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MINIREVIEW

Brain-Eating Amoebae: Predilection Sites in the Brain and Disease Outcome

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ABSTRACT *Acanthamoeba* spp. and *Balamuthia mandrillaris* are causative agents of granulomatous amoebic encephalitis (GAE), while *Naegleria fowleri* causes primary amoebic meningoencephalitis (PAM). PAM is an acute infection that lasts a few days, while GAE is a chronic to subacute infection that can last up to several months. Here, we present a literature review of 86 case reports from 1968 to 2016, in order to explore the affinity of these amoebae for particular sites of the brain, diagnostic modalities, treatment options, and disease outcomes in a comparative manner.

KEYWORDS brain, free-living amoebae, meningoencephalitis, central nervous system infections

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Acanthamoeba spp., *Balamuthia mandrillaris*, and *Naegleria fowleri* are pathogenic free-living amoebae (1). They are well known to produce fatal central nervous system (CNS) infections, and pathogenic *Acanthamoeba* spp. can also produce blinding keratitis, which is often associated with the inappropriate use of contact lenses. All three genera are known as amphizoic amoebae, due to their ability to exist as parasitic organisms and to inhabit natural environments as free-living organisms. In nature, *Acanthamoeba* seems to be most ubiquitous; it can inhabit a variety of environments and has been isolated from soil, water, and air. *B. mandrillaris* is rather selective, living in the soil, and it has been rarely isolated from water (1–3). *Naegleria fowleri*, being a thermophilic protist, prefers warm water such as hot springs in temperate zones and lakes in the tropics (4, 5). *Acanthamoeba* spp. and *B. mandrillaris* are known to have two stages in their life cycles, i.e., a vegetative trophozoite stage and a dormant cyst form. *N. fowleri* exhibits a transient flagellate form in addition to the trophozoite and cyst forms (1–6). These forms are interchangeable, depending on the environmental conditions. Among the various forms, the trophozoite form is often the infectious one. These amoebae cause two distinct clinical entities, namely, granulomatous amoebic encephalitis (GAE), caused by pathogenic *Acanthamoeba* spp. and *B. mandrillaris*, and primary amoebic meningoencephalitis (PAM), caused by *N. fowleri*. GAE and PAM are distinguished by their etiologies, risk factors, duration of illness, clinical features, and laboratory and imaging findings (6). *N. fowleri* is the only known pathogenic species in the genus *Naegleria*, which consists of over 40 species, that causes human disease, while *B. mandrillaris* is the only isolated species in the genus *Balamuthia*. The genus *Acanthamoeba* is classified into 20 genotypes (T1 to T20) (1–3, 7, 8). These amoebae and their associated infections have garnered increasing scientific and medical attention in recent years due to their poor prognoses, i.e., less than 5% of patients survive if early intervention is not initiated (1, 6). In addition to poor prognoses, cases of amoebic meningoencephalitis are often underreported, misreported, and underrecognized globally, due to lack of awareness, lack of available diagnostic tools, lack of wide distribution of knowledge regarding public health issues and risk factors, especially in developing countries, and the similarity of symptoms to those of other common CNS infections, such as viral and bacterial meningitis. In addition, the pathogenesis and

pathophysiology of CNS infections due to the aforementioned free-living amoebae are incompletely understood. For example, PAM is an acute infection that lasts only a few days, while GAE is a chronic to subacute infection that lasts up to several months. Given the nasal route of entry, *N. fowleri* is likely to have an intimate correlation with the frontal lobe, due to the anatomical proximity of the olfactory bulb to the frontal lobe; the olfactory bulb is terminal to the olfactory neuroepithelium of the nasal passage, traversing the cribriform plate to the brain (1, 6). Although the intranasal route is the route of infection, current administration of drugs (such as amphotericin B) against PAM is via the intravenous route, which causes significant toxicity to other tissues and requires high doses to reach the site of infection at sufficient concentrations to kill the parasite. In contrast, pathogenic *Acanthamoeba* and *B. mandrillaris* spread hematogenously and possibly distribute in the frontal lobe, the temporal lobe, and the parietal lobe, likely through the middle cerebral artery, as these cortices are among the main regions for middle cerebral artery supply (9). By comparing the available reported cases of CNS infections due to free-living amoebae, the aim of the present study was to determine the principle sites of infection within the brain, the diagnostic methods employed (premortem and postmortem), and the available treatment regimens, with examples of successful outcomes, with the goal of increasing awareness for the improved management of amoebic meningoencephalitis.

