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*. *N. fowleri* usually cause acute fulminant meningoencephalitis in young immunocompetent individuals, whereas *Acanthamoeba* species usually cause chronic but fatal encephalitis in immunocompromised hosts that is often granulomatous amebic encephalitis (GAE). Timely diagnosis of GAE is often not made because of its rarity and unawareness of the clinicians. Although ~150 patients with GAE have been reported worldwide, < 10 survived. 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 32 mg/dL. Gram stain and 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 DNA from 250 μL of the supernatant according to the manufacturer’s instructions. PCR was performed on the CSF specimens after DNA extraction. Two diagnostic primer sets, Nae3-For/Nae3-Rev and AcantF900/AcantR1100, were used to detect *Naegleria* spp. and *Acanthamoeba* spp., respectively. *A. castellanii* Neff strain (ATCC 30010) and *N. fowleri* Carter strain (ATCC 22758) were used as positive controls. Thermal cycling conditions were 95°C for 10 minutes, followed by 35 cycles of 94°C for 45 seconds, 42°C for 45 seconds, 72°C for 1 minute, and a final extension of 72°C for 10 minutes for *Acanthamoeba* spp., and 95°C for 10 minutes, followed by 40 cycles of 94°C for 30 seconds, 50°C for 45 seconds, 72°C for 45 seconds, and a final extension of 72°C for 10 minutes for *Naegleria* spp. The PCR products were visualized after electrophoresis on 2% agarose gels. As shown in Figure 2A, only a 180-bp amplicon of *Acanthamoeba* spp. was detected in the first CSF specimen (Lanes 1–3) but negative for *Naegleria* spp. (Lanes 4–6). The other four CSF specimens remained negative for both.

For further confirmation, the primer set JDP1/JDP2 that targets the variable region of a small subunit rRNA gene frag-
results indicated that the men are from the same strain needs further clarification, the when he fell into the gully and choked on the water.

The rice field, and it is likely that the patient became infected 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 specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of the CSF specimen or brain tissue, detection and isolation of

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 agammaglobulinemia, 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 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. 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 castellani 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.

Acanthamoeba 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.

Acanthamoeba spp. 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.

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.
Therapeutic options for GAE are limited. In vitro susceptibility tests suggest that ketoconazole, pentamidine, polymyxin, trimethoprim-sulfamethoxazole, sulfadiazine, flucytosine, amphotericin B, and rifampin may be active against *Acanthamoeba*, but the activities of these agents showed variable results. Animal studies have shown sulfadiazine, rifampin, and flucytosine to be effective and, if given earlier, particularly before the infection enters the central nervous system, death may be averted. 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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