Case report

A case report of primary amebic meningoencephalitis in North Florida

Saad K. Anjum, Karna Mangrola, Garrett Fitzpatrick, Kimberly Stockdale, Laura Matthias, Ibne Karim M. Ali, Jennifer R. Cope, Kevin O’Laughlin, Shelley Collins, Stacy G. Beal, Frances M. Saccocio

Department of Pediatrics, University of Florida, 1600 SW Archer Rd, Gainesville, FL, 32610, USA
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, 1600 SW Archer Rd, Gainesville, FL, 32610, USA
Division of Disease Control and Health Protection, Florida Department of Health, 200 Ringling Blvd, Sarasota, FL, 34237, USA
National Center for Emerging and Zoonotic Infectious Diseases, Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA, 30329, USA
Epidemic Intelligence Service, Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA, 30329, USA
Department of Pediatrics, Division of Hospital Medicine, University of Florida, 1600 SW Archer Rd, Gainesville, FL, 32610, USA
Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Florida, 1600 SW Archer Rd, Gainesville, FL, 32610, USA

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Primary amebic meningoencephalitis is a rare, usually fatal disease, caused by Naegleria fowleri. This case highlights the challenging clinicopathologic diagnosis in a 13-year-old boy who swam in freshwater in northern Florida where a previous case had exposure to a body of water on the same property in 2009.

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Introduction

Primary amebic meningoencephalitis (PAM) is a rapid and usually fatal disease of the central nervous system caused by Naegleria fowleri, a free-living ameba that lives in warm freshwater. The ameba enters the body through the nasal passage, crosses the cribiform plate, and travels to the brain [1]. The Centers for Disease Control and Prevention (CDC) identified 148 cases in the United States from 1962 through 2019. The majority of these cases occurred in the southern United States in males (76%) with a median age of 12 years [2]. Recently, CDC estimated that there are 16 cases of PAM in the United States annually [3].

Case report

A 13-year-old Caucasian boy with a history of headaches had been on a camping trip in July 2020 that included swimming at a water park in northern Florida. Three days after he returned, he developed a severe headache, fevers and intractable emesis. On the day of symptom onset, he did not respond to acetaminophen and ibuprofen. His parents sought evaluation at an urgent care provider where he tested negative for COVID-19. He then went to a hospital where he received ondansetron, acetaminophen, ibuprofen, diphenhydramine, ketorolac, chlorpromazine, and benzylpenicillin for a tonsillolith. He remained symptomatic and was brought to our hospital. Initial workup included a comprehensive metabolic panel within normal limits, a complete blood count with a white blood cell count of 12,700/cu mm with segmented neutrophil predominance (87.8%), and a C-reactive protein (CRP) of 14.9 mg/L. His emesis continued along with poor oral intake. Thus, he was admitted for further workup.

He began demonstrating hemodynamic instability, altered mental status, and meningeal signs approximately 6 h into admission. Vital signs suggested increased intracranial pressure (ICP) prompting an urgent computed tomography (CT) scan. Fluid resuscitation was initiated, and ceftriaxone was given. His care was escalated to the Pediatric Intensive Care Unit (PICU). A lumbar puncture was performed. Opening pressure was >36 cm H2O; cerebrospinal fluid (CSF) was turbid with a glucose of 26 mg/dL, protein of 389 mg/dL, and leukocyte count of 670/cu mm (90% segmented neutrophils). This raised concern for bacterial etiology that may have been partially treated with benzylpenicillin. The preliminary pathology report described a predominance of mature neutrophils with scattered lymphocytes and...
macrophages. Acyclovir and vancomycin were started in addition to ceftriaxone for suspected severe meningitis. A repeat CRP was performed on day 4 of admission and showed an increase to 285 mg/L.

