Original Article

Diagnosis, Clinical Course, and Treatment of Primary Amoebic Meningoencephalitis in the United States, 1937–2013

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Received July 15, 2014; accepted July 22, 2014; electronically published October 23, 2014.

Background. Primary amoebic meningoencephalitis (PAM) is a rapidly progressing waterborne illness that predominately affects children and is nearly always fatal. PAM is caused by *Naegleria fowleri*, a free-living amoeba found in bodies of warm freshwater worldwide.

Methods. We reviewed exposure location, clinical signs and symptoms, diagnostic modalities, and treatment from confirmed cases of PAM diagnosed in the United States during 1937–2013. Patients were categorized into the early (ie, flu-like symptoms) or late (ie, central nervous system signs) group on the basis of presenting clinical characteristics. Here, we describe characteristics of the survivors and decedents.

Result. The median age of the patients was 12 years (83% aged ≤18 years); males (76%) were predominately affected (N = 142). Most infections occurred in southern-tier states; however, 4 recent infections were acquired in northern states: Minnesota (2), Kansas (1), and Indiana (1). Most (72%) of the patients presented with central nervous system involvement. Cerebrospinal fluid analysis resembled bacterial meningitis with high opening pressures, elevated white blood cell counts with predominantly neutrophils (median, 2400 cells/μL [range, 5–26 000 cells/μL]), low glucose levels (median, 23 mg/dL [range, 1–92 mg/dL]), and elevated protein levels (median, 365 mg/dL [range, 24–1210 mg/dL]). Amoebas found in the cerebrospinal fluid were diagnostic, but PAM was diagnosed for only 27% of the patients before death. Imaging results were abnormal in approximately three-fourths of the patients but were not diagnostic for amoebic infection. Three patients in the United States survived.

Conclusions. To our knowledge, this is the first comprehensive clinical case series of PAM presented in the United States. PAM is a fatal illness with limited treatment success and is expanding into more northern regions. Clinicians who suspect that they have a patient with PAM should contact the US Centers for Disease Control and Prevention at 770–488–7100 (available 24 hours/day, 7 days/week) to discuss diagnostic testing and treatment options (see cdc.gov/naegleria).

Key words. amoebic meningoencephalitis diagnosis and treatment; *Naegleria* infection; water-borne disease.

Primary amoebic meningoencephalitis (PAM) (0–8 infections/year in the United States) is a rapidly progressive illness with a case-fatality rate that is greater than 97%. The causative agent for PAM is *Naegleria fowleri*, a ubiquitous, thermophilic, free-living amoeba that is found in many warm freshwater ponds, lakes, streams, and canals. Historically, most US cases have occurred in the southern-tier states, with most case-patients being exposed to freshwater from a lake, pond, or reservoir; a canal, ditch, or puddle; or a river or stream [1]. However, the epidemiology of *N. fowleri* seems to be changing; a case of PAM associated with local recreational water exposure recently occurred in the northern state of Minnesota [2].

N. fowleri exists in 3 morphological forms: trophozoite (amoeba), flagellate, and cyst [3]. Trophozoites are the infectious form of *N. fowleri* [4, 5]. Water containing trophozoites and flagellates may be inhaled into the nasal cavity [6, 7]. Amoebas penetrate the nasal mucosa and migrate

via the cribriform plate into the central nervous system (CNS) through the olfactory nerves [7, 8]. The spread of amoebas through the CNS causes cerebral edema and necrosis, cerebellar or uncal herniation, and ultimately death [3, 7, 9].

Although definitive diagnosis of *N. fowleri* infection requires specialized experience, immunohistochemical staining, or polymerase chain reaction (PCR) testing, presumptive diagnosis can be made before death through microscopic examination of the cerebrospinal fluid (CSF) immediately after collection. Actively motile trophozoites can be observed on a wet mount of the CSF (Figure 1). CSF smears stained with Giemsa or Wright stains can be useful in trophozoite identification (Figure 2) [3]. PCR can be used to detect *N. fowleri* nucleic acid in CSF [10].

Treatment for PAM has been largely unsuccessful, and the prognosis is poor. We present here the largest (to our knowledge) clinical case series of patients with PAM in the United States and discuss the exposure location, clinical presentation, diagnostic modalities, and clinical management.

