Early Detection and Identification of Amphizoic Amoebae from Nasal Exudates of a Symptomatic Case

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A man visited the Out Patient Department of the hospital for Tropical Diseases in February 2004 with low grade fever and severe headache for a week. He had the history of diving in a natural pond 2-3 days before the onset of the disease. A thick bloody mucous was observed from the nasal discharge. Fresh microscopic observation of the exudates in 0.85% sodium chloride revealed numerous active amoeba trophozoites. Two groups of the trophozoites were observed. The first group was 10 sized amoeba with active directional movement by lobopodia and the second group was 15-30 sized amoeba with active multiprogressive movement by filopodia. Few flagellate forms were observed after exflagellation in distilled water and some polygonal cysts were also found. Giemsa' stain was used to differentiate the amoeba trophozoites from the leukocytes. It was concluded that this patient was infected by both Naegleria spp.and Acanthamoeba spp. This is the first report of double infection of free-living amoeba in a symptomatic and non-fatal patient.

Keywords: *Naegleria spp., Acanthamoeba spp., Lobopodium, Filopodium, Enflagellation, PAM, GAE*

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Free-living amoebae, *Naegleria fowleri* and *Acanthamoeba* spp. were notified as the causative agents of Primary Amoebic Meningoencephalitis (PAM) and Granulomatous Amoebic Encephalitis (GAE) in humans[1,2]. The infection almost invariably resulted in death[3]. *Naegleria* causing PAM was given the specie name *N. fowleri*[4]. The disease typically occurred in healthy children and young adults with a recent history of swimming in fresh-water, the source of human infection[5]. Diagnosis was difficult and dependent upon recognition of the amoebae in the cerebrospinal fluid; not infrequently, trophozoites were dismissed as leukocytes. All reported cases have been described as rapidly onset, leading to death and usually were diagnosed at autopsy[5,6]. PAM was characterized by the sudden onset of headache, fever (38-40°C), nausea, vomiting, signs of meningeal irritation and encephalitis. Pharyngitis and symptoms of nasal discharge might be present in some cases[7]. The symptoms of GAE were low grade fever, stiff neck, nausea and vomiting. The route of invasion and penetration to the CNS in cases of GAE was hematogenous, probably from a primary focus in the lower respiratory tract or the skin[8,9]. The authors reported here the first case of early detection and double infections of *Naegleria* spp. and *Acanthamoeba* spp. in the sinus cavity of a symptomatic patient. Amoeba trophozoites were well differentiated by fresh preparation under a compound microscope at 40x objective.

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Materials and Methods

A man visited the Out Patient Department of the hospital for Tropical Diseases in February 2004 with low grade fever and severe headache for a week. He had the history of diving in a natural pond 2-3 days before the onset of the disease. A thick bloody mucous was observed from the nasal discharge. Blood examination revealed WBC 5.1 x 10^9/l, RBC 4.81 x 10^{12}/l, Hct 0.41, Plt count 203 x 10^9 / l. Blood chemistry and urinalysis were normal. Computed Tomography of the brain and sinuses showed normal study of the brain

and left maxillary/ ethmoid sinusitis with complete left OMU occlusion. Nasal discharges were collected and promptly sent to the laboratory for culture and microscopic observation. The culture from the nasal discharge was positive for gram negative bacilli and numerous WBC. Gross observation demonstrated the blood with high viscosity mucous. Fresh preparation was prepared using normal saline solution. The specimens were examined at 40X objective under a compound microscope and phase-contrast optics. Some parts of the specimens were also smeared, fixed and stained by Giemsa's stain. Enflagellation was performed by mixing the exudates with two ml distilled water. Then they were gently shaken and incubated at 37°C for two to three hours.

Results

From fresh observation, the movement of amoeba trophozoites and their pseudopodia were divided into two groups. The first group was 10 amoebae, directional movements by lobopodia and the second group was 15-30 amoebae, multiprogressive movement by filopodia or acanthopodia (Fig. 1, 2). The majority of trophozoites were amoebae with filopodia and most of them were still active. Heavy bacterial infection was documented. Some amoeba trophozoites attached and also invaded into the epithelial cells (Fig.3). Ingested reticulocytes were also observed.

