

NAEGLERIA FOWLERI IN THAILAND, 2003

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Abstract. Between 1988 and 2003, *Naegleria fowleri* was studied extensively in Thailand. The distribution of *N. fowleri* has been studied in many provinces since 1988. During 2001-2003, we received financial support from Mahidol University to study the distribution of pathogenic *Naegleria* spp in water reservoirs in central, northern, and western parts of Thailand. With these funds, research was conducted in Bangkok (Taling Chan District), Saraburi, Nakhon Nayok, Nakhon Sawan, Sukhothai, Prachuap Khiri Khan, Surat Thani and Chumphon Provinces. Studies indicated that pathogenic strains of *Naegleria* belonging to the species *fowleri* were found in Saraburi and Surat Thani, and could be identified by external morphology, molecular weight, isoenzyme patterns and cytopathogenicity. However, the development of therapeutic drug treatment is still problematic because of the high mortality rate and rapid progression of disease. Since 1970, the effects of numerous drugs and chemical agents have been studied; there are continuing efforts to develop new drugs *in vitro* and *in vivo*, because the current treatment of primary amebic meningoencephalitis remains ineffective for most patients. A study of the *in vitro* effect of various antifungal drugs and the drug combination 5-fluorouracil and amphotericin B on pathogenic *Naegleria* spp was published in 2002. It was concluded that amphotericin B, in combination with 5-fluorouracil was still the most effective treatment for pathogenic *Naegleria* spp infection. In addition, the IC₅₀ of this drug combination was significantly lower than that of amphotericin B and 5-fluorouracil alone. Moreover, this drug combination has high synergistic activity and few side-effects in patients. These results emphasize the need for prompt treatment at an adequate minimum dosage by intrathecal route to arrest the disease.

INTRODUCTION

The free-living ameba, *Naegleria fowleri*, is the causative agent of primary amebic meningoencephalitis (PAM), a fatal disease of the central nervous system. *Naegleria fowleri* infection is acquired while swimming or diving in freshwater lakes, ponds and man-made pools (Sugita *et al*, 1999). Although PAM is rare, patients almost always die within 5-7 days of infection. The speed with which the disease progresses stresses the need for an overview study of its distribution (Tiewcharoen *et al*, 2001) identification, pathogenicity (Jarolim *et al*, 2000) and of the amebicidal drugs used to treat it (Tiewcharoen *et al*, 2003). Accurate information supplies the basic knowledge the Thai Department of Public Health needs to plan effective strategies to prevent and control this disease.

DISTRIBUTION AND IDENTIFICATION

The initial description of *Naegleria fowleri* was published in 1970 (Carter, 1970). The first case of PAM in Thailand was reported from Si Sa Ket Province

(Jariya *et al*, 1983). During the period 1983-2003, five cases were found in Thailand: 1 in Bangkok, 2 in Trat, 1 in Nakhon Pathom, and 1 in Suphan Buri Province (Somboonyosdech *et al*, 1987; Sirinavin *et al*, 1989; Pongvarin and Jariya, 1991). From the available information, it is reasonable to conclude that *Naegleria fowleri* is found primarily in central, eastern, and northeastern parts of Thailand (Jariya *et al*, 1988) corresponding to the provinces where PAM occurred. According to published survey reports, a thermophilic *Naegleria* spp (42°C) was found in Lopburi (Jariya *et al*, 1997) and in Bangkok (Taling Chan District) (Tiewcharoen *et al*, 2004). After this survey, isolation and specific identification of the pathogenicity of *Naegleria* spp in central, northern, and western parts of Thailand (Saraburi, Nakhon Nayok, Nakhon Sawan, Sukhothai, Prachuap Khiri Khan, Chumphon, and Surat Thani provinces) was carried out. Isoenzyme patterns of the ameba isolates were significantly positive for malic, malate dehydrogenase and esterase enzymes when compared with those of the reference strains (Figs 1-5). These results showed that the distribution of thermophilic *Naegleria* spp in Saraburi was 0.39% (1/258), and 0.77% (2/258) in Surat Thani Province. It was concluded that biochemical analysis using the selection of isoenzymes was necessary to distinguish *Naegleria fowleri* from many strains of thermophilic *Naegleria* spp. However, the isoenzyme method of analysis yielded a correlation of intraspecific isoenzyme variations. Currently, there are plans to use