METHODS

The US Centers for Disease Control and Prevention (CDC) Free Living Ameba Laboratory registry and other data sources listed in an earlier epidemiological *N. fowleri* review [1] were used to identify and describe patients with PAM who were diagnosed and reported in the United States through 2013. Medical records and autopsy reports of individual cases shared with the CDC as part of diagnostic and clinical consultations were also reviewed.

To describe the clinical status of patients on initial presentation and to explore disease progression, we categorized patients into 1 of 2 groups on the basis of their signs and symptoms on initial presentation to a healthcare facility (ie, first time seeking care at a primary care provider, local health clinic, urgent care clinic, emergency department, or hospital). A patient was categorized in the early group when he or she presented with vague symptoms resembling a flu-like prodrome, including headache, nausea, vomiting, fever, fatigue, or earache. A patient was categorized in the late group when he or she presented with any sign of CNS involvement, including nuchal rigidity, altered mental status (including confusion, disorientation, agitation, combativeness, lethargy, drowsiness, obtundity, stupor, or coma), seizures, abnormal deep tendon reflexes, vision or eye abnormalities (including photophobia, blurred vision, nystagmus, anisocoria), cranial nerve abnormalities (including ageusia), abnormal gait/inability to walk, syncope, or hallucinations.

RESULTS

Patient Characteristics

From 1937 to 2013, 142 patients with PAM were reported in the United States. Patients were predominately male (76%) and children (83% aged ≤18 years), with a median age of 12 years (range, 8 months to 66 years). Exposure location was predominantly in the south and west regions of the United States (Figure 3). Five cases were reported in the Midwest (1 in Kansas, 2 in Minnesota, 1 in Missouri, and 1 in Indiana); the 4 northernmost cases occurred in or after 2010. There were 99 (70%) patients with some clinical information available for analysis, including clinical signs/symptoms, laboratory testing, radiologic imaging, and treatment. All the patients were healthy before the *Naegleria* infection except for 3 patients with reported

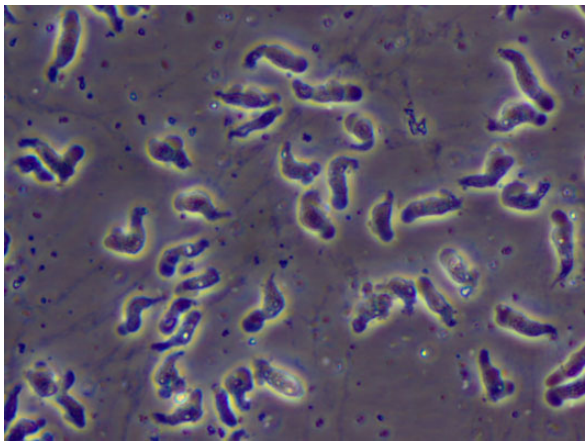


Figure 1. A wet mount of *Naegleria fowleri* trophozoites cultured from the CSF of a patient with primary amebic meningoencephalitis (PAM) viewed using phase contrast microscopy. Magnification, x600.

