limited success.

At present, miltefosine, an anti-leishmanial, is the most effective and safe drug for the treatment of PAM (Cope *et al*, 2016). The Centers for Disease Control and Prevention, USA recommends miltefosine

for PAM treatment as successful treatment instead of amphotericin B or antimicrobial agents (CDC, 2017). However, it is worth bearing in mind miltefosine also shows serious adverse reactions, such as Stevens-Johnson syndrome, renal and liver toxicity (Sunder and Olliaro, 2007).

In Thailand, *in vitro* studies on the effects of antifungal drugs against *Naegleria* trophozoites demonstrated ketoconazole (0.125 µg/ml) has higher activity than amphotericin B (0.5 µg/ml) (Tiewcharoen *et al*, 2002). However, ketoconazole has the adverse effect on liver (Yan *et al*, 2013). Chlorpromazine shows higher efficacy and rapid activity against *Naegleria* trophozoites than amphotericin B, causing a down regulation of *nfa1* and *Mp2CL5* expression (Tiewcharoen *et al*, 2011). The side effects of chlorpromazine; acute dystonia and dyskinesia, are more frequent in children and young adults, appearing within the first four days of treatment (Brunton *et al*, 2010).

As a result of these adverse effects from the use of synthetic drugs, traditional herbs were recommended as alternatives (WHO, 2000). *Andrographis paniculata*, *Curcuma longa*, *Momordica charantia*, and *Zingiber officinale* were tested for anti-amebic activity. The minimal non-toxic dose of *A. paniculata* (0.5 mg/ml) and *M. charantia* (5 mg/ml) have 100% inhibitory activity against *N. fowleri* trophozoites in human neuroblastoma SK-N-MC or rhesus LLC-MK2 cells (Luangboribun *et al*, 2014). Diosgenin purified from *M. charantia* has a number of mechanisms attributed to its amebocidal activity: (i) inhibition of food cup formation, (ii) down-regulation of a cysteine protease gene expression, and (iii) anti-inflammation activity on human monocyte-derived macrophage U937 after exposure with LPS or *N. fowleri* lysate

through blocking TNF- α synthesis (Rabablert *et al*, 2015; *ibid*, 2016; Tiewchareon *et al*, 2017, in press).

CONCLUSION

The presence of *N. fowleri* in Thailand in aquatic water resources poses health risk to people. Obviously, considering the limited understanding of life cycle of *N. fowleri* cannot be excluded that other cases escaped diagnosis. The increasing knowledge of *N. fowleri*: cytopathogenesis, identification, distribution and chemotherapy can be an approach to alert the health professionals. The health authorities concerned should be aware of the possible hazards and provide guidelines to ensure safety of Thai people.

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