CASE STUDIES OF AMOEBIC MENINGOENCEPHALITIS

Predilection for sites in the brain. In this review, we examined cases of brain infections due to free-living amoebae, i.e., *Acanthamoeba* spp., *B. mandrillaris*, and *Naegleria fowleri*. In total, we examined 86 case reports, from 1968 to 2016, that were available at PubMed, in order to explore the affinity of these three types of amoebae for particular sites of the brain. For GAE due to pathogenic *Acanthamoeba*, a total of 46 cases reported in 35 publications were reviewed. For GAE due to *B. mandrillaris*, a total of 29 cases reported in 16 publications were reviewed. For PAM due to *N. fowleri*, a total of 11 cases reported in 10 publications were reviewed. Most cases (up to 90%) were reported from the United States. PAM due to *N. fowleri* was observed in immunocompetent individuals, while GAE was observed in both immunosuppressed individuals (mostly *Acanthamoeba* cases) and immunocompetent individuals (mostly *B. mandrillaris* cases). The cases were stratified based on the year of the report, the patient's age and gender, the place of origin, the chief complaints, relevant positive and negative findings, laboratory findings (cerebrospinal fluid [CSF] and blood profiles, serological data, and culture results), the diagnosis, neuroimaging results, definitive treatments, and the disease outcome. In literature from 1960 to 1970, *B. mandrillaris* was recognized as *Leptomyxid* genus when taxonomical categorization was not clear (10); however, those cases were included in this review as *B. mandrillaris* infections. Cases with imaging studies included magnetic resonance imaging (MRI) (27 cases), computed tomographic (CT) scanning (24 cases), and a combination of CT scanning and MRI (16 cases). Because this was a study of preferential sites, the first imaging studies for the first admission were selected for analyses unless stated otherwise. Moreover, when two imaging modalities were used simultaneously during the first admission, MRI was considered superior to CT scanning in terms of demonstrating focal lesions that were evolving over time. Therefore, we prioritized MRI images and descriptions over CT images and descriptions (11). MRI availability is limited in some parts of the world, however, and CT scanning was used as standard imaging in such instances.

Neuroimaging of GAE typically shows multiple, well-defined, focal, ring-enhancing, space-occupying lesions, with perilesional edema and leptomeningeal enhancement if meninges are involved (12–14). PAM in neuroimaging had a single focus of infection, with diffuse cerebral edema, signs of increased intracranial pressure (midline shift and effacement of ventricles and cisterns), and basilar meningeal enhancement (12–14). For GAE due to pathogenic *Acanthamoeba* spp., 12 cases (26.1%) were reported to have lesions in the frontal lobe, 11 cases (23.9%) were reported to have lesions in the parietal lobe, 12 cases (26.1%) were reported to have lesions in the temporal lobe, and 9 cases