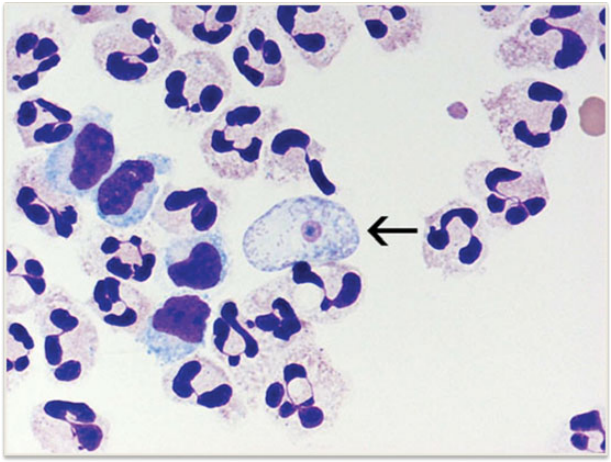
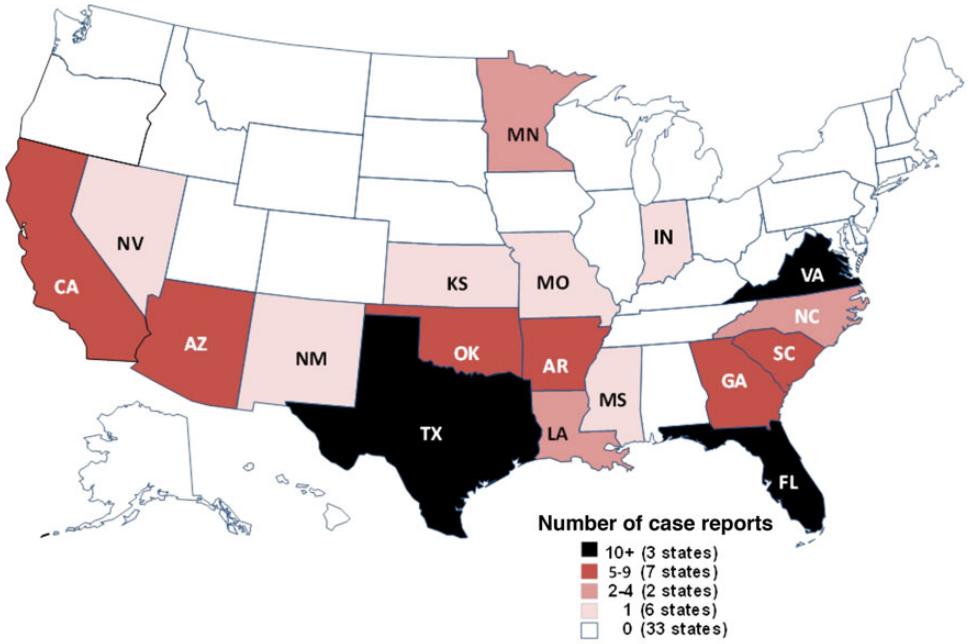


Figure 2. A cytospin of fixed CSF showing an *N. fowleri* trophozoite (arrow) stained with Giemsa-Wright amidst polymorphonuclear leukocytes and a few lymphocytes. Within the trophozoite, the nucleus and nucleolus can be seen. Magnification, ×1000.



*State of exposure unknown for 4 cases
†11/12 Virginia (VA) cases that occurred during 1937–1957 were identified as part of a retrospective pathology study.

Figure 3. Number of case reports of PAM caused by *N. fowleri* (N = 142) by state* of exposure— United States, 1937–2013†.

Table 1. Characteristics of Patients With PAM—United States, 1937–2013

Variable	Value
Median age (range) (N = 142)	12 y (8 mo to 66 y)
Sex (n [%]) (N = 142)	
Male	108 (76)
Female	34 (24)
Region[a] of exposure (N = 138) (n [%])	
Midwest	5 (4)
Northeast	0 (0)
South	117 (85)
West	16 (12)
Median (range) incubation period (N = 44) (days)	5 (1–9)
Median (range) time from onset of symptoms to hospitalization (N = 86) (days)	2 (0–7)
Median (range) time from onset of symptoms to initiation of PAM-specific treatment (N = 32) (days)	3 (0.75–8)
Median (range) time from hospitalization to initiation of PAM-specific treatment (N = 32) (days)	0.75 (0–4)
Median (range) duration of hospital stay (days)	
Decedents (N = 94)	2 (0–17)
Survivors (N = 3)	55 (30–76)
Median (range) time from onset of symptoms to death (N = 103) (days)	5 (1–18)

[a]Regions defined by the US Census Bureau.

chronic ear infections. The median incubation period, based on patients with a known single exposure (n = 44), was 5 days (range, 1–9 days), and there were 3 survivors in our case series, 2 of whom fully recovered and 1 who had residual neurologic deficits (Table 1). All the survivors had *N. fowleri* infection confirmed by PCR [10].