In the PICU, 12 h into admission, he continued to show signs of increased ICP. He was treated with mannitol and 3% normal saline due to concern for cerebral edema. His mentation improved momentarily, but he developed respiratory failure and was intubated. A brain magnetic resonance imaging (MRI) study revealed leptomeningeal enhancement without herniation. Neurosurgery was consulted for the increased ICP and placed an external ventricular drain (EVD). His symptoms persisted, and another CT scan suggested early transtentorial herniation. Neurosurgery thus placed a second EVD. A CSF sample from this EVD showed protein of >1gm/dL, and a Wright-Giemsa stain showed amebic trophozoites with exuberant neutrophilic response (Fig. 1). Per CDC recommendations, he was started on miltefosine, amphotericin B, fluconazole, rifampin, azithromycin, and dexamethasone approximately 62 h after symptom onset. Despite aggressive intervention, there was no response on repeat neurological examination. His parents withdrew care four days after presentation.

A real-time PCR performed at the CDC on a CSF sample confirmed _N. fowleri_. The strain belonged to genotype I based on the internal transcribed spacer 1 sequence [4]. CDC and the Florida Department of Health (FDOH) collaborated to investigate the patient’s water exposures. The most probable exposure was a campground in Madison County, FL, with treated and untreated bodies of water. The treated water bodies included a water slide, swimming pool, and human-made river. The untreated areas included a lake for fishing, retention pond, and another lake with an obstacle course. The family reported the patient swam in both treated and untreated water.

FDOH conducted a site visit on August 5, 2020. Maintenance logs for the pool and lazy river indicated that the chlorine level and pH were acceptable on the days of exposure. Testing during the site visit also showed appropriate free chlorine levels and a pH of 7.3. The recreational lake is human made, has a sand bottom, and has a surface area of 2.2 acres with the greatest depth measured at 9 feet. Water chemistry parameters were measured at two locations; both had a temperature of 90°F, pH of 8+, and an undetectable chlorine level. A dye was added to give the lake a bluer appearance. No other chemicals were added and there was no thermal pollution of the lake. The lake had not been drained recently and the water level was normal. The owners were informed and closed the recreational lake. At the time of assessment, there were no plans to reopen and signs prohibiting swimming were posted.

**Discussion**

PAM presents a multifaceted clinicopathologic challenge. Early diagnosis and treatment have the potential to prevent a nearly universal fatal outcome. This depends on communication between clinicians and the clinical laboratory. While practices vary, knowledge of how CSF samples are processed by the institution’s laboratory is essential to understanding the diagnostic challenges.

Several studies are routinely ordered on CSF samples in cases of suspected meningitis, including culture and gram stain, molecular

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*Fig. 1.* Wright-Giemsa stain of CSF demonstrating _N. fowleri_ organisms and inflammatory cells. A) Three organisms (arrowheads) adjacent a segmented neutrophil showing prominent vacuolation (arrow). B) Three vacuolated trophozoites are pictured on high-magnification. A single small, pale nucleus can be identified in each organism, each of which is smaller than the individual segments of surrounding segmented neutrophils. C) A single trophozoite is present at the bottom of the field (arrowhead), not to be confused with a macrophage (black arrow) or vacuolated lymphocyte (white arrow) at the top of the image. D) A cluster of organisms (arrowheads) and inflammatory cells of multiple types. Multiple similar clusters were present on the slide, some of which showed nearly completely obscured trophozoites.
diagnostic assays, cell count with differential, and chemistry studies. CSF chemistry in PAM mimics that of bacterial meningitis with increased protein and decreased glucose. Amoebae are not reliably detected on gram stain or other routine studies. Furthermore, N. fowleri does not grow on standard culture medium, as it requires bacteria-enriched agar.

Without access to rapid confirmatory molecular testing, microscopic examination of the CSF plays a key role in identification with inherent challenges. Microscopy is only reliable when “clearly motile” N. fowleri trophozoites are seen on wet prep of a fresh CSF sample. Motility can commonly be diminished by inappropriate handling of the CSF (for example, refrigeration of CSF), or anti-amebic treatment. Additionally, early in the infection, there may be too few organisms to detect. These challenges can be overcome by molecular assays, such as real-time PCRs, which can provide reliable diagnosis.