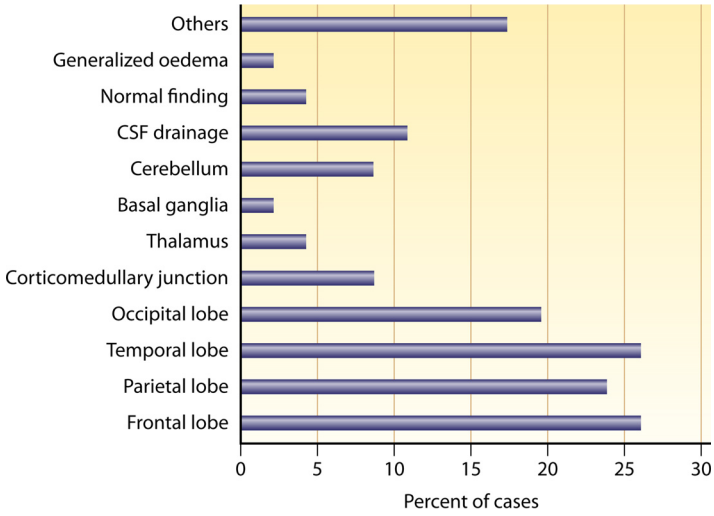

FIG 1 Sites of infection of GAE due to *Acanthamoeba* spp. The majority of cases involved the cerebral cortices, with the frontal lobe and the temporal lobe being most affected, followed by the parietal lobe and the occipital lobe. Among extracortical sites, the cerebellum and the corticomedullary junction were the most favored sites. Hydrocephalus, which results from blockage of CSF drainage, was observed in a few cases. Other affected sites included the thalamus, the caudate nucleus, and the brainstem. Infection can also present as normal findings in early neuroimaging.

(19.6%) were reported to have lesions in the occipital lobe. For sites beyond the cerebral cortices, the corticomedullary junction and the cerebellum represented most of the cases (17.4% and 8.7%, respectively). In 2 cases (4.3%), the thalamus was also affected. The CSF drainage system was favored in 5 cases (10.9%) (with hydrocephalus), while generalized edema was found in 1 case (2.2%) (Fig. 1; also see Table S1 in the supplemental material). There were possible false-negative findings in 2 cases (4.3%), in which normal findings were observed in early imaging. Other sites made up 8 cases (17.4%) of GAE due to *Acanthamoeba*. Overall, the frontal lobe, parietal lobe, temporal lobe, and occipital lobe (constituting 56% of the total cases reviewed in this study) were affected most in cases of GAE due to *Acanthamoeba*.

For GAE due to *B. mandrillaris*, 12 cases (41.4%) were reported to have lesions in the frontal lobe, 10 cases (21.7%) were reported to have lesions in the parietal lobe, 15 cases (51.7%) were reported to have lesions in the temporal lobe, and 9 cases (31%) were reported to have lesions in the occipital lobe. Sites beyond the cerebral cortices included the corticomedullary junction, the thalamus, the basal ganglia, and the cerebellum (Fig. 2; also see Table S2). Notably, one case manifested as an aneurysm, while two cases affected the CSF drainage. In one case, coinfection with HIV (advanced infection), *Acanthamoeba*, and *B. mandrillaris* with cerebral toxoplasmosis was observed. Overall, the frontal lobe, parietal lobe, temporal lobe, and occipital lobe (constituting 54% of the total cases reviewed in this study) were affected most in cases of GAE due to *B. mandrillaris*, which appears consistent with the findings for GAE due to *Acanthamoeba*.

For PAM due to *N. fowleri*, it was observed that the parasite favors the frontal lobe, followed by the parietal lobe. Among the reported cases of PAM due to *N. fowleri*, involvement of the frontal lobe was reported for 36% of cases (Fig. 3; also see Table S3). Sites beyond the cerebral cortices included the corticomedullary junction, while the CSF drainage system was targeted in 27% of cases. Three cases (27%) showed signs of hydrocephalus. Notably, one case of PAM showed normal neuroimaging findings. In comparison to GAE due to *Acanthamoeba* spp. and *B. mandrillaris*, the frontal lobe was affected most in cases of PAM due to *N. fowleri*.

Diagnosis. Among cases of GAE due to *Acanthamoeba* spp., 34.5% were diagnosed postmortem and 65.5% were identified premortem (Table 1). Among the postmortem cases, microscopy was used successfully in 10.9% of cases, immunofluorescence assays (IFAs) were used effectively in 18.2% of cases, and PCR was used positively in 5.4% of