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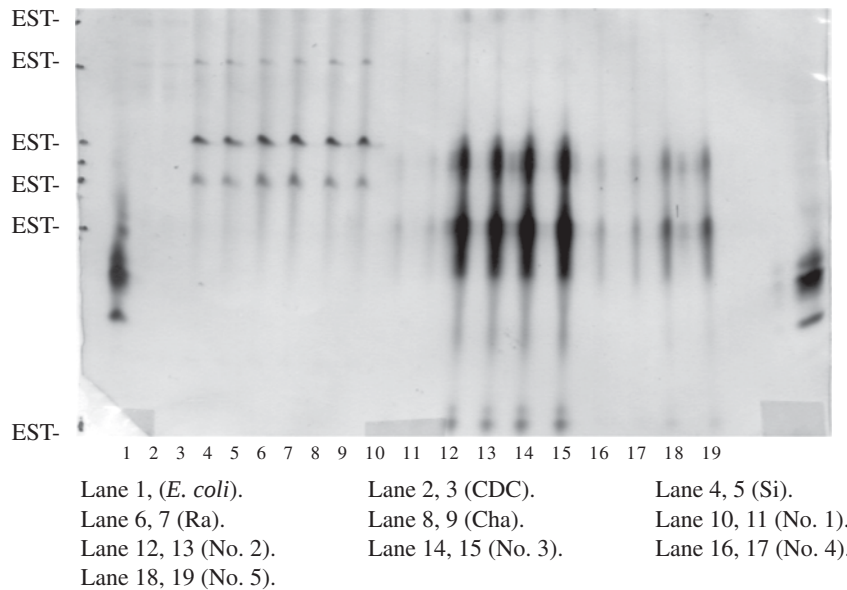


Fig 1- Zymogram pattern of esterase of four strains of *Naegleria fowleri* and *Naegleria* species isolated from natural water sources in Bangkok.

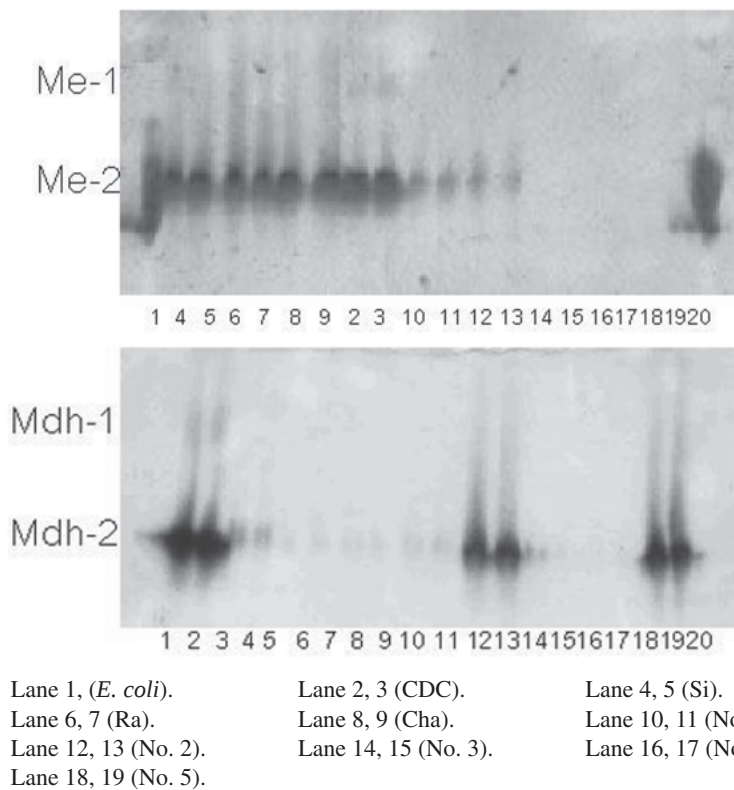
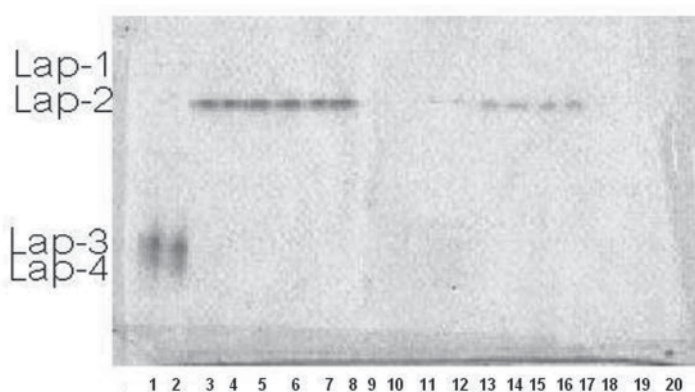