If a Wright–Giemsa stain is performed on centrifuged CSF, non-motile N. fowleri trophozoites may be identified by their distinct cytoclogic appearance: a small nucleus accompanied by pale nuclear staining, a rim of enhancement at the periphery, and clustering of organisms. Additionally, the size of N. fowleri trophozoites (10–20 microns) overlaps with different types of leukocytes. Their cytoplasmic color and vacuolation may also resemble that of macrophages and lymphocytes.

Importantly, neither wet prep nor Wright-Giemsa stained slides are routinely reviewed by pathologists. In cases of suspected meningitis, cell counts and differential are typically performed by medical technologists who may alert the pathologist about concerning findings. The experience and vigilance of medical technologists are paramount. Given the rarity of N. fowleri infections, diagnostic challenges, and importance of rapid diagnosis, communication with the clinical laboratory is critical if PAM is suspected.

Within the context of this case, the aforementioned challenges were encountered before accurate diagnosis. Initial microscopic review was difficult to discern amoeba as their motility or counts were not seen. It was not until re-sampling of the CSF that amoeba were visualized by Wright-Giemsa staining. However, given the aggressive nature of PAM, diagnosis needs to be prompt for the best outcomes. The intrinsic difficulty of this case resided in the arduous nature of diagnosis and the presentation mimicking a typical bacterial meningitis at onset.

The site visit showed a facility that met requirements for operating a treated swimming pool. However, the facility also maintained an untreated lake with an obstacle course, diving platform, and slide. These features allow people to enter the water with more speed and force, increasing the possibility of water entering the nose. The warm, untreated water forcibly entering the nose most likely allowed exposure to N. fowleri. A previous case was investigated at the same facility in 2009 when the campground was under a different name. In the 2009 case, the patient was a 13-year-old boy who swam in a human-made body of water. When comparing aerial views from 2009 and present, that lake appears to no longer exist. Although the two cases separated by 11 years were likely in different human-made bodies of water, the bodies were both on the same property, supplied with well water, treated with blue dye, and had built-in features such as water slides.

The CDC estimates that 16 cases of PAM occur annually [3]. Appropriate history-taking and knowledge of the pathology of PAM can allow for earlier diagnosis. A previous case report denotes successful treatment with amphotericin B, azithromycin, fluconazole, rifampin, miltefosine, and dexamethasone [5]. The case presented here demonstrates the unusual nature of N. fowleri infections. Despite N. fowleri being frequently found in the environment, and presumably frequent exposures given the millions of swimming visits every year, infections remain rare. Further investigation is needed to explain the two cases of PAM resulting from water exposure on the same property.

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Author contribution
SKA, KM, SC, and FMS provided direct patient care. GF and SGB provided pathological review for the patient and developed Fig. 1. KS and LM conducted the site visit. IK, JRC, and KO provided confirmatory molecular diagnostic testing. All authors participated in writing and editing of this report.

Author statement
Saad K Anjum: conceptualization, writing – original draft.
Karna Mangrola: conceptualization, writing – original draft, review & editing.
Garrett Fitzpatrick: Investigation (pathological diagnosis), visualization (designed figure).
Kimberly Stockdale: Investigation (epidemiologic, including site visit), writing – original draft, review & editing.
Laura Matthias: Investigation (epidemiologic, including site visit), writing – original draft.
Ibn Karim M Ali: Investigation (molecular diagnosis), writing – original draft.
Jennifer R Cope: Investigation (molecular diagnosis), writing – original draft.
Kevin O’Laughlin: Investigation (molecular diagnosis), writing – original draft, review & editing.
Shelley Collins: conceptualization, writing – review & editing.
Stacy G Beal: Investigation (pathological diagnosis), visualization (designed figure).
Frances M Saccoccio: conceptualization, supervision, writing – review & editing.

Disclaimer
The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Declaration of Competing Interest
The authors report no declarations of interest.

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