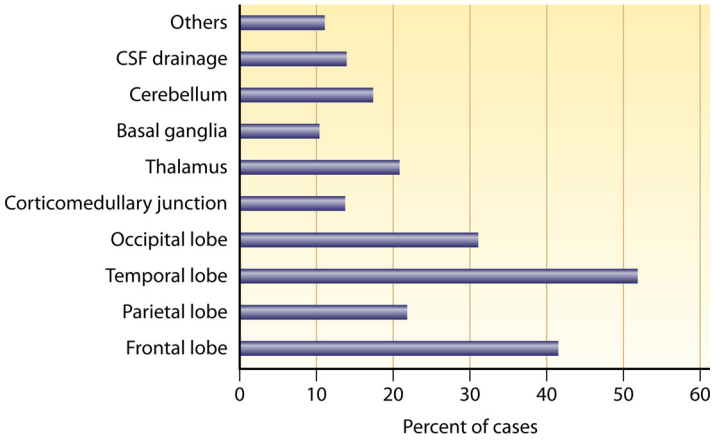

FIG 2 Sites of infection of GAE due to *Balamuthia mandrillaris*. Involvement of the temporal lobe was observed in most cases, followed by involvement of the frontal, parietal, and occipital lobes. Among extracortical sites, the thalamus was most affected, followed by the corticomedullary junction, the cerebellum, and the basal ganglia.

cases. Among the premortem cases, CSF observations of amoebae were made in 38.1% of cases (using microscopy [14.5% of cases], culture of parasites [20%], and PCR [3.6%]) and brain biopsy samples were assessed in 30.41% of cases (using microscopy [15.21%], culture [4.34%], PCR [4.34%], and IFAs [6.52%]). Overall, for GAE due to *Acanthamoeba* spp., observation of parasites in CSF samples using culture or microscopy was the most widely used premortem diagnostic method.

Among cases of GAE due to *B. mandrillaris*, 31% were diagnosed postmortem and 68.9% were identified premortem (Table 1). Among the postmortem cases, microscopy was used successfully in 10.34% of cases and IFAs were used effectively in 20.68% of cases. Among the premortem cases, CSF observations of amoebae were made in 3.44% of cases (using PCR) and brain biopsy samples were assessed in 44.81% of cases (using microscopy [20.68%], PCR [10.34%], and IFAs [13.79%]). Overall, for GAE due to *B. mandrillaris*, observation of parasites in brain biopsy samples using microscopy or IFAs was the most widely used premortem diagnostic method.

Among cases of PAM due to *N. fowleri*, 63.7% were diagnosed postmortem and 36.3% were identified premortem (Table 1). Among the postmortem cases, microscopy was used successfully in 36.4% of cases, IFAs were used effectively in 18.2% of cases, and PCR was used positively in 9.1% of cases. Among the premortem cases, CSF observations of amoebae were made in 36.4% of cases (using microscopy [18.2%] and culture [18.2%]). Overall, for PAM due to *N. fowleri*, observation of parasites in CSF samples using microscopy or IFAs was the most widely used premortem diagnostic method.

Treatment. The findings for the compiled cases indicated that, despite the establishment of clinical guidelines for amoebic meningoencephalitis, the physicians were

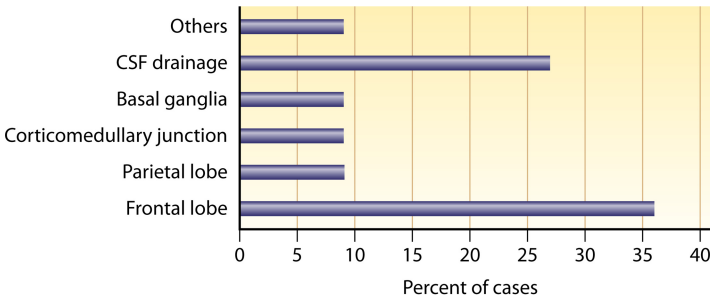

FIG 3 Sites of infection of PAM due to *Naegleria fowleri*. More cases involved the frontal lobe, followed by the parietal lobe and the corticomedullary junction. Hydrocephalus was observed in 27% of cases.