Fig 2- Zymogram pattern of malic enzyme and malate dehydrogenase of four strains of *Naegleria fowleri* and *Naegleria* species isolated from natural water sources in Bangkok.

Lane 1, 2 (*E. coli*).

Lane 3,4 (CDC).

Lane 5, 6 (Si).

Lane 7, 8 (Ra).

Lane 9, 10 (Cha).

Lane 11, 12 (No. 1).

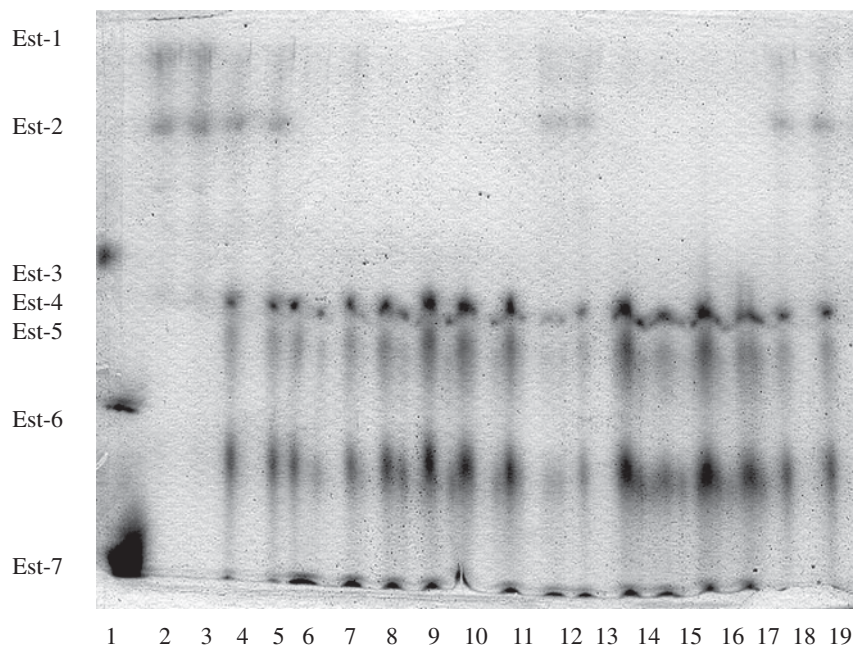
Lane 13, 14 (No. 2).

Lane 15, 16 (No. 3).

Lane 17, 18 (No. 4).

Lane 19, 20 (No. 5).

Fig 3- Zymogram pattern of leucine aminopeptidase of four strains of *Naegleria fowleri* and *Naegleria* species isolated from natural water sources in Bangkok.

Lane 1, (*E. coli*).

Lane 2, 3 (CDC).

Lane 4, 5 (Ra).

Lane 6, 7 (N1).

Lane 8, 9 (N2).

Lane 10, 11 (N3).

Lane 12, 13 (C1).

Lane 14, 15 (C2).

Lane 16, 17 (S1).

Lane 18, 19 (S2).

Fig 4- Esterase banding patterns of thermophilic *Naegleria* spp from central, northern, and southern parts of Thailand.

