Brain-eating amoebae: Predilection sites in the brain and disease outcome

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Short title: CNS infections and free-living amoebae

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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 lasting 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 affinity of these amoebae towards particular sites of the brain, diagnostic modalities, treatment options and the disease outcome in a comparative manner.

Introduction

Acanthamoeba spp., *Balamuthia mandrillaris* and *Naegleria fowleri* are pathogenic free-living amoebae (1). They are well-known to produce fatal central nervous system infections, however pathogenic *Acanthamoeba* spp., can also produce blinding keratitis that 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 as well as inhabit natural environment as free-living. In nature, *Acanthamoeba* seems to be most ubiquitous that can inhabit a variety of environments and has been isolated from soil, water, and air, whereas *B. mandrillaris* is rather selective, living in the soil and has been rarely isolated from water (1-3). *Naegleria fowleri*, being thermophilic protist, prefers warm water such as hot spring in temperate zone and lakes in the tropics (4, 5). *Acanthamoeba* spp. and *B. mandrillaris* are known to have two stages in their life cycle, including a vegetative trophozoite stage and a dormant cyst form, while *N. fowleri* exhibits an additional transient flagellate form in addition to the trophozoite and cyst form (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 including, granulomatous amoebic encephalitis (GAE) caused by pathogenic *Acanthamoeba* spp., and *B. mandrillaris*, and primary amoebic meningoencephalitis (PAM) caused by *N. fowleri*. Both GAE and PAM are distinguished by their aetiology, risk factors, duration of illness, clinical features, laboratory and imaging findings (6). *N. fowleri* is the only known pathogenic species that causes human disease in the genus *Naegleria* that consists of over 40 species, while *B. mandrillaris* is the only species isolated in the genus *Balamuthia*. Genus *Acanthamoeba* is classified into 20 genotypes (T1 – T20) (1-3, 7, 8). These amoebae and associated infections have garnered increasing scientific/medical interests in recent years due to poor prognosis, i.e., less than 5% patients survive if early intervention is not initiated (1, 6). In addition to

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poor prognosis, cases of amoebic meningoencephalitis are often under-reported and under-recognized globally due to lack of awareness, absence of availability of diagnostic measures, lack of access to wide distribution of knowledge on public health issues especially in developing countries and similarity of symptomatology with other common causes of central nervous system (CNS) infections such as viral and bacterial meningitis. In addition, a complete understanding of the pathogenesis and pathophysiology of CNS infection due to aforementioned free-living amoebae is incompletely understood. For example, PAM is an acute infection lasting only a few days, while GAE is a chronic to subacute infection lasting 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 anatomical proximity of olfactory bulb to the frontal lobe, of which the olfactory bulb is terminal to the olfactory neuroepithelium of the nasal passage, traversing through the cribriform plate to the brain (1, 6). Although intranasal route is the mode of infection, current administration of drugs (such as amphotericin B) against PAM is *via* the intravenous route that causes significant toxicity to other tissues and require high dosage to reach the site of infection at sufficient concentration to kill the parasite. In contrast, pathogenic *Acanthamoeba* and *B. mandrillaris* spread haematogenously 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 studying the available reported cases of CNS infection due to free-living amoebae comparatively, the aim of the present study is to determine the principle sites of infection within the brain, diagnostic methods employed, pre-mortem and post-mortem, and available treatment regimens with a examples of successful prognosis, with an eye to increase awareness for the improved management of amoebic meningoencephalitis.

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Case studies of amoebic meningo-encephalitis: Predilection sites in the brain

In this review, we examined cases presenting brain infections due to free-living amoebae, *Acanthamoeba* spp., *B. mandrillaris* and *Naegleria fowleri*. In total, we examined 86 case reports that are available on Pubmed from 1968 to 2016, in order to explore the affinity of these three amoebae towards particular sites of the brain. For GAE due to pathogenic *Acanthamoeba*, a total of 46 cases were reviewed that were reported in 35 publications; GAE due to *B. mandrillaris*, a total of 29 cases were reviewed from 16 publications, while for PAM due to *N. fowleri*, 11 cases were reviewed from 10 publications. The majority of cases were reported in the America (up to 90%). PAM due to *N. fowleri* was reported in immunocompetent individuals, while GAE was reported in both immunosuppressed (mostly *Acanthamoeba* cases) as well as immunocompetent individuals (mostly *B. mandrillaris* cases). The cases were stratified based on the year of the report, patient's age and gender, place of origin, chief complaints, relevant positive and negative findings, laboratory findings (cerebrospinal fluid, blood profiles, serology and cultures), diagnosis, neuroimaging, definitive treatments and disease outcome. In earlier literature dated from 1960-1970, *B. mandrillaris* was recognized as *Leptomyxid* genus when taxonomical categorization was not clear (10), however these cases have been included in this review as *B. mandrillaris* infections. Cases with imaging studies included MRI imaging (27 cases), CT scans (24 cases), and a combination of CT and MRI (16 cases). As it is a study on preferential sites, first imaging studies on first admission was selected for analyses unless stated otherwise. Moreover, if two imaging modalities were done at once during first admission, MRI is considered superior to CT in terms of demonstrating focal lesions that are evolving over time. Therefore, we prioritize MRI images and descriptions over CT images and descriptions (78). However, MRI availability is limited in some parts of the world, hence CT images were used as standard imaging in such instances.