TABLE 1 Use of various methods for the diagnosis of GAE due to *Acanthamoeba* spp. or *Balamuthia mandrillaris* and PAM due to *Naegleria fowleri*

Disease (total cases reviewed)	Diagnostic modality	Method of analysis	% of cases (no. of cases)[a]
GAE due to *Acanthamoeba* spp. ($n = 46$)	Brain biopsy	Microscopy	15.21 (7)
		PCR	4.34 (2)
		IFA	6.52 (3)
		Culture	4.34 (2)
	CSF	Microscopy	17.39 (8)
		Culture	23.9 (11)
		PCR	4.34 (2)
	Postmortem	Microscopy	13.04 (6)
		IFA	21.7 (10)
		PCR	6.52 (3)
	Skin biopsy		2.17 (1)
GAE due to *B. mandrillaris* ($n = 29$)	Brain biopsy	Microscopy	20.68 (6)
		PCR	10.34 (3)
		IFA	13.79 (4)
	CSF	PCR	3.44 (1)
	Postmortem	Microscopy	10.34 (3)
		IFA	20.68 (6)
	Skin biopsy		6.9 (8)
PAM due to *N. fowleri* ($n = 11$)	Postmortem	Microscopy	36.4 (4)
		IFA	18.2 (2)
		PCR	9.1 (1)
	CSF	Microscopy	18.2 (2)
		Culture	18.2 (2)

[a]The data are presented as percentages of cases reviewed in this study. Some cases may involve more than one diagnostic modality.

liberal with combinations of several classes of drugs with different mechanisms of action and individualized the regimens according to age, gender, availability of chemotherapy, and underlying medical conditions that might affect the metabolism of drugs; therefore, we examined the results according to classes of chemotherapeutic agents instead of combinations of agents. Percentages were determined separately for cases of GAE (due to *Acanthamoeba* or *Balamuthia*) and cases of PAM. For determination of disease outcomes, cases of survival were deemed successful, while the cases that resulted in death (including brain death) were considered poor outcomes.

When the reported cases of amoebic meningoencephalitis were reviewed, it was clear that no drug was effective against GAE or PAM and, as a result, the majority of cases resulted in death. Various types of drugs and their combinations were tested but the prognoses remained poor. For example, for the cases of GAE due to *Acanthamoeba* spp. reviewed here, the most commonly used drugs included azole compounds, sulfonamides, amphotericin B, sulfadiazine, macrolides, miltefosine, pentamidine, flucytosine, and rifampin (Table 2). In contrast, azole compounds, sulfadiazine, pentamidine, miltefosine, and amphotericin B were most commonly used for cases of GAE due to *B. mandrillaris*. For cases of PAM due to *N. fowleri*, the most commonly used drugs included amphotericin B, azole compounds, sulfadiazine, and rifampin (Table 2). Among cases with successful prognoses, there appeared to be combinations of several compounds (Table 3). For some of those cases, a combination of amphotericin B, sulfamethoxazole-trimethoprim, and rifampin was given for the treatment of GAE due to *Acanthamoeba* spp. (Table 3). In contrast, a combination of flucytosine, fluconazole, azithromycin, pentamidine, sulfadiazine, azithromycin, and miltefosine was used for the majority of cases of GAE due to *B. mandrillaris* (Table 3). In recent years, a combination of amphotericin B, fluconazole, rifampin, azithromycin, dexamethasone, and miltefosine was given for PAM (Table 3).

CHALLENGES AND OPPORTUNITIES

Free-living pathogenic amoebae are now well-recognized agents of brain infections leading to GAE and PAM. GAE is a chronic infection that can last up to several months,

TABLE 2 Use of various individual drugs for the treatment of GAE due to *Acanthamoeba* spp. or *Balamuthia mandrillaris* and PAM due to *Naegleria fowleri*