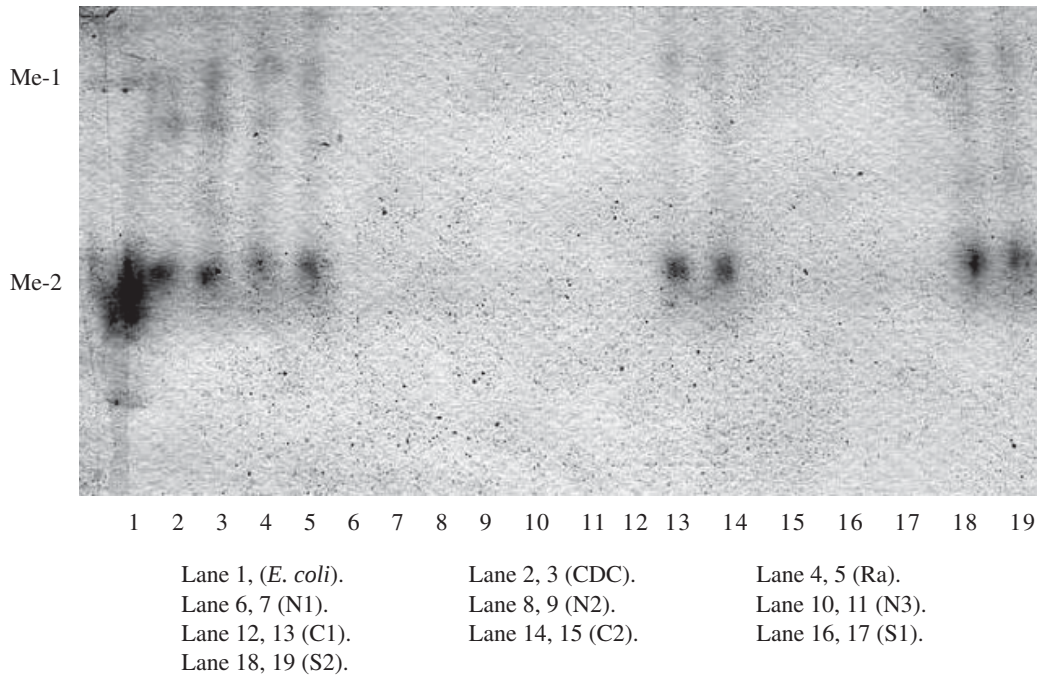


Fig 5- Malic enzyme banding patterns of thermophilic *Naegleria* spp isolated from central, northern, and southern parts of Thailand.

polymerase chain reaction, a highly sensitive and specific technique to identify *Naegleria fowleri* species isolated from environment.

CYTOPATHIC ACTION OF *N. FOWLERI* 3081, THAI STRAIN ON *GLIOBLASTOMA* *MULTIFORMAE*

Various mammalian cell lines have been used to study cell-cell interactions with *Naegleria* and other free-living amoebae (Brown, 1980; Fulford *et al*, 1985). The results from previous studies recorded that *N. fowleri* injure rat neuroblastoma target cells by two alternative mechanisms, trophocytosis or contact-dependent lysis (Marciano-Cabral *et al*, 1990). In 1994, an *in vitro* study of cytopathogenicity of known *Naegleria* spp on African green monkey kidney (vero)-cell cultures was done. The results demonstrated that cytopathic effect (CPE) could not distinguish nonpathogenic from pathogenic *Naegleria fowleri* (John and John, 1994). Cell-culture studies of *N. fowleri* on *Glioblastoma multiformae* have led to a better understanding of the activity of *N. fowleri* 3081 and the Thai strains' ability to destroy cultured mammalian cells (Figs 6-7). In summary, we have demonstrated that four species of *Naegleria fowleri* are able to eliminate *Glioblastoma* and that CPE

depends on incubation temperature and the amoeba: target-cell ratio (1:10). A temperature of 30°C is optimal for incubation and *Glioblastoma* cells provide a good model for studying the cytopathogenicity of all *Naegleria* species isolated from the environment.