Clinical Presentation

In total, 89 patients (1951–2013) had data available to determine their clinical status on initial presentation to a healthcare facility. Of these patients, 25 (28%) and 64 (72%) presented with early symptoms and late symptoms, respectively (Table 2). The 2 survivors with known clinical status on initial presentation presented with late symptoms. Forty-four patients (49%) presented with altered mental status (late group), and 7 (8%) patients presented in an advanced state, including 2 (2%) with obtundity, 2 (2%) with stupor, and 3 (3%) in a coma. All the patients became comatose before death. The early signs/symptoms lasted for a median of 2 days (range, 0–8 days). Patients spent a median of 3 days (range, 0–10 days) with CNS symptoms, although the duration depended on how long the patient was maintained on life support.

Initial Discharge and Misdiagnosis

Twenty-four (27%) of 89 patients with PAM were initially sent home after first presentation to the primary care provider, local health clinic, urgent care clinic, emergency department, or hospital. Nineteen (79%) of these patients had early symptoms, and 5 (21%) had late symptoms. The majority (14 [74%]) of the discharged patients presenting as the early group complained of persistent frontal headaches not alleviated with medication and were diagnosed with a flu-like or viral illness; 2 (11%) of these patients complained of earache and were sent home with antibiotics for ear infection. Discharged patients in the early group

also experienced fevers (11 [58%]), vomiting (11 [58%]), fatigue (4 [21%]), and nausea (2 [11%]). Of 5 patients who initially presented with late symptoms and were treated as outpatients, 4 (80%) had altered mental status (including 1 with lethargy, 2 with agitation, 1 with confusion, and 1 with restlessness), 1 (20%) had nuchal rigidity, 1 (20%) had photophobia, and 1 (20%) had ageusia.

Table 2. Clinical Status on Initial Presentation to a Healthcare Facility for Patients With PAM (N = 89)—United States, 1951–2013

Group and Symptoms	n (%)
Early (flu-like prodrome symptoms only)	25 (28)
Headache	82 (92)
Nausea/vomiting	58 (65)
Fever	75 (84)
Fatigue	14 (16)
Earache	2 (2)
Late (CNS involvement)	64 (72)
Nuchal rigidity	33 (37)
Lethargy	26 (29)
Confusion/disorientation	22 (25)
Anorexia	15 (17)
Irritation/combativeness	11 (12)
Photophobia	10 (11)
Drowsiness	8 (9)
Seizures	8 (9)
Blurred vision	4 (4)
Cranial nerve abnormalities	4 (4)
Myalgia	4 (4)
Abnormal deep tendon reflexes	3 (3)
Comatose	3 (3)
Stuporous	2 (2)
Obtunded	2 (2)
Abnormal gait/inability to walk	2 (2)
Syncope	2 (2)
Hallucinations	2 (2)

Clinical Laboratory Testing

Abnormalities were found in the patients' complete blood counts (CBCs) on admission, most commonly a leukocytosis (median white blood cell [WBC] count, 17×10^3 cells/μL [range, 8×10^3 to 31×10^3 cells/μL) predominated by neutrophils (median, 87% [range, 31%–98%]) and bands (median, 8% [range, 1%–34%]) (Table 3). CSF findings were also abnormal in most of these patients (Table 3). The opening pressures were markedly elevated (median, 385 mm H_2O [range, 230–560 mm H_2O]). Large numbers of red blood cells (RBCs) were commonly present in the CSF (median, 265 cells/μL [range, 0–30 750 cells/μL]). The WBC counts were usually elevated (median, 2400 cells/μL [range, 5–26 000 cells/μL]) and predominated by neutrophils (median, 83% [21%–98%]). The CSF also generally showed increased protein concentrations (median, 365 mg/dL [range, 24–1210 mg/dL]) and low glucose concentrations (median, of 23 mg/dL [range, 1–92 mg/dL]).

Microscopic Diagnosis

PAM was diagnosed before death (or in the case of the survivors, discharge) in 27% (39 of 142) of the patients. In nearly all (38 of 39) cases, motile amoebas were seen in a wet mount of the CSF and subsequently identified by Wright-Giemsa staining. For 35% (15 of 43) of the patients with CSF stain information, only a Gram stain was performed on CSF; amoebas were not identified in the CSF of any of these patients, and a diagnosis of PAM was not made before death. Of 34 patients with information available, the median time from admission to first identification of amoebas in the CSF was 1 day (range, 0–4 days). Of 30

Table 3. Initial Laboratory Findings Reported on Admission for Patients With PAM—United States, 1937–2013.