Drug or drug class[a]	% of cases (no. of cases)		
	GAE due to *Acanthamoeba* ($n = 46$)	GAE due to *B. mandrillaris* ($n = 29$)	PAM due to *N. fowleri* ($n = 11$)
Nonspecific	19.5 (9)	20.7 (6)	18.2 (2)
Miltefosine	15.2 (7)	13.8 (4)	0
Pentamidine	13 (6)	31 (9)	0
Sulfadiazine	19.5 (9)	34.5 (10)	18.2 (2)
Flucytosine	13 (6)	24.1 (7)	0
Macrolides (azithromycin or clarithromycin)	17.4 (8)	31 (9)	0
Azoles	41.3 (19)	48.3 (14)	18.2 (2)
Carbapenems	4.3 (2)	3.4 (1)	0
Sulfonamides (trimethoprim-sulfamethoxazole)	34.8 (16)	3.4 (1)	0
Rifampin	37 (17)	6.9 (2)	18.2 (2)
Chloramphenicol	6.5 (3)	0	9.1 (1)
Pyrimethamine	2.2 (1)	6.9 (2)	9.1 (1)
Amphotericin B	30.4 (14)	10.3 (3)	27.3 (3)
Glycopeptides (vancomycin)	2.2 (1)	0	0
Tetracyclines	0	3.4 (1)	0

[a]Nonspecific treatment included general measures to reduce intracranial pressure and inflammation (mannitol, decompressive craniotomy, and corticosteroids) and treatment for differential diagnosis (cephalosporins for bacterial meningitis). In cases of combinations of drugs, the therapeutic agents were calculated independently.

while PAM is an acute fulminant infection that lasts a few days (1, 6). It is intriguing to see the distinctive difference of the chronicity in pathogenicity of these amoebae. For example, *Acanthamoeba* and *B. mandrillaris* likely enter the host via the lower respiratory tract and/or skin breaks (1, 6). In contrast, *N. fowleri* enters the host via the nasal route. Recently, another route of entry has been included, i.e., via organ transplantations, with recipients of organ donations acquiring amoebic meningoencephalitis from the donor diagnosed postmortem with amoebic meningoencephalitis of the same genotype (15–18). This is important because amoebae are ubiquitous and nonresponsive to antibiotics, and organ recipients are already rendered immunosuppressed; therefore, any entry of these pathogenic free-living amoebae may have devastating consequences. Although data on risk factors were not available for all cases reviewed in this study, some factors were observed to dictate the susceptibility of patients to amoebic meningoencephalitis. For GAE due to *Acanthamoeba*, immunosuppression appeared to be a factor (1, 6, 19, 20), while *B. mandrillaris* was shown to infect immunocompetent individuals in addition to immunocompromised patients (1, 3). Preceding cutaneous lesions are often liable to GAE caused by both types of amoebae. PAM usually occurred in immunocompetent children and young adults (1, 6, 7). However, all patients had history of activities in or near fresh water sources such as swimming pools or hot springs, including recreational activities and religious practices such as ablution, or health care practices such as the use of neti pots. Eliciting a thorough patient history is absolutely paramount for the accurate diagnosis of PAM, and public health preventive measures such as water treatment should be undertaken for high-risk populations.

Neuroimaging studies revealed the locations of lesions in the frontal, parietal, and temporal lobes in most cases of GAE, but lesions in the frontal lobe were much more frequent for *N. fowleri*. Neuroimaging modalities can have false-negative results, however, and the specificity of neuroimaging for the diagnosis of amoebic meningoencephalitis has yet to be evaluated. In the absence of accurate diagnoses and effective treatments, both diseases often result in death. *N. fowleri* was found in the CSF more often than the other two types of amoebae, most likely due to its motile flagellated form. However, diagnosis with biopsy samples may be hindered by the inoculum size and the magnitudes of inflammation and necrosis in the tissue sections. In addition to the aforementioned factors, to untrained eyes the morphology of trophozoites in tissue sections bears a close resemblance to that of macrophages, which are also common in acute inflammatory responses. The other challenges in diagnosis include a wide

