Special Articles

Amphizoic Amoebae: Pathogenic Free-living Protozoa; Review of the Literature and Review of Cases in Thailand

Chutatip Siripanth, MSc (Trop Med)*

** Department of Protozoology, Faculty of Tropical Medicine, Mahidol University*

A large variety of species of free-living amoebae (FLA) caused an indefinite form of these protozoa. Non-fixed form, as indicated by amoeboid movement and possed the bacteria to survive in nature. Two species of pathogenic FLA: Naegleria fowleri and Acanthamoeba spp. were identified as the causative agents of Primary Amoebic Meningoencephalitis (PAM) and Granulomatous Amoebic Encephalitis (GAE) respectively. They were suggested to amphizoic protozoa, capable of living as parasites or as free-living and they were also considered to be distributed worldwide. These amoebae were detected in lakes, rivers and ponds. The first case of meningoencephalitis was observed in 1961 by Fowler. Many cases were reported later on and the pathogenicity was tested by nasal inoculation of mice. In fact, quite a number of FLA were isolated but only a few species were pathogenic to humans. The three typical features which allow recognition of Naegleria spp. flagellate stage, round cyst and promitotic trophozoite. This promitosis distinguishes the Naegleria genus from Acanthamoeba spp. The disease caused by PAM usually occurs with acute onset, whereas chronic for GAE. The GAE cases mentioned are mostly in debilitated patients, chronic alcoholics or patients under treatment with immunosuppressive methods. About 6 cases of PAM were reported in Thailand during 1982-1997. Four cases of GAE were reported in 1994 and two isolated cases of Acanthamoeba from keratitis patients were reported in 2000. Finally one case of PAM and one case of GAE were reported in 2001. The surveys of FLA were set up to study the distribution of these pathogenic amoebae and determine the prevalence of amoebae in aquatic habitats of human environments. About 40% were identified as Acanthamoeba spp., 30% were Naegleria spp., 20% were Hartmanella and 10% were Vahlkampfia. Only 10% of Naegleria spp. belonged to Naegleria fowleri.

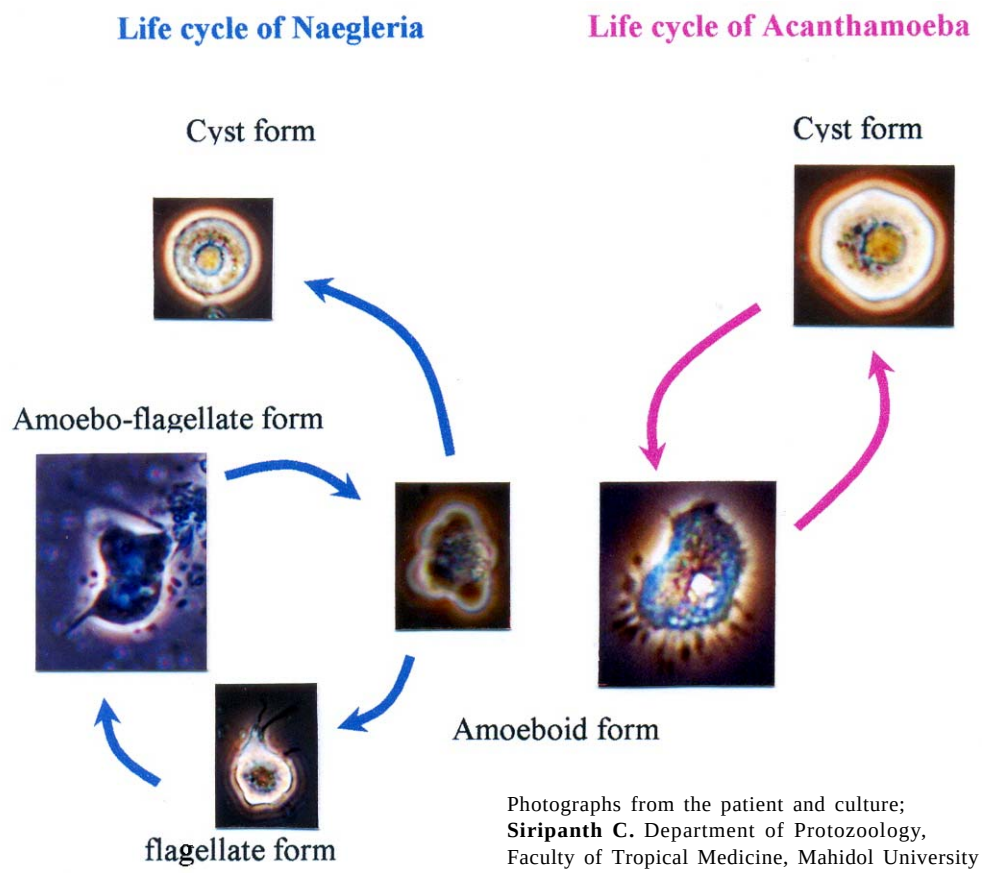
Keywords: *Naegleria fowleri, Lobopodia, Acanthamoeba spp., Acanthopodia, Pathogenic free-living amoebae (FLA), Primary amoebic meningoencephalitis (PAM), Granulomatous amoebic encephalitis (GAE)*