Amoeba trophozoites stained pale blue and dark pink by Giemsa'stain. Ectoplasm stained pale blue, endoplasm and nucleus stained dark pink (Fig. 4). The nuclear structure was not clearly demonstrated. Red blood cells and bacteria were observed in the cytoplasm of some amoeba trophozoites. Amoeba trophozoites characterized as described above were present at the margin of the epithelium cell by Giemsa' stain (Fig. 5). After three hours of the enflagellation, few biflagellates were observed under phase-contrast optics (Fig. 6). The flegellate stage was used to confirm the life cycle of *Naegleria* spp. However, the sp. of *Naegleria* could not be specified by microscopic examination.

Discussion

From the observation, *Acanthamoeba* trophozoites appeared more aggressive and predominant than *Naegleria*. Trophozoites were abundant and active when the specimen was freshly collected from nasal exudates. On the contrary, their movements were slowed down when the specimen was collected from the sputum. It might be caused by the source of the specimen affecting the survival of parasites for example enzyme from saliva. Only a few flagellates were observed, which might suggest the correlation between the amount of *Acanthamoeba* and *Naegleria* trophozoites from fresh observation. Numerous *Acanthamoeba* trophozoites observed were compared to *Naegleria* trophozoites therefore; it was possible that the dividing stage of both protozoa should not be equal because of the bacterial association involved. *Acanthamoeba* spp. preferred to feed on

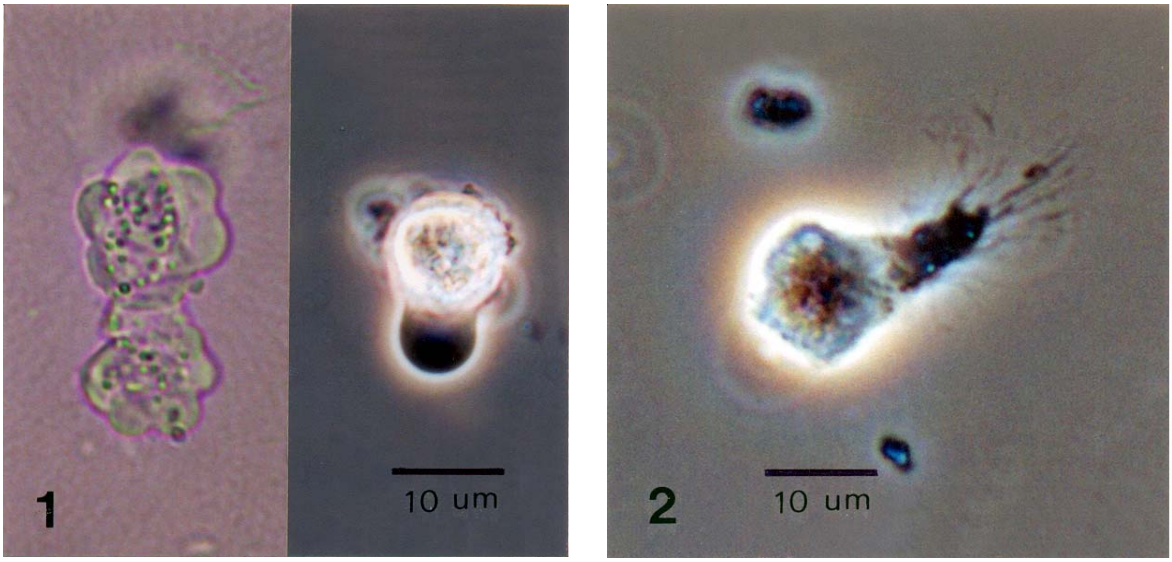

Fig. 1, 2 Free-living amoebae from nasal exudates of patient, bright field and phase contrast obtics, typical characters of *Naegleria* spp. and *Acanthamoeba* spp. pseudopodia were noted