TABLE 3 Selected cases of amoebic meningoencephalitis with successful outcomes

Year	Patient description	Causative agent	Treatment
2000	33-yr-old man	*Acanthamoeba* spp.	Sulfadiazine, pyrimethamine, and fluconazole; left homonymous hemianopia (visual field defects)
2002	45-yr-old woman	*Acanthamoeba* spp.	Rifampin, co-trimoxazole, fluconazole, and ceftriaxone for 4 wk; monitored for 1 yr for facial nerve palsy
2006	10-yr-old boy	*Acanthamoeba* spp.	Ketoconazole and rifampin; duration of therapy is unknown
2008	25-yr-old man	*Acanthamoeba* spp.	Miltefosine; seronegative for *Acanthamoeba* after treatment but neurological deficits did not improve in monitoring for 24 mo
2009	63-yr-old man with history of contact with contaminated water	*Acanthamoeba* spp.	Amphotericin B and rifampin; discharged after 78 days of hospitalization
2011	2-yr-old boy with underlying acute lymphoblastic leukemia	*Acanthamoeba* spp.	Meropenem, teicoplanin, fosfomycin, metronidazole, and liposomal amphotericin B; symptom resolution
2012	Immunocompetent 38-yr-old man	*Acanthamoeba* spp.	Voriconazole and miltefosine; radiological and clinical relief after 6 days of treatment, with subsequent monitoring for refractory seizure complications
2012	2-yr-old boy	*Acanthamoeba* spp.	Co-trimoxazole, rifampin, and ketoconazole; improvement after 2 days
2014	30-yr-old man	*Acanthamoeba* spp.	Rifampin, sulfamethoxazole-trimethoprim, and fluconazole for 2 wk; asymptomatic after 2 wk of monitoring
2016	2-yr-old boy	*Acanthamoeba* spp.	Ceftazidime, metronidazole, fluconazole, and rifampin for 3 wk
2016	11-yr-old girl	*Acanthamoeba* spp.	Amphotericin B, sulfamethoxazole-trimethoprim, and rifampin
2016	12-yr-old boy	*Acanthamoeba* spp.	Amphotericin B, sulfamethoxazole-trimethoprim, and rifampin
2016	9-mo-old girl	*Acanthamoeba* spp.	Amphotericin B, sulfamethoxazole-trimethoprim, and rifampin
2003	64-yr-old man	*Balamuthia mandrillaris*	Amphotericin B, flucytosine, fluconazole, and sulfadiazine for 5 yr, clarithromycin for 2 yr, and pentamidine for 18 days
2003	5-yr-old girl	*Balamuthia mandrillaris*	Flucytosine and fluconazole for 2 yr, pentamidine for 34 days, and clarithromycin for 2 yr
2004	72-yr-old woman	*Balamuthia mandrillaris*	Pentamidine, sulfadiazine, fluconazole, and clarithromycin; hospitalized for 13 days
2004	72-yr-old man	*Balamuthia mandrillaris*	Fluconazole, sulfadiazine, clarithromycin, and pentamidine isethionate; duration of therapy is unknown
2006	10-yr-old girl	*Balamuthia mandrillaris*	Albendazole, itraconazole, and sulfamethoxazole-trimethoprim for 6 mo
2006	8-yr-old boy	*Balamuthia mandrillaris*	Albendazole and itraconazole for 14 mo
2010	21-yr-old woman	*Balamuthia mandrillaris*	Albendazole and fluconazole for 7.5 mo and miltefosine for 7 mo
2010	2-yr-old boy	*Balamuthia mandrillaris*	Pentamidine (stopped after 2 mo), sulfadiazine, flucytosine, clarithromycin, and fluconazole
2010	27-yr-old man	*Balamuthia mandrillaris*	Sulfadiazine, azithromycin, and miltefosine for unspecified duration
2011	27-yr-old male organ recipient	*Balamuthia mandrillaris*	Pentamidine, sulfadiazine, flucytosine, fluconazole, azithromycin, and miltefosine
2011	80-yr-old woman	*Balamuthia mandrillaris*	Pentamidine, itraconazole, azithromycin, sulfadiazine, flucytosine, and liposomal amphotericin
2013	5-yr-old girl	*Balamuthia mandrillaris*	Flucytosine, fluconazole, azithromycin, pentamidine, and sulfadiazine, changed to final regimen of azithromycin, fluconazole, and miltefosine
2013	4-yr-old immunocompetent girl with history of water contact from floods around her residence	*Balamuthia mandrillaris*	Flucytosine, fluconazole, azithromycin, pentamidine, and sulfadiazine