J Med Assoc Thai 2005; 88(5): 701-7
Full text. e-Journal: *http://www.medassocthai.org/journal*

Signs, Symptoms and Clinical manifestation

The pathogenic FLA, *Naegleria fowleri* and *Acanthamoeba* spp. cause human diseases[1]. This disease is characterized by extensive brain tissue destruction when the amoebae penetrate the brain from the nasal passages. PAM is characterized by a sudden onset of headache, fever (38-40°C), nausea, vomiting, signs of meningeal irritation and encephalitis[2]. Pharyngitis and symptoms of nasal discharge may be present in some cases. PAM resembles acute purulent bacterial meningitis and in the early stages can not be distinguished from this disease. The olfactory neuroepithelium is the route of invasion. The pathogenic amoebae probably enter the nasal cavity by inhalation or aspiration of water or dust containing the trophozoites or cysts. *N. fowleri* produces scant purulent exudates. GAE or the infections due to *Acanthamoeba* spp. are more insidious at onset and with a prolonged clinical course. *Acanthamoeba* spp. caused a wide range of clinical symptoms, particularly in immunocompromised hosts. The central nervous system is usually involved. Several localizing neurological signs, depending on the area of brain are involved. The symptoms of GAE are low grade fever,

Correspondence to : Siripanth C, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand. Phone: 0-2354-9100 ext. 1830, E-mail: tmcsr@mahidol.ac.th



Photographs from the patient and culture;
Siripanth C. Department of Protozoology,
Faculty of Tropical Medicine, Mahidol University

Fig. 1 Life Cycle of pathogenic free-living amoeba

stiff neck, nausea and vomiting. The route of invasion and penetration to CNS in cases of GAE is hematogenous, probably from a primary focus in the lower respiratory tract or the skin[3,4].

Pathogenesis

N. fowleri has been implicated in human PAM. Other species such as *N. lovaniensis* and *N. jadini* have not been found to produce disease in humans. The cause of death in human PAM was probably related to the excessive increase of intracranial pressure due to cerebral edema. In GAE the cause of death was probably multifactorial because those patients might had additional[5].

Diagnosis and Differential diagnosis

PAM usually occurrs in children and young adults who had a previous history of good health and had been swimming in warm water or had contacted with stagnant water or mud a few days before the onset of sickness[6]. These history is helpful in making a presumptive diagnosis of PAM. GAE is an uncommon infection that usually occurs in an immunocompromised host[7,8]. The isolation and identification of the trophozoites of *N. fowleri* and *Acanthamoeba* spp. and their cysts from CSF provide the only means of a positive diagnosis. The CSF should be promptly sent to a laboratory for culture to isolate the amoebae and examination by wet mount preparation or staining methods. The differential diagnosis between both PAM and GAE infection were not only based on the history of patients but also the morphology of trophozoites. *N. fowleri* demonstrated lobopodia, whereas *Acanthamoeba* spp. presented filopodia or acanthopodia. The wet mount should be immediately examined for motile amoebae under a compound microscope with 10 and 40 objectives. The trophozoites of *N. fowleri* moved by producing eruptive, smooth, and hemispherical bulges. The trophozoites were in active translational movement, 10 to 35 m. The cytoplasm could be differentiated as an outer, hyaline ectoplasm and an inner, coarsely granular

endoplasm. *Acanthamoeba* trophozoites were slightly larger, 15 to 45 m, and produced from the surface of the body fine, tapering clear projections called "acanthopodia". The amoebae moved sluggishly and could easily be mistaken as leukocytes. *Acanthamoeba* had no flagellate stage but produced double-walled cysts, with a wrinkled outer wall called ectocyst and a stellate, polygonal or even round inner wall called endocyst[9,10]. Both *Naegleri*a and *Acanthamoeba* were uninucleate, and the nucleus was characterized by a large, dense, centrally located nucleolus.