Test (reference value)	n	Median (Range)
Complete blood count		
WBCs[a] (4.5×10^3–11.0×10^3 cells/μL)	47	16.8×10^3 (8.4–31.4)
% lymphocytes (25%–35%)[a]	32	4 (2–28)
% neutrophils (40%–80%)[a]	37	87 (31–98)
% bands (0%–5%)[a]	20	8 (1–34)
% monocytes (2%–10%)[a]	21	4 (1–8)
Hemoglobin (12–17 g/dL)[b,c]	24	13 (10.3–16.8)
Hematocrit (36%–51%)[b,c]	22	39 (32–48)
Platelets (150×10^3–350×10^3 cells/μL)[b]	11	288 (89–585)
CSF		
Opening pressure (100–200 mm H_2O)[b]	12	385 (230–560)
RBCs (0 cells/μL)[b]	47	265 (0–30 750)[d]
WBCs (0–5 cells/μL)[b]	79	2400 (5–26 000)
% lymphocytes (62% ± 34%)[e]	43	15 (2–90)
% neutrophils (2% ± 5%)[e]	61	83 (21–98)
% monocytes (36% ± 20%)[e]	26	8 (1–66)
Glucose (40–80 mg/dL)[b]	66	23 (1–92)
Protein (15–60 mg/dL)[b]	67	365 (24–1210)

[a]Reference values were obtained from reference [30].
[b]Reference values were obtained from reference [31].
[c]Range values combined for male and female patients.
[d]The number of those with 0–250 RBCs was 23; 251–500, 6; 501–750, 3; 751–1000, 3; >1000, 12.
[e]Reference values were obtained from reference [32].

patients with information available, the median time from symptom onset to the first identification of amoebas in the CSF was 3 days (range, 1–8 days). The remaining patients were diagnosed with PAM after death through hematoxylin and eosin (H&E) or polyclonal antibody staining of brain tissue for *N. fowleri*, postmortem CSF culture, or reanalysis of premortem CSF samples using immunofluorescence, real-time PCR, or Wright-Giemsa stain.

Radiologic Imaging

Forty-seven patients (33%) had documented computed tomography (CT) or magnetic resonance imaging (MRI) scans of the brain, or both, totaling 71 scans. The earliest case to have documented brain imaging occurred in 1978. Fifty-five (79%) of the total brain scans were read as abnormal. Interpretations included descriptions of diffuse cerebral edema with effacement of the cortical sulci and basilar cisterns, leptomeningeal enhancement, decrease in the size of ventricles, areas of hemorrhage or necrosis, and/or herniation.

Therapeutic Management of PAM

Treatment data for 70 of 142 (49%) patients in the United States were available. Of these patients, 36 (51%) were treated for PAM, 3 (8%) of whom survived (Table 4). All 36 patients treated for PAM received amphotericin B (7 [19%] received only intravenous [IV] therapy, 5 [14%] received only intrathecal [IT] therapy, and 24 [67%] received a combination of IV and IT therapy). We identified 7 patients who received a non-deoxycholate

Table 4. Clinical Outcome and Therapeutic Management of Patients Treated for PAM (N = 36)—United States, 1969–2013