(Continued on next page)

TABLE 3 (Continued)

Year	Patient description	Causative agent	Treatment
2002	26-yr-old woman	*Naegleria fowleri*	Rifampin, amphotericin B, and ornidazole for 2 wk
2008	8-mo-old boy	*Naegleria fowleri*	Amphotericin B, chloramphenicol, and rifampin; afebrile at day 7 of treatment
2013	12-yr-old girl and 8-yr-old boy	*Naegleria fowleri*	Amphotericin B, fluconazole, rifampin, azithromycin, dexamethasone, and miltefosine for both

spectrum of differential diagnoses, e.g., brain tumors, multiple sclerosis, lupus encephalitis, progressive multifocal leukoencephalopathy, stroke, meningitis from other causes (viral, tuberculous, or pyogenic), and cerebral toxoplasmosis (1, 6). A recent case of cerebral toxoplasmosis complicated by GAE caused by both *Acanthamoeba* and *B. mandrillaris* highlighted the complex nature of the disease, especially because both types of amoebae are known to act as reservoir hosts for many microorganisms (1, 6, 21–23). It is more intriguing that *Acanthamoeba* and *B. mandrillaris* meningoencephalitis cases present as vascular diseases (masquerading as cerebral vascular occlusions or aneurysms). This is most likely due to the ability of amoebae to produce endothelial damage, resulting in cytokine release, crossing of the blood-brain barrier, granulomatous inflammation, thromboembolic events, increased vascular permeability, and ultimately necrosis.

For chemotherapeutic strategies, current available delivery routes include intravenous, oral, and intrathecal administration. Systemic antimicrobial treatment has its limitations, however, due to its adverse effects and reduced delivery, together with delayed diagnosis. Other concerns include poor pharmacodynamic and pharmacokinetic profiles of available drugs, solubility, CNS penetration, drug-drug interactions, patient medical conditions, patient tolerance, and *Acanthamoeba* susceptibility to amoebicidal agents (24). In the case of PAM, the amphotericin B deoxycholate preparation is preferable to the liposomal formulation against *N. fowleri* infections, although it has no effect on *Acanthamoeba* or *B. mandrillaris* (25, 26). More recently, miltefosine has shown promising results with respect to bioavailability and drug-drug interactions (25). Of note, the major groups of azoles and macrolides are amoebistatic rather than amoebicidal. Additionally, nephrotoxic and hepatotoxic effects due to the use of drugs among patients with compromised renal and liver functions (such as transplant patients) may further complicate treatment. Potential drug delivery systems that directly target the inoculation sites of amoebae by circumventing the need for optimal blood-brain barrier penetration should be the focus of future studies, to increase the odds of survival for patients with PAM while minimizing adverse effects and disease complications. Overall, a complete understanding of the pathogenic mechanisms and the role of the immune system and development of novel chemotherapeutic approaches to drug delivery (27, 28) are important for the rational development of antiamoebic therapy.

CONCLUDING REMARKS

Despite advances in clinical recognition, diagnostic methods, and treatment approaches, the mortality rates associated with CNS infections due to amoebae have remained high. Although neuroimaging findings reveal common areas for lesions, the locations may not be consistent and may vary depending on the causative agent. High levels of clinical suspicion are important, especially for refractory cases of meningoencephalitis, for rapid diagnosis of the infection, which is a prerequisite for successful treatment. The fact that only a few individuals, among all of the hosts exposed to these amoebae, develop infections suggests the possible presence of underlying predisposing factors. Future research is needed to define genetic, immunological, pathogenic, and environmental factors that contribute to deadly amoebic meningoencephalitis. Moreover, the abilities of pathogenic amoebae to host other microbial pathogens as

reservoirs and to act as hyperparasites have enhanced their capacity as pathogens of increasing importance to human and animal health.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.02300-16.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 2, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 3, PDF file, 0.1 MB.

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