Case Report: First Case of Granulomatous Amebic Encephalitis Caused by *Acanthamoeba castellanii* in Taiwan


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**Abstract.** We report the first case of granulomatous amebic encephalitis caused by *Acanthamoeba* in a previously healthy farmer in Taiwan who fell into a ditch. The DNA amplicons of amebas identified by polymerase chain reaction in the cerebrospinal fluid specimen and the ditch water specimens were identical and shared strong homology with *A. castellanii*. He survived after treatment with amphotericin B, rifampin, and corticosteroids.

### INTRODUCTION

Free-living amebas that cause infections of the central nervous system (CNS) in humans include *Naegleria fowleri*, Acanthamoeba species, and *Balamuthia mandrillaris*.1 *N. fowleri* usually cause acute fulminating meningoencephalitis in young immunocompetent individuals, whereas *Acanthamoeba* species usually cause chronic but fatal encephalitis in immunocompromised hosts that is often called granulomatous amebic encephalitis (GAE).2 Timely diagnosis of GAE is often not made because of its rarity and unawareness of the clinicians.3 Although ~150 patients with GAE have been reported worldwide, < 10 survived.4 In this report, we describe a case of GAE in a previously healthy Taiwanese farmer after choking on muddy water. By polymerase chain reaction (PCR) and DNA sequence analysis, DNA amplicons from the cerebrospinal fluid (CSF) and the ditch water specimens were identical and shared strong homology with *A. castellanii*.

### CASE REPORT

A 63-year-old farmer had been previously healthy until 2 weeks earlier when he fell into a gully and aspirated muddy water. General weakness and difficulty of defecation and urination developed 10 days after the incident, for which he was admitted to an outside hospital. On Day 8 of hospitalization, nausea, vomiting, and severe headache developed. An examination of the CSF specimen showed 438 cells/μL with 66% eosinophils, protein 91 mg/dL, and glucose 28 mg/dL. Gram stain, India ink smear, and acid-fast stains of the CSF specimen remained non-diagnostic. Brain imaging showed multiple scattered hyperintensities along the pial surface of the brain and left periventricular white matter with abnormal leptomeningeal enhancement (Figure 1B). A CSF specimen was sent to Centers for Disease Control (CDC), Taiwan, where *Acanthamoeba* infection was confirmed (see below). Amphotericin B and rifampin were continued with dexamethasone, mannitol, and furosemide for increased intracranial pressure. His consciousness gradually improved. Dexamethasone, amphotericin B, and rifampin were continued for 4 weeks, with continuing improvement of clinical status. On Day 78, the patient was discharged with clear orientation to time, place, and person but with slow response.

Five CSF specimens obtained during hospitalization were examined at the CDC, Taiwan. Each specimen was mixed into 2.0 mL 5.3 mol/L guanidine thiocyanate. The mixture was heated at 95°C while shaking for 30 minutes and cooled to room temperature before centrifuging for 5 minutes at 20,000g. The MagNA Pure Systems (Roche Diagnostics, Mannheim, Germany) were used to isolate a total of 100 μL of both lower extremities. His white blood cell count was 10,940 cells/μL with 14.6% eosinophils. Repeat lumbar puncture yielded turbid, sticky CSF. The analysis of CSF showed 87 cells/μL (32 eosinophils/μL), protein 171 mg/dL, and glucose 28 mg/dL. Gram stain, India ink smear, and acid-fast stains of the CSF specimen remained non-diagnostic. Brain imaging showed multiple scattered hyperintensities along the pial surface of the brain and left periventricular white matter with abnormal leptomeningeal enhancement (Figure 1B). A CSF specimen was sent to Centers for Disease Control (CDC), Taiwan, where *Acanthamoeba* infection was confirmed (see below). Amphotericin B and rifampin were continued with dexamethasone, mannitol, and furosemide for increased intracranial pressure. His consciousness gradually improved. Dexamethasone, amphotericin B, and rifampin were continued for 4 weeks, with continuing improvement of clinical status. On Day 78, the patient was discharged with clear orientation to time, place, and person but with slow response.

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For further confirmation, the primer set JDP1/JDP2 that targets the variable region of a small subunit rRNA gene frag-
ment was used for phylogenetic analysis of Acanthamoeba spp. in the CSF specimen and a water specimen from the gully that the patient fell into. The sequence alignments and cluster analysis were performed using the programs CLUSTALW and the program MEGA4. Phylogenetic construction produced gene trees by using neighbor-joining distance trees with a generation of 1,000 bootstrapped replicates. Sequences of Acanthamoeba castellanii, B. mandrillaris, Entamoeba histolytica, and N. fowleri were included for phylogenetic analysis that are shown in Figure 2B. The DNA amplicons from the CSF and the water specimen were identical and shared strong homology with A. castellanii (Figure 2B). Although whether the Acanthamoeba isolates from the CSF and the water specimen are from the same strain needs further clarification, the results indicated that the Acanthamoeba sp. indeed existed in the rice field, and it is likely that the patient became infected when he fell into the gully and choked on the water.