Outcome	Year	Age (y)	Sex	IV Amphotericin B (Duration of Treatment [days])	IT Amphotericin B (Duration of Treatment [days])	-Azole IV/PO[a] (Duration of Treatment [days])	-Azole IT[b] (Duration of Treatment [days])	Rifampin IV/PO (Duration of Treatment [days])	Steroid IV (Duration of Treatment [days])	Symptom Onset to Start of Treatment (days)
D	1969	24	M	X (3)	X (3)				X (3)	2
D	1969	14	M	X (3)	X (3)				X (3)	3
D	1977	14	F	X (1)						2
S	1978	9	F	X (9)	X (10)	X (9)	X (9)	X (9)	X	3
D	1978	8	M	X (2)	X (2)	X (2)	X (2)	X (2)		3
D	1978	14	M	X (5)	X (5)	X (5)	X (5)			2
D	1978	10	M	X (3)	X (3)					0.75
D	1979	6	M	X (3)						3
D	1980	10	M	X (9)	X (9)	X (9)	X (9)	X (9)		3
D	1980	14	F	X (10)	X (10)	X (10)	X (10)		X	4
D	1982	12	M	X (7)	X (7)	X (7)	X (7)	X (7)		1
D	1983	13	M	X (1)	X (1)	X (1)	X (1)	X (1)		5
D	1984	3	M	X (5)	X (5)	X (5)	X (5)	X (5)		1
D	1984	12	M	X	X	X	X			1
D	1985	8	F		X (1)					Unknown
D	1987	11	M		X (5)			X (5)	X (6)	6
D	1991	29	M	X (1)	X (1)			X (1)		Unknown
D	1998	14	M		X (1)		X (1)			3
D	2001	9	F		X (2)			X (2)		Unknown
D	2002	11	M	X (2)		X (2)		X (2)		2
D	2005	7	F		X (7)					2
D	2007	10	M	X (2)[c]	X (2)[c]	X (2)		X (2)		2
D	2007	11	M	X (1)	X (1)	X (1)	X (1)	X (1)		5
D	2007	12	M	X (4)	X (4)			X (4)		Unknown
D	2007	22	M	X (1)[c]				X	X (4)	3
D	2008	14	F	X (1)[d]	X (1)[d]	X (1)	X (1)	X (1)		5
D	2008	9	M	X (2)[c]	X (2)[c]	X (2)		X (2)	X	3
D	2009	13	M	X (1)[c]						7
D	2009	22	M	X (2)[c]					X (3)	4
D	2010	7	M	X (9)[c]	X (7)	X(9)		X(9)		3
D	2011	28	M	X (1)[c]				X(1)		3
D	2011	16	F	X (3)	X (2)	X(3)		X(3)	X (3)	3
D	2012	9	M	X (2)	X (2)	X (2)		X(2)	X (3)	3
D	2013	12	M	X (15)[d]	X (13)	X (16)		X(16)	X (18)	1.5
S	2013	12	F	X (14)	X (10)	X (26)		X(26)	X (4)	1.5
S	2013	8	M	X (19)	X (5)	X (19)		X(19)	X (29)	3.5

Abbreviations:
S, survival, D, death.
[a]This included miconazole, fluconazole, or ketoconazole.
[b]This included miconazole or fluconazole.
[c]Non-deoxycholate amphotericin B formulation.
[d]Combination liposomal and deoxycholate amphotericin B.

amphotericin B formulation (which includes liposomal and lipid complex formulations). In addition to amphotericin B treatment, 21 (58%) patients also received IV or IT azole therapy, and 23 (64%) patients were treated with oral (PO) or IV rifampin. Information on time from onset to initiation of PAM therapy was available for 89% (32 of 36) of the patients who received PAM treatment. The median time from onset to the start of PAM therapy was 3 days (range, 0.75–7 days). The median time from hospitalization to the start of PAM therapy was 0.75 days (range, 0–4 days). For those patients not treated for PAM, multiple therapeutic agents were tried. Antibiotics targeting pathogens of bacterial meningitis were used in 94% of the patients during their hospital stay.

DISCUSSION

The clinical course of *N. fowleri* infection is fulminant with a rapid progression from onset to death occurring in a median of 5 days. PAM is nearly always fatal, with only 3 documented survivors in the United States through 2013. This clinical case series of PAM occurring in the United States from 1937 to 2013 demonstrates that *N. fowleri* infections are difficult to detect and effectively treat, and the geographic range is expanding northward. Since the first appearance of PAM in a northern state in 2010 [2], there have been 3 additional cases in the northern states of Kansas, Minnesota, and Indiana. This apparent geographic shift necessitates increased provider awareness and improvement in case detection among a broader range of clinicians so that PAM will be considered early in the differential diagnosis of meningoencephalitis, identification is attempted, and amoebic-specific therapy is initiated more rapidly.

The majority of patients with PAM categorized in the early group who were discharged after initial presentation to a healthcare facility (74%) complained of headache and were diagnosed with a flu-like or viral illness. Without knowledge of freshwater exposure, suspicion of PAM with early symptoms is lower. In addition, patients with PAM categorized in the late group in this case series most closely resembled patients with acute bacterial meningitis. Ninety-four percent of the patients were empirically treated for bacterial meningitis before their diagnosis of PAM. The clinical resemblance of *N. fowleri* infection to acute bacterial meningitis has been reported in the literature [7, 11] and further complicates the early diagnosis of PAM.