Confirmation of the presence of amoebae in fresh preparation was obtained by staining the smears in appropriate stains; Wright's or Giemsa stains were found most commonly in hospital laboratories. In those stains, the cytoplasm of the amoebae appeared sky-blue and the nucleus faded pink[11,12]. The best stains for demonstrating the definitive nuclear morphology of the amoebae were the iron-hematoxylin and trichrome techniques[13]. Amoebae showed their characteristic nuclear morphology. In iron-hematoxylin-stained smears, the nucleus stains black while the cytoplasm was coloured grayish or bluish. In the trichrome-stained preparations the nuclear elements of the amoebae stain red while the cytoplasm was coloured bluish green (Fig. 2, 3).

The isolation of FLA by culturing methods was useful for further studies such as biology or genetic diversity. Non-nutrient agar plates, liquid medium and cell culture were recommended[14] (Fig 4). Non-nutrient agar plates made up of amoeba saline and an 18- to 24-hour-old culture of *Escherichia coli* or *Enterobacter aerogenes*. The various liquid media were suggested to use for axenic culture such as proteose peptone-yeast extract-glucose medium[15], or Bacto-casitone[16]. *Acanthamoeba* could easily be grown without the addition of serum or host tissue, in a variety of media. A basic medium that supported growth of *Acanthamoeba* spp. consisted of Proteose peptone, yeast extract and glucose[17].

Enflagellation of amoeba trophozoites would be helpful in differentiation between *Naegleri*a spp. and *Acanthamoeba* spp. A drop of CSF or a loopful of amoebae scraped from the surface of the agar plate was mixed with about 1 ml of warmed (37 C) sterile distilled water and gently shaken and incubated at 37 C for several hours. A wet mount preparation was made and examined microscopically for free-swimming flagellates. *N. fowleri* had flagellate stage but *Acanthamoeba* spp. did not[18].

Mice were generally recommended as animal models for the isolation and propagation of pathogenic FLA, because of their susceptibility to these