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Neuroimaging of GAE typically showed multiple well-defined focal ring-enhancing space occupying lesions with perilesional edema and leptomeningeal enhancement if meninges are involved (11). PAM in neuroimaging has 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 (11). For GAE due to pathogenic *Acanthamoeba* spp., 12 cases (26.1%) were reported to have lesions in the frontal lobe, 11 cases (23.9%) in the parietal lobe, 12 cases (26.1%) reported lesions in the temporal lobe, 9 cases (19.6%) in the occipital lobe respectively. While for sites beyond cerebral cortices, cortico-medullary junction and cerebellum made up most of the cases (17.4% and 8.7% respectively). In 2 cases (4.3%), the thalamus was also affected. The cerebrospinal fluid (CSF) drainage system is favored in 5 cases (10.9%) (with hydrocephalus), while generalized edema was found in 1 case (2.2%) (Fig. 1; supplementary Table 1). There are possible false negative findings in 2 cases (4.3%) where normal findings on early imaging were observed. Other sites made up 8 cases (17.4%) of GAE due to *Acanthamoeba*. Overall, frontal lobe, parietal lobe, temporal lobe and occipital lobe (constituted 56% of total cases reviewed in this study) were affected in most cases of GAE due to *Acanthamoeba*.

For GAE due to *B. mandrillaris*, 12 cases (41.4%) reported the involvement of the frontal lobe, 10 cases (21.7%) reported lesions in the parietal lobes, 15 cases (51.7%) reported lesions in the temporal lobe, and 9 cases (31%) reported lesions in the occipital lobe, respectively. The sites beyond the cerebral cortices included the involvement of cortico-medullary junction, thalamus, basal ganglia, and the cerebellum (Fig. 2; supplementary Table 2). Notably, one case was manifested as an aneurysm, while two cases affected the CSF drainage. In one case, co-infection of advanced HIV infection, *Acanthamoeba* and *B. mandrillaris* with cerebral toxoplasmosis was observed. Overall, the frontal lobe, parietal lobe, temporal lobe and occipital lobe (constituted 54% of total cases reviewed in this study)

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were affected in most cases of GAE due to *B. mandrillaris*, which appears consistent with GAE due to *Acanthamoeba*.

For PAM due to *N. fowleri*, it was observed that the parasite favours the frontal lobe, followed by the parietal lobe. Among the reported cases of PAM due to *N. fowleri*, 36% cases reported the involvement of the frontal lobe (Fig. 3; supplementary Table 3). The sites beyond the cerebral cortices included cortico-medullary 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 findings on neuroimaging. In comparison to GAE due to *Acanthamoeba* spp., and *B. mandrillaris*, the frontal lobe constituted 37% of total cases reviewed in this study) were affected in most cases of PAM due to *N. fowleri*.

Case studies of amoebic meningo-encephalitis: Diagnosis

Among GAE due to *Acanthamoeba* spp. cases, 34.5% cases were diagnosed at post-mortem and 65.5% cases were identified pre-mortem (Table 1). Among the post-mortem cases, microscopy was used successfully in 10.9% of cases, immunofluorescence assays (IFA) were used effectively in 18.2% of cases, and polymerase chain reaction (PCR) was used positively in 5.4% of cases. In pre-mortem cases, CSF observation of amoebae were made in 38.1% of cases [using microscopy (14.5% cases), culture of parasites (20% cases), and PCR (3.6%)] and brain biopsies were made in 30.41% of cases [using microscopy (15.21%), culture (4.34%), PCR (4.34%), and IFA (6.52%)]. Collectively, in GAE due to *Acanthamoeba* spp., observation of parasites in CSF samples using culture and microscopy was the most widely used diagnostic method reported pre-mortem.

For GAE due to *B. mandrillaris* cases, 31% cases were diagnosed at the post-mortem stage and 68.9% cases were identified pre-mortem (Table 1). Among the post-mortem cases,

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158 microscopy was used successfully in 10.34% of cases, and IFA was used effectively in 20.68%
159 of cases reported. In pre-mortem cases, CSF observation of amoebae was made in 3.44% of
160 cases [using PCR], and brain biopsies were made in 44.81% of cases [using microscopy
161 (20.68%), PCR (10.34%), and IFA (13.79%)]. Overall, among GAE due to *B. mandrillaris*
162 cases, observation of parasites in brain biopsies using microscopy and IFA was the most
163 widely used diagnosis pre-mortem.