In addition, the diagnosis of PAM through clinical laboratory analysis can be challenging, because CSF findings in PAM cases are generally nonspecific and also mirror those of acute bacterial meningitis. However, this review highlights some common CSF findings that might be useful for PAM diagnosis. In some patients, large numbers of RBCs were found in the CSF (median, 265 cells/μL). Subarachnoid or cerebral hemorrhage was likely, because neuropathologic changes from *N. fowleri* infection include hemorrhagic necrotizing encephalitis with extensive destruction of cerebral hemispheres and arachnoid and pia mater [7, 9, 12]. Although elevated numbers of RBCs may be the result of a traumatic lumbar puncture (which is not possible to discern from these data), the presence of RBCs might also be a diagnostic clue. The number of erythrocytes found in the CSF may correlate with the level of brain necrosis and inflammation [13].

In addition, suspicion of PAM should be raised on the basis of visualization of motile amoebas in a wet mount of the CSF or identification of the trophozoite by Giemsa or Wright staining [3]. Confirmatory testing with immunohistochemical staining, PCR, or culture is needed for antemortem diagnosis of PAM if amoebas are detected in the CSF [7, 8, 14]. The visualization of *Naegleria*-like organisms in the CSF is a relatively common finding (27%) among patients with PAM and might be more common if all initial CSF specimens from suspect PAM cases were visualized using the proper staining described above. In 15 patients for whom the diagnosis of PAM was not made until after death, only a Gram stain was performed on antemortem CSF, and no amoebas were visualized in these specimens before death occurred. Gram stains are not diagnostic, because *N. fowleri* amoebas will be destroyed upon heat fixation [15]. It is therefore important for healthcare providers to inquire about risk factors for amoebic infections (eg, recent freshwater swimming), particularly in cases of meningoencephalitis with negative CSF Gram stains, and to consider performing a wet mount early in these cases [3, 8]. Hospitals, particularly those in southern-tier states, should consider having the capacity to perform CSF wet mounts and Wright-Giemsa staining in a timely manner to facilitate early diagnosis.

At least three-fourths of the patients with PAM who had CT scans or MRI had abnormal findings. Abnormal findings on CT scans and MRI include massive cerebral edema and are generally found in late-stage disease, suggesting a poor outcome with rapid progression to death secondary to herniation [8, 9]. Therefore, although radiologic imaging is not helpful for diagnosing early disease, it can be important for assessing complications associated with cerebral edema in patients with PAM. It is critical to closely monitor intracranial pressure and control edema to prevent herniation. In this case series, 19 patients received steroids to treat cerebral edema. All 3 US survivors received dexamethasone [16]; another documented survivor from Mexico also received dexamethasone [17].

Treatment of PAM has evolved recently with the addition of 2 survivors during 2013—the first US survivors in 35 years. Although amphotericin B is still considered the drug of choice for treating PAM [11, 18], it has limited efficacy. Only 3 (8%) of 36 patients who were treated with this drug survived. From in vitro drug-sensitivity studies of amoebas acquired from the original US survivor in 1978, amphotericin B and miconazole had an additive effect, whereas rifampin did not inhibit amoeba growth [16]. In a separate in vitro study, miconazole alone was shown to inhibit *Naegleria* growth [19]. In total, 25 (78%) of 32 decedents with available data (Table 4) received PAM therapy within 3 days from the onset of symptoms. Thus, other factors are likely involved in the survival of patients with PAM in addition to treatment regimen and time of diagnosis, including infectious dose and virulence of the amoeba [18], which we could not assess. The treatment of the 2 survivors in 2013 included all of the drugs given to the 1978 survivor (fluconazole was used in place of miconazole) with the addition of an investigational drug called miltefosine, along with azithromycin. The 2013 survivors also were aggressively managed for their elevated intracranial pressure with interventions including external ventricular drain placement, therapeutic hypothermia, and administration of steroids.

