INTRODUCTION

Free-living amebas that cause infections of the central nervous system (CNS) in humans include Naegleria fowleri, Acanthamoeba species, and Balamuthia mandrillaris. Acanthamoeba species usually cause chronic but fatal encephalitis in immunocompromised hosts, whereas Naegleria fowleri usually cause acute fulminant meningoencephalitis in young immunocompetent individuals. 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. He survived after treatment with amphotericin B, rifampin, and corticosteroids.

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 and Prevention (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 20,000×g. The MagNA Pure Systems (Roche Diagnostics, Mannheim, Germany) were used to isolate a total of 100 μL DNA from 250 μg 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-
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.1 Predisposing conditions to *Acanthamoeba* infections include AIDS, diabetes, receipt of steroids or chemotherapy, systemic lupus erythematosus, lymphoproliferative disorders, and agammaglobulinemia,13 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.10

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.1 Imaging studies in GAE usually show multifocal hypodense lesions with enhancement.11 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.14,15 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.4,6 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,15 earlier diagnosis of GAE should be made and might alter the outcome of treatment.
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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