THERAPEUTIC AGENTS AGAINST *N. FOWLERI* *IN VITRO*

Finding effective amoebicidal drugs for *N. fowleri* is still problematic, because a wide range of therapeutic agents have limited efficacy against the protozoan (Goswick and Brenner, 2003). Continuing *in vitro* studies of therapeutic agents is still important for finding an effective cure. Until now, amphotericin B is the only drug with established clinical efficacy against PAM; at least 7 patients have been successfully treated with amphotericin B alone or in combination with other drugs (Wang *et al*, 1993). Because the efficacy of amphotericin B alone is not successful in all patients, it is reasonable to investigate the activity of other drugs. Studies of the following drugs at sensitivity (MIC₅₀) against PAM have been published: gentamicin (160 µg/ml) (Tiewcharoen *et al*, 1999), amphotericin B (0.5 µg/ml), ketoconazole (0.125 µg/ml), itraconazole (10 mg/ml), and 5-fluorouracil (2 mg/ml) were carried out *in vitro*

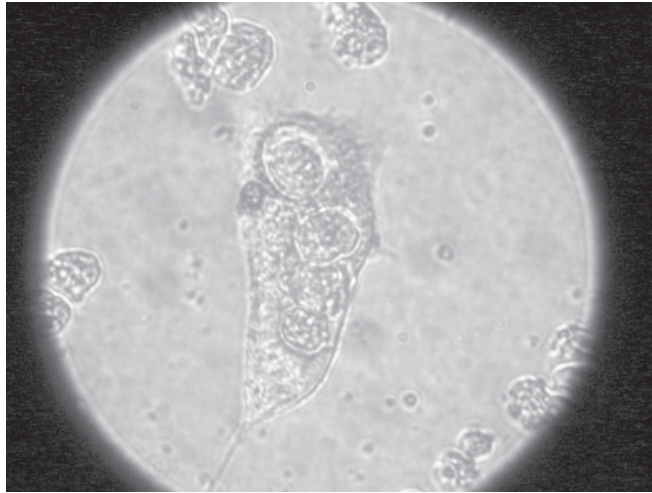


Fig 6- Cytopathogenicity of *Naegleria fowleri*-invaded human *Glioblastoma multiformae* by 8 hours, post-inoculation.

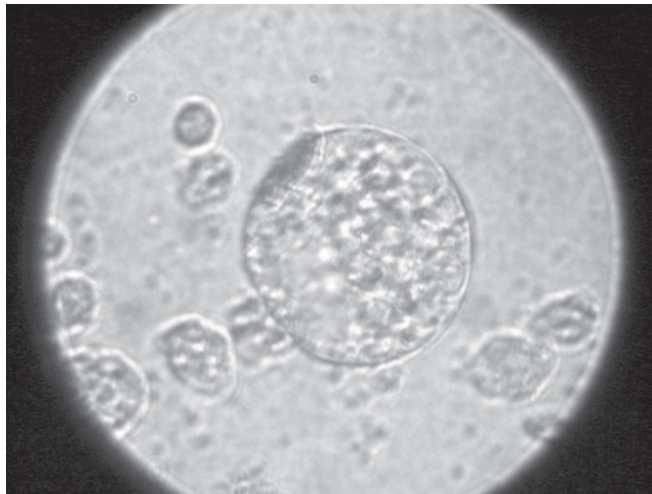


Fig 7- Human *Glioblastoma multiformae* with round-up and deformity of the extra- and intra-structure, evidenced by trypan blue exclusion and light microscopy examination.

(Tiewcharoen *et al*, 2002, 2003). We also conclude that amphotericin B and ketoconazole are the two most chemically effective drugs for treating patients with PAM, even though these drugs have many side-effects, especially relating to kidney and liver toxicity. It is reasonable to try drug combinations in PAM cases since one drug alone is not enough to eliminate the ameba. Currently, more research is needed into the efficacy of various drug combinations and minimum dosages. *In vitro* susceptibility testing of pathogenic *Naegleria* spp Thai strain to the drug combination of 5-fluorouracil

and amphotericin B has been published, showing that MIC₅₀ of 5-fluorouracil used alone needed a much higher concentration than it did when used in combination with amphotericin B (Tiewcharoen *et al*, 2002). Coadministration of amphotericin B and 5-fluorouracil should be valuable for *in vitro* screening of drugs with potentially antiamebic activity. We plan to continue research on the result, emphasizing the need for prompt treatment, and improved treatment methods using minimum drug dosages that have few side effects.

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