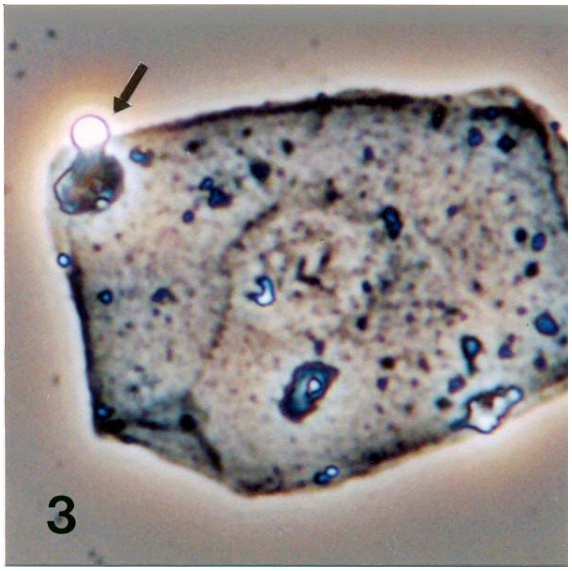

Fig. 3 Typical character of lobopodium of *Naegleria spp.*, amoeba trophozoite attached epithelium cell (arrow), phase-contrast optics

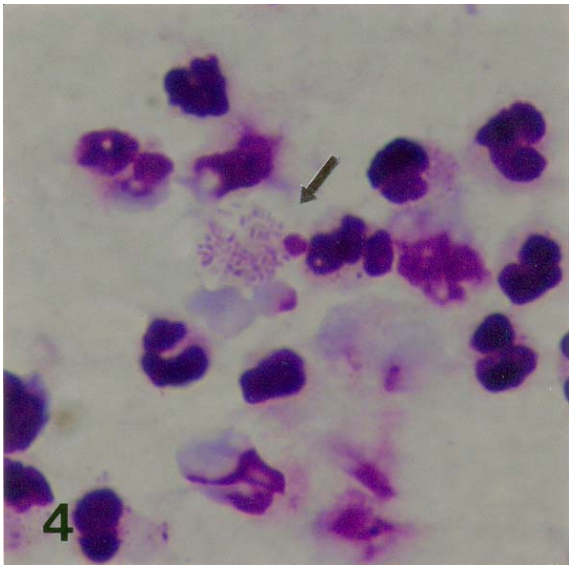

Fig. 4 *Naegleria spp.* and *Acanthamoeba spp.*, notice bactheria were phagocytosed by amoeba trophozoites (arrow), Giemsa' stain

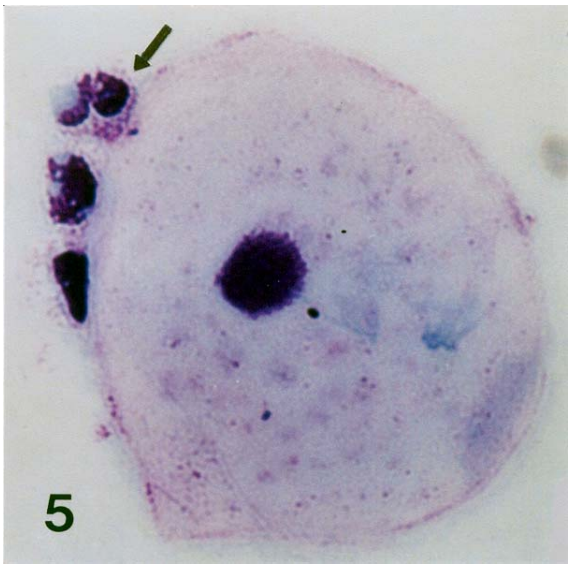

Fig. 5 Free-living amoeba attached epithelial cell, Giemsa' stain

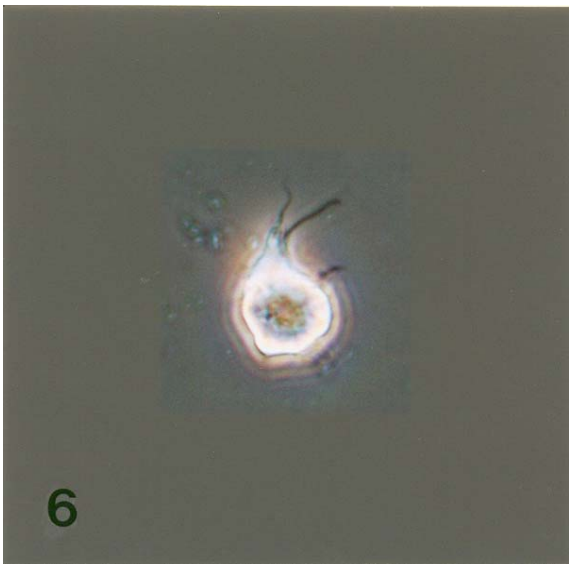

Fig. 6 Flagellate stage of Naegleria spp. after enflagellation, flagella were observed, phase-contrast optics