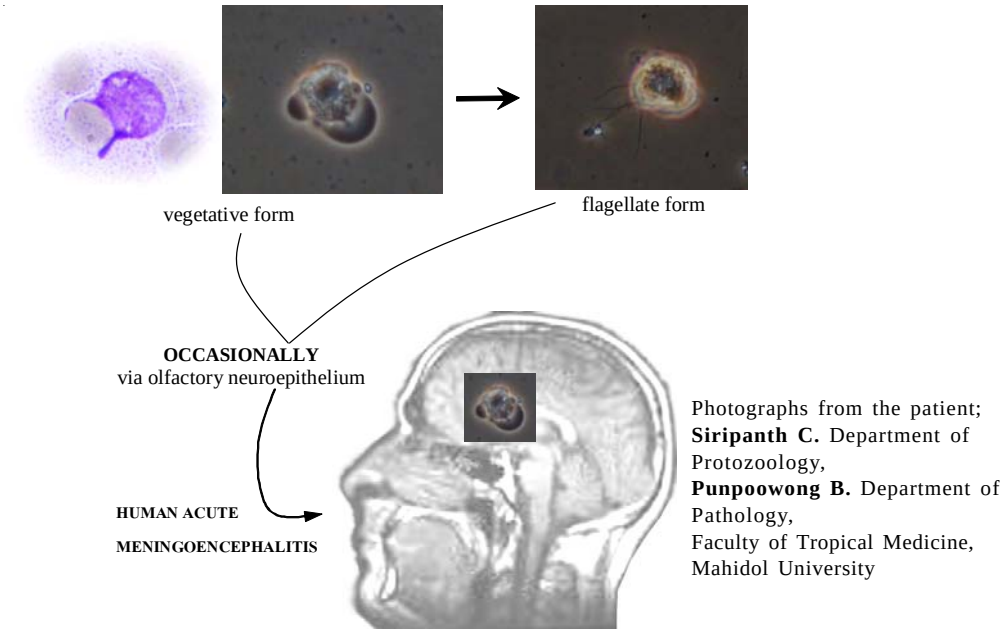
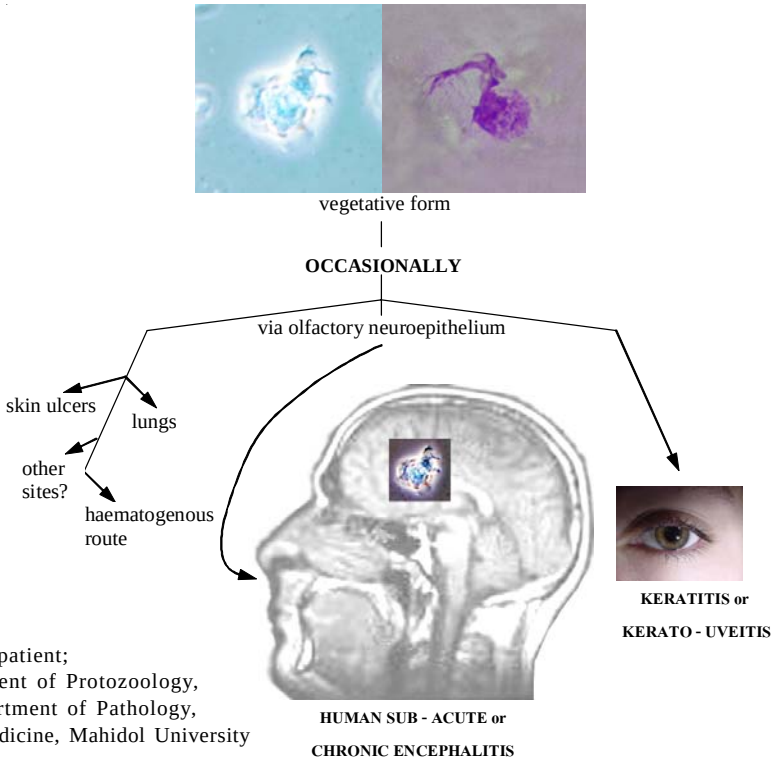
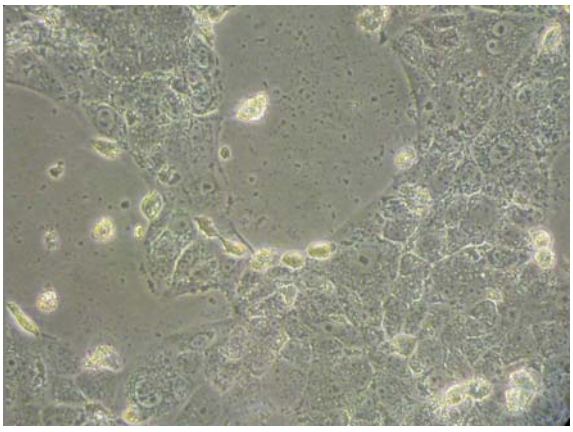


Fig. 2 Route of infection and diagnosis of *Naegeria fowleri*



Photographs from the patient;
Siripanth C. Department of Protozoology,
Punpoowong B. Department of Pathology,
Faculty of Tropical Medicine, Mahidol University

Fig. 3 Route of infection and diagnosis of *Acanthamoeba* spp.



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Siripanth C. Department of Protozoology,
Faculty of Tropical Medicine, Mahidol University

Fig. 4 The cytopathic effect (CPE) in cell culture

amoebae, manageability, and low cost. Suspected CSF containing amoebae or amoebae suspension from culture was instilled into their nostrils. The infected mice developed characteristic signs of disease and died within 5-7 days for *N. fowleri*[2,19,20] or longer (7-30 days) if they were *Acanthamoeba* spp.[21]. The amoebae could be demonstrated in the brains of mice, either by culture or histological methods.