164 Among PAM due to *N. fowleri* cases, 63.7% cases were diagnosed at post-mortem
165 and 36.3% cases were identified pre-mortem (Table 1). Among post-mortem cases,
166 microscopy was used successfully in 36.4% of cases, IFA was used effectively in 18.2% of
167 cases, and PCR was used positively in 9.1% of cases reported. In pre-mortem cases, CSF
168 observation of amoebae was made in 36.4% of cases [using microscopy (18.2%), and culture
169 (18.2)]. Overall, among PAM due to *N. fowleri* cases, observation of parasites in CSF
170 samples using microscopy and IFA was the most widely used diagnosis pre-mortem.

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172 **Case studies of amoebic meningo-encephalitis: Treatment**

173 With all the treated case studies compiled, despite establishment of clinical guidelines
174 on amoebic meningoencephalitis, the physicians had been liberal with combinations of
175 several classes of drugs with different mechanisms of action and individualized according to
176 age, gender, availability of chemotherapy and underlying medical conditions which may
177 affect metabolism of drugs, therefore we examined accordingly by classes of
178 chemotherapeutic agents instead of combinations of the agents. The percentage was
179 determined by cases of GAE (*Acanthamoeba* and *Balamuthia*) and PAM separately. In
180 determination of outcomes in diseases, survival cases were deemed successful while the cases
181 that result in death which include brain death was considered as poor outcome.

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When reviewing reported cases of amoebic meningoencephalitis, it is clear that there is no effective drug against GAE or PAM and as a result, the majority of cases resulted in death. Various types of drugs and their combinations have been tested but the prognosis remained poor. For example, in the GAE due to *Acanthamoeba* spp., cases reviewed here, the most commonly used drugs include the Azole compounds, Sulfonamides, Amphotericin B, Sulfadiazine, Macrolides, Miltefosine, Pentamidine, Flucytosine, and Rifampicin (Table 2). In contrast, Azole compounds, Sulfadiazine, Petamidine, Miltefosine and Amphotericin B were most commonly used in GAE cases due to *B. mandrillaris*. For PAM due to *N. fowleri*, the most commonly used drugs included Amphotericin B, Azole compounds, Sulfadiazine, and Rifampicin (Table 2). Among cases with successful prognosis, there appears to be a combination of several compounds (Table 3). In some of these cases, a combination of Amphotericin B, Sulfamethoxazole and Trimethoprim, and Rifampicin was given in the treatment of GAE due to *Acanthamoeba* spp. (Table 3). In contrast, combination of Flucytosine, Fluconazole, Azithromycin, Pentamidine, Sulfadiazine, Azithromycin, and Miltefosine was given in the majority of GAE cases due to *B. mandrillaris* (Table 3). For PAM, in recent years, a combination of Amphotericin B, Fluconazole, Rifampin, Azithromycin, Dexamethasone, Miltefosine was given (Table 3).

Challenges and opportunities

Free-living pathogenic amoebae are now well recognized agents of brain infection leading to GAE and PAM. GAE is a chronic infection that can lasts up to several months, while PAM is an acute, fulminant infection lasting few days (1, 6). It is intriguing to see the distinctive difference of 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* enter the host via the nasal route. Recently, another

route of entry has been included, i.e., *via* organ transplantations, leading to recipients of organ donations in acquiring amoebic meningoencephalitis from the donor who was diagnosed with amoebic meningoencephalitis post-mortem of the same genotype (22-25). This is important as amoebae are ubiquitous, non-responsive to antibiotics, and organ recipients are already rendered immunosuppressed, thus any entry of these pathogenic free-living amoebae may lead to devastating consequences. Although risk factors data was not available for all cases reviewed in this study, there are factors that were observed to dictate susceptibility of patients to amoebic meningoencephalitis. For GAE due to *Acanthamoeba*, immunosuppression appeared to be a factor (1, 6, 26, 27), 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 amoebae. Primary amoebic meningoencephalitis usually occurred in immunocompetent children and young adults (1, 6, 7). However, all patients had history of activities in proximity to fresh water sources such as swimming pools, hot springs, recreational activities, religious practices such as ablution, and healthcare 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 taken for high risk populations.

Neuroimaging studies revealed the location of lesions in the frontal, parietal and temporal lobes in most cases of GAE, but the lesions were much more frequent in the frontal lobe for *N. fowleri*. Neuroimaging modalities however can have false negative results, therefore specificity of neuroimaging in diagnosis of amoebic meningoencephalitis is yet to be evaluated. In the absence of accurate diagnosis and effective treatment, both diseases often result in death. *N. fowleri* was found more often in the CSF than the other two amoebae, most likely due to its motile flagellated form. However, the diagnosis in biopsy may be hindered by the inoculum size and magnitude of inflammation and necrosis in the tissue section. In

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addition to factors above, morphology of trophozoites in tissue section bears a close resemblance to macrophages under untrained eyes which are also common in acute inflammatory response. The other challenge in diagnosis include wide spectrum of differential diagnosis ranging from brain tumors, multiple sclerosis, lupus encephalitis, progressive multifocal leukoencephalopathy, stroke, meningitis of 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* has highlighted the complex nature of the disease, especially as both amoebae are known to act as