all inclusion including bacteria[10] but *Naegleria fowleri* ate tissue[11]. The nasal cavity was an unusual site of infection[5,10,12] therefore most of them slowly multiplied, compared to *Acanthamoeba.* Both *Naegleria* spp. and *Acanthamoeba* spp. were reported as pathogenic and non pathogenic in humans[13]. Although the molecular technique was not done for the identification of species of both free-living amoebae from this specimen but microscopic examination is still valuable for the early diagnosis of PAM and GAE cases before death. In conclusion, early detection might be possible in the hands of experienced physicians and laboratory technicians. The correlation between the history and symptoms should be noted including differential diagnosis of parasites from other biological materials in the specimens. All information may be useful for prevention of death in PAM and GAE patients.

Conclusion

The patient came to the Out Patient Department of the Tropical Medicine Hospital, Faculty of Tropical Medicine Mahidol University with the symptoms of severe headache and low grade fever for several days. He gave the history of swimming and diving in a pond before the onset of the disease. The nasal exudate was collected and sent to the Department of Protozoology for microscopic examination. Numerous amoeba trophozoites were found by fresh preparation at 40X objective and phase-contrast optics under a compound microscope. Their movements with pseudopodia were observed and identified as two characters. The majority was filopodia with sluggish movement. Only a few of them showed directional movement of lobopodia. A few polygonal cysts were also observed. Enflagellation was performed to confirm either *Naegleria* spp. or *Acanthamoeba* spp. Some flagellates were observed after a few hours of enflagellation. The nasal exudates were also smeared and stained by Giemsa. The trophozoites revealed the characters of free-living amoebae. From microscopic examination, it was concluded that the patient was infected from both *Naegleria* spp. and *Acanthamoeba* spp. This was the first report of double infection of two pathogenic free-living amoebae which was detected ante mortem and had no cerebral symptoms.

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การตรวจวินิจฉัยเบื้องต้นสำหรับผู้ป่วยที่ติดเชื้อ Naegleria และ Acanthamoeba จากตัวอย่างน้ำมูกและเสมหะ

จุฑาทิพ ศิริพันธุ์, เบญจนี พันธ์ภูวงศ์, มาเรียว ริกันตี

ผู้ป่วยชายสุขภาพแข็งแรงมาพบแพทย์ด้วยอาการปวดศีรษะ และมีประวัติดำน้ำมา 2-3 วันก่อนมีอาการจากตัวอย่างน้ำมูกมีลักษณะเหนียวข้น มีเลือดปนเล็กน้อย ผลการตรวจโดยการดูสดด้วยการป้ายบนสไลด์ ในน้ำเกลือ 0.85% พบเม็ดเลือดขาวและเม็ดเลือดแดงจำนวนมาก ตรวจพบเชื้ออะมีบาขนาด 20-30 ไมครอน มีการเคลื่อนไหวค่อนข้างเร็ว ลักษณะของขาเทียมมี 2 ชนิด คือ ชนิดแหลมและชนิดป้าน พบเชื้อระยะซิสต์ มีลักษณะเหลี่ยม ขนาด 10-15 ไมครอน ทำ enfragellation พบเชื้อระยะ fragellate จำนวนหนึ่ง ผลการตรวจด้วยวิธีย้อมสี Giemsa พบอะมีบาในระยะแบ่งตัวรวมทั้งแบคทีเรียชนิด Cocci และ Bacilli จำนวนมาก สรุปผลการตรวจทางห้องปฏิบัติการ พบว่าผู้ป่วยมีการติดเชื้อ free-living amoeba 2 ชนิด คือ Naegleria และ Acanthamoeba ร่วมกับการติดเชื้อแบคทีเรีย ซึ่งเป็นรายงานแรกของการติดเชื้อร่วมกัน 2 ชนิดในโพรงจมูก และผู้ป่วยไม่มีอาการผิดปกติทางสมอง