Although this case series highlights the fact that an effective treatment for this devastating disease is not yet known, the 2 survivors in 2013 provide hope for more positive outcomes. Mainstay therapy of IV/IT amphotericin B, IV/IT azole drugs, with or without PO rifampin, continues to be recommended because of in vitro antiamoeba activities against *N. fowleri* and the survival of the US patients and a Mexico patient using this treatment [16, 17]. It should be noted that the original US survivor and the 2013 survivors received deoxycholate amphotericin B. The liposomal formulation of amphotericin B, AmBisome, was first approved for use in the United States in 1997 by the US Food and Drug Administration [20]. Patients receiving amphotericin B treatment for *Naegleria* after 1997 may have been more likely to receive the liposomal preparation, because fewer renal adverse effects have been reported with this preparation [21]. However, liposomal amphotericin B has been found to be less effective against *N. fowleri* in vitro and in a mouse model than deoxycholate amphotericin B, although these data came from 2 different studies in which the deoxycholate formulation was given at a higher dose than what is routinely used in patients [22, 23]. We identified 7 patients with *N. fowleri* infection who received a non-deoxycholate formulation. Thus, because of the extremely poor prognosis of PAM caused by *N. fowleri*, healthcare providers might consider using deoxycholate amphotericin B instead of the liposomal or lipid complex formulation. However, if deoxycholate amphotericin B is not immediately available, treatment should be initiated with a non-deoxycholate formulation to facilitate prompt treatment of the patient.

Miltefosine also seems to be beneficial for the treatment of PAM because it was used to treat both of the 2013 survivors. Miltefosine has shown in vitro efficacy against *Naegleria* and has been used with some success to treat infection caused by the related amoebas *Acanthamoeba* and *Balamuthia* [24–27]. Miltefosine is now available in the United States under an investigational new drug protocol through the CDC and is rapidly accessible to US health facilities for urgent treatment of *Naegleria* infection [28]. The addition of azithromycin to the treatment regimen should also be considered, because azithromycin has shown efficacy against *N. fowleri* infection in both a mouse model and in vitro and was used in the treatment regimens of the 2013 US survivors. Furthermore, azithromycin and amphotericin B are synergistic, and their combined effects may be a useful treatment for PAM [29]. For diagnostic assistance and treatment recommendations, clinicians who suspect PAM should contact the CDC Emergency Operations Center at 770–488–7100.

This case series is likely limited by some inherent selection bias, because health facilities in different states vary in their experience with PAM and in their capacity to identify and report cases. Surveillance bias is also likely; annual case counts have increased since the 1970s, but that may reflect improvement in surveillance and not necessarily an increase in infection rates [1]. Because PAM is such a rare disease, the conclusions drawn from this case series are limited because of the small sample size. However, it is likely that cases are still largely underreported as a result of common misdiagnoses of bacterial meningitis and the difficulty in diagnosing *Naegleria fowleri* after death. Therefore, a greater awareness of PAM could improve detection and reporting of cases and thereby improve our knowledge about this disease.

Furthermore, because of the severity of illness, a higher index of suspicion is warranted among healthcare providers to rule out bacterial meningitis and facilitate early diagnosis and treatment of PAM. This is especially important in the summer months in Florida and Texas, which account for more than half the cases [1]. In addition, increased awareness is now needed by all healthcare providers throughout the United States because of the recent detection of cases in northern states and because of travel (eg, a person is exposed in 1 state when traveling but disease onset and management occurs in their state of residence upon their return home). Healthcare providers should

routinely inquire about recreational exposure to warm freshwater (eg, swimming in a lake) even if flu-like illness is suspected or bacterial meningitis seems clinically apparent. In addition, because nearly all cases of PAM are fatal, continued testing of new therapies is warranted to identify drugs with increased effectiveness against *N. fowleri*.

Acknowledgments

We thank state public health departments for providing PAM case reports, members of the CDC/Council of State and Territorial Epidemiologists *Naegleria* Workgroup for their review of case reports and recommendations, and all those who assisted in data collection.

Financial support. This work was supported by the US Centers for Disease Control and Prevention.

Disclaimer. The findings and conclusions in this report are those of the authors and do necessarily represent the official position of the CDC.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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