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Isolation of *Acanthamoeba culbertsoni* from a Patient with Meningitis

M. K. LALITHA,[1]* V. ANANDI,[1] A. SRIVASTAVA,[2] K. THOMAS,[2] A. M. CHERIAN,[2] AND S. M. CHANDI[3]

Departments of Microbiology,[1] Internal Medicine, Unit II,[2] and Pathology,[3] Christian Medical College Hospital, Vellore 632 004, South India

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A case of amoebic meningitis, presumably primary, was encountered in the Christian Medical College Hospital, Vellore, South India, in November 1983. The patient, a 40-year-old man, had cerebrospinal fluid rhinorrhea before the meningitis developed. *Acanthamoeba culbertsoni* was repeatedly demonstrated in and cultured from the cerebrospinal fluid. The patient responded dramatically to a combination therapy of penicillin and chloramphenicol.

Free-living amoebae were established as being pathogenic for animals by Culbertson et al. (5) in 1959 and for humans by Fowler and Carter (6) in 1965. The pathogenic genera were designated as *Naegleria* and *Acanthamoeba* (*Hartmannella*) (8, 12). Most of the reported cases of disease (14) have been caused by *Naegleria* species, and a majority of them have met with a fatal outcome.

The documented cases of *Acanthamoeba* infections in humans were mainly diagnosed after an autopsy by immunoperoxidase and immunofluorescent-antibody techniques (7). So far, amoebae have been cultured from the cerebrospinal fluid (CSF) in only two instances (3, 4). They were identified as *Acanthamoeba astronyxis* and *A. rhysodes*.

Primary amoebic meningoencephalitis was first encountered in India in 1971 (9). Subsequently, one more case, apparently caused by a *Naegleria* species, was recorded (2). The case reported here is presumably the first case of primary amoebic meningitis caused by *A. culbertsoni* in India.

Subject. A 40-year-old man was admitted to the internal medicine unit with an acute onset of fever, a headache, and pain in the neck preceded by 2 days of lethargy. On the day of admission, he was found to be lethargic. There was no history of convulsions. For the last 8 months, he had had a continuous watery discharge from the left nostril which increased on sneezing and coughing. The patient had a frequent history of bathing in ponds because he was a truck driver.

On examination, the patient was febrile (temperature, 39.9°C), his pulse was 104/min, and his blood pressure was 108/80 mmHg. He had neck stiffness, and Kering's sign was present. Fundi oculi showed no papilledema. There was no neurological deficit. A tentative diagnosis of acute pyogenic meningitis with CSF rhinorrhea was made.

Laboratory findings. A peripheral blood examination revealed a total leukocyte count of 20,000/mm^3, with 80% neutrophils. A lumbar puncture revealed opalescent CSF with 2,960 leukocytes per mm^3 (94% neutrophils and 6% lymphocytes). There were 10 erythrocytes per mm^3. The protein concentration was 110 mg/dl and the glucose concentration was 88 mg/dl. The concomitant blood glucose concentration was 134 mg/dl. A computed axial tomographic scan of the skull was normal.

Microbiological findings. The Gram stain smear of the CSF showed many pus cells but no bacteria. A wet preparation revealed many cells with amoeboid movement. They contained a single nucleus centrally or slightly eccentrically located. Within the nucleus was a centrally located, large karyosome surrounded by a clear halo. The cytoplasm was vacuolated. Various staining procedures, including periodic acid-Schiff, Papanicolaou, and iron hematoxylin, were done to rule out macrophages and malignant cells as well as to confirm the presence of amoebae.

The CSF sample was inoculated onto culture media for bacteria and fungi. It was also cultured on a nonnutrient agar medium with *Escherichia coli* as the associate, in accordance with the method described by Singh (11). For animal pathogenicity testing, 0.01 ml of the material was injected intracerebrally into adult white mice.