Case review of free-living amoebae in Thailand

Primary Amoebic Meningoencephalitis

The first case of PAM was reported by Jariya et al in 1982[22]. A 5-year old boy with a history of swimming in a pond along a rice field before onset of his illness was admitted to Sisaket Hospital and referred to Siriraj Hospital for specific diagnosis and treatment. Microscopic examination from the CSF revealed progressive motility by pseudopodia of amoeba trophozoites which were characteristic of *Naegleri*a. The trophozoites form had flagella when kept at room temperature for more than one hour the rounded form of cysts were also present. The second case was reported in 1986 by Charoenlarp et al[23]. A young man aged 17 years was admitted to Siriraj Hospital with a history of headache for 3 days and semiconciousness for one day. He had no history of swimming but 2 weeks before admission he was exposed to water during Songran, the traditional

Thai festival. The investigation of CSF showed many moving amoebae. CSF culture for amoeba was positive and there was also a flagellate form. The patient died on the second day of admission and numerous amoebae were found from postmortem brain tissues. Three PAM cases were reported during 1886-1887, two cases from Trat and another case from Nakhon Prathom[24]. The patient from Nakhon Prathom was referred to Ramathibodi Hospital. A few amoeba trophozoites were detected from the CSF of the patient from this patient who finally died on the fifth day of illness. A survivor of PAM was reported in 1991 by Poungvarin et al[25]. The patient was successfully treated with triple combination of drugs. Numerous active slug-like movement trophozoites of *Naegleri*a with blunt projection pseudopodia were observed. Iron henatoxylin staining demonstrated characteristic nucleus of *Naegleri*a with large central karyosome surrounded by halo. In 1997, the first case of *Naegleri*a meningomyeloencephalitis was reported by Viriyavejakul et al[26]. The patient was a 12 year-old boy with a history of swimming in the canal. He died on the fifth day after admission. Clusters of *Naegleri*a trophozoites were seen within purulent exudates and in the necrotic areas of the cerebral cortex including cerebellum and also in the area of the pericentral canal of the spinal cord. The trophozoites were 8-10 m with prominent nucleoli and peri-nucleolar were observed. Four cases of GAE were reported in immunocompromised hosts from 1990 to 1992. All cases were treated at Siriraj Hospital. One case was antemortem, from brain biopsy. The other three cases were diagnosed as GAE postmortem. One patient had chronic skin ulcers in which FLA were found[27]. *Acanthamoeba*e were isolated from two keratitis patients from 1988 to 1990. The patients developed decreased vision, severe photophobia, severe eye pain and foreign body sensation after minor corneal trauma[28]. During the period January to February 2001, two patients with GAE and PAM were diagnosed at Siriraj Hospital. The first patient came from Suphanburi Province with chronic progressive headache and deterioration of neurological symptoms due to brain parenchymal involvement and increased intracranial pressure. She had a history of frequent swimming in a dam before her illness. The histology from brain biopsy and autopsy confirmed the diagnosis of GAE from *Acanthamoeba* spp. infections. The second patient was a case of *N. fowleri* infection. A man from Nakornpratom province presented with high fever, sudden severe headache and signs of miningeal irritation. PAM was comfirmed by the demonstration of numerous trophozoits of *N. fowleri* in a fresh examination of the CSF. He died from increased intracranial pressure with brain herniation[29].

The distribution of *N. fowleri* in Thailand was carried out from 1985 to 1987. The result showed that 10% of pathogenic *Naegleri*a belonged to *N. fowleri*[30]. The survey of FLA was performed from in 1999 to 2001[31]. Amoeba of 49 isolates from water were identified as *Acanthamoeba* (36.7%), *Naegleri*a (28.6%), *Hartmannella* (20.4%), *Vahlkampfia* (12.5%) and *Vannella* (2%). Those findings supported the evidence of pathogenic FLA infections in Thailand.

In conclusion, although both PAM and GAE in Thailand are rare, recognized causes of fatal infection in both immunocompetent and immunocompromised persons. Both causes of PAM and GAE were diagnosed at Siriraj Hospital, which brought this condition to the public attention and stimulated researchers to find out the relationship between the causative agents and prevalence of free-living amoebic infection in Thailand. Several patients may be misdiagnosed and only a few doctors in Thailand have direct experience with these conditions. Thus, a good opportunity exists to alert medical personnel to the existence of these rare but fatal infections[32].