**DISCUSSION**

Acanthamoeba is ubiquitous in nature and can be found in such diverse places as surface waters, swimming pools, bottled mineral water, contact lens solutions, human and animal bodies, and the dust in the air. Predisposing conditions to Acanthamoeba infections include AIDS, diabetes, receipt of steroids or chemotherapy, systemic lupus erythematosus, lymphoproliferative disorders, and amaggllobulinemia, and the skin and upper respiratory tract are the most common portals of entry. In this case, the patient likely acquired Acanthamoeba through aspiration, and the ameba may reach the central nervous system through hematogenous route or olfactory nerves.

There are no characteristic clinical or radiographic features for GAE. Clinical manifestations, ranging from headache, focal neurologic deficit to impaired cognition and even coma, may be subacute to chronic. CSF findings may comprise lymphocytic pleocytosis, moderately elevated protein, and low or normal glucose levels; trophozoites are generally absent. Imaging studies in GAE usually show multifocal hypodense lesions with enhancement. Although definitive diagnosis of GAE relies on demonstration of trophozoites or cysts in the CSF specimen or brain tissue, detection and isolation of Acanthamoeba from the CSF specimen may be difficult because acanthamoebae usually reside deep in the tissue, mainly around blood vessels. In this case, microscopy of the CSF specimens identified a free-living ameba, and the diagnosis was subsequently confirmed by PCR for Acanthamoeba 18S rDNA and phylogenetic analysis. These latter newer and rapid diagnostic methods may be helpful in earlier identification and differentiation of Acanthamoeba from other free-living amebas. Interestingly, Acanthamoeba DNA was only detected in acute-phase CSF specimens; four follow-up specimens were all negative. This may be because of the fact that Acanthamoeba only appeared in the CSF in the early phase of GAE or that sensitivity of PCR diagnosis for Acanthamoeba in the CSF was reduced by treatments. Because earlier diagnosis together with virulence of the agent, infection dose, and host immunity all play a role in determining the outcome of GAE, earlier diagnosis of GAE should be made and might alter the outcome of treatment.

**Figure 1.** A, Wet-mount smear of CSF specimen on day 17 showed trophozoite-like organisms; magnification, ×1,000. B, Brain MRI shows multiple scattered hyperintensity along pial surface of brain and left periventricular white matter with abnormal leptomeningeal enhancement (arrow).

**Figure 2.** A, PCR diagnosis of the CSF specimen for free-living amebas. Lanes 1 and 4, the first CSF specimen from the patient for Naegleria spp. and Acanthamoeba spp. The 180-bp band in Lane 4 was subsequently sequenced and proven to be Acanthamoeba using BLAST at NCBI. Lanes 2 and 3, positive and negative control for Naegleria spp.; Lanes 5 and 6, positive and negative control for Acanthamoeba spp.; Lane 7: 100-bp DNA ladder. B, The phylogenetic tree of Acanthamoeba DNA from the patient (CSFTw) and the water specimen based on the 18S rDNA variable region. The tree was constructed using neighbor-joining distance trees with a generation of 1,000 bootstrapped replicates. Acanthamoeba castellanii Castellani, U07413; Acanthamoeba castellanii CDC, 0981; V006; U07400; Acanthamoeba healyi, AF019070; Acanthamoeba culbertsoni Lilly A-1, AF019067; Entamoeba histolytica, AB426549; Balamuthia mandrillaris, AF477022; Naegleria fowleri, AF338423; Giardia lamblia, AACB02000118.1; CSFTw, FJ707372; water sample, FJ707373.
Therapeutic options for GAE are limited. In vitro susceptibility tests suggest that ketoconazole, pentamidine, polymyxin, trimethoprim-sulfamethoxazole, sulfadiazine, fluycosine, amphotericin B, and rifampin may be active against *Acanthamoeba*, but the activities of these agents showed variable results.\textsuperscript{16,17} Animal studies have shown sulfadiazine, rifampin, and fluycosine to be effective and, if given earlier,\textsuperscript{18,19} particularly before the infection enters the central nervous system, death may be averted.\textsuperscript{20} However, clinical experience with these drugs is limited. In this case, mortality was averted by combination therapy with rifampin, amphotericin B, and steroids for 4 weeks. More experience is necessary to confirm our findings.

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