Routine culturing did not yield any bacteria or fungi. In the nonnutrient medium, after 48 h, there was macroscopic evidence of lysis of *E. coli* in the region where the CSF sample was spot inoculated. A wet preparation from this area revealed many motile trophozoites (Fig. 1) with characteristic lobopodium and acanthopodia (13). Later, cystic forms with a double wall and a wrinkled appearance were also demonstrated. The CSF samples examined on days 2 and 3 after admission also revealed similar findings; the number of motile amoebae decreased gradually. The fluid collected from the left nostril showed a few amoebae, but these did not grow in cultures.

The inoculated mice developed typical signs of paralysis after 1 week. Just before death, the mice were sacrificed, and a wet preparation of the purulent fluid present on the brain surface was made. Many motile trophozoites morphologically suggestive of *Acanthamoeba* spp. were seen, and their presence was confirmed by culturing. A histopathological examination of the mouse brain revealed acute meningitis and microabscess formation. Associated with the latter in adjacent areas were several structures resembling amoebae; they were confirmed as amoebae with the periodic acid-Schiff technique. The strains were referred to B. N. Singh of the Central Drug Research Institute, Lucknow, India, for confirmation and identification of the species. The strains were identified as *A. culbertsoni*.

Treatment in the hospital and follow-up. Treatment for pyogenic meningitis was started with penicillin G (2.0×10^6 U every 3 h intravenously) and chloramphenicol (500 mg every 6 h orally). Because there was remarkable improve-

* Corresponding author.

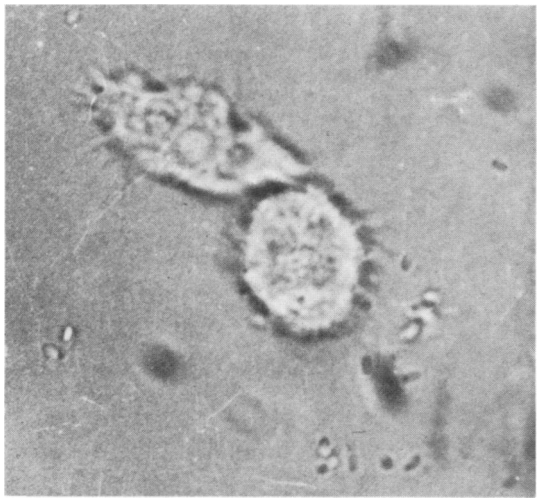

FIG. 1. Photomicrograph of a culture showing amoeba with acanthopodia (magnification, ×1,500).

ment, no separate therapy for an amoebic infection was instituted after the culture results became available.

The patient was afebrile from day 3 of admission on and was asymptomatic within 10 days, except for the CSF rhinorrhea. A follow-up examination done 2 months later revealed the cessation of the CSF rhinorrhea. A lumbar puncture revealed clear CSF with one lymphocyte per mm^3 and no amoebae. The protein content was 10 mg/dl, and the sugar content was 76 mg/dl. There was no neurological deficit.

Discussion. Humans usually acquire amoebic meningitis by swimming or bathing in ponds, lakes, or streams. In the case reported here, the patient had a history of frequent bathing in ponds. The CSF rhinorrhea could have precipitated the infection.

There have been only a few survivors among the classically described cases of *Acanthamoeba* meningitis. The similarity between the case recorded in 1968 (3) and ours is striking. The history of exposure to water collections, the absence of any other underlying disease, the short duration of the symptoms, the predominantly meningitic features at presentation, the marginal elevation of the protein concentration and the normal glucose concentration in the CSF, and the complete recovery after a course of antibiotics were the same in both cases.

No specific therapy has been described so far for the treatment of *Acanthamoeba* meningitis. However, amphotericin B has been used for the treatment of *Naegleria* infections with some success (1, 10). In the case of an *Acanthamoeba* infection reported by Cleland et al. (4), the patient experienced a partial recovery over a period of time with sulfamethazine therapy. The efficacy of a penicillin-chloramphenicol combination, the recommended regime for pyogenic meningitis, as instituted in our case is yet to be determined.

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