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อมีบาอิสระ: โรคติดเชื้อระบบประสาทกลาง: ทบทวนวารสารและรายงานผู้ป่วยในประเทศไทย

จุฑาทิพ ศิริพันธุ์

อมีบาอิสระที่เป็นสาเหตุก่อให้เกิดการติดเชื้อของระบบประสาทกลาง มีสองกลุ่มคือ Naegleria fowleri และ Acanthamoeba spp. เชื้อ N. fowleri ก่อให้เกิดการติดเชื้อที่มีอาการเยื่อหุ้มสมองและสมองอักเสบปฐมภูมิ (PAM) ส่วนเชื้อ Acanthamoeba spp. ทำให้เกิดการอักเสบของสมองชนิดแกรนุโลมา (GAE) ในปัจจุบันมีรายงานการติดเชื้อทั้งสองชนิดทั่วโลก ซึ่งรายงานการติดเชื้อครั้งแรกในปีพ.ศ. 2504 โดย Fowler และได้มีการศึกษาต่อเนื่องมาโดยตลอดเกี่ยวกับพยาธิสภาพของโรคและสายพันธุ์ของเชื้อที่ก่อโรค พบว่ามีเชื้ออมีบาอิสระเพียงไม่กี่ชนิดที่เป็นสาเหตุก่อโรคในคน แต่การตรวจวินิจฉัยเพื่อแยกเชื้ออมีบาเหล่านี้จากเชื้อชนิดอื่น ๆ และแยก N. fowleri จาก Acanthamoeba spp. จำเป็นต้องเข้าใจถึงวงจรชีวิต ลักษณะเฉพาะ ของตัวเชื้อทั้งสอง เช่น N. fowleri มีระยะโทรโฟซอยต์ ที่มีทั้งอมีบา และ flagellate มีขาเทียมลักษณะป้าน ต่างกับ Acanthamoeba spp. ที่มีระยะอมีบาเพียงอย่างเดียว และขาเทียมมีลักษณะแหลม ส่วนระยะซีสต์ทั้งสองชนิดจะมีลักษณะแตกต่างกัน เป็นต้น รายงานการติดเชื้อ N. fowleri ในประเทศไทยระหว่างปีพ.ศ. 2525-2540 มีทั้งหมด 6 ราย เป็นการติดเชื้อจาก Acanthamoeba spp. 4 ราย ในปีพ.ศ. 2537 และอีก 2 ราย ในปีพ.ศ. 2543 ส่วนในปีพ.ศ. 2544 มีรายงานการติดเชื้อ N. fowleri และ Acanthamoeba spp. อีกชนิดละ 1 ราย จากการสำรวจแหล่งน้ำจืดพบว่า ประมาณ 40% ตรวจพบเชื้อ Acanthamoeba spp. 30% พบเชื้อ N. fowleri 20% พบเชื้อ Hartmanella และอีก 10% พบเชื้อ Vahlkampfia. ในจำนวน 30% ของเชื้อ Naegleria spp. มีเพียง 10% ที่พิสูจน์ได้ว่าเป็นเชื้อ N. fowleri ดังนั้นจึงกล่าวโดยสรุปได้ว่าทั้ง PAM และ GAE มีอุบัติการณ์ของโรคเกิดขึ้นในประเทศไทยมาตั้งแต่ปีพ.ศ. 2525 เป็นต้นมา นอกจากนี้ ยังสามารถตรวจพบเชื้อที่เป็นสาเหตุก่อโรคทั้งสองชนิดได้ในแหล่งน้ำต่าง ๆ โดยทั่วไป ดังนั้นโอกาสติดเชื้อจึงมีโอกาสเป็นไปได้ ด้วยเหตุนี้การตรวจวินิจฉัยที่แม่นยำจึงเป็นสิ่งจำเป็น ทั้งนี้เพื่อช่วยชีวิตผู้ป่วยได้ทันท่วงที เพราะทั้งสองโรคนี้มีอัตราตายสูงมาก ถ้าไม่ได้รับการตรวจวินิจฉัยและให้การรักษาอย่างถูกต้อง รวดเร็วจากแพทย์และเจ้าหน้าที่ผู้ที่มีประสบการณ์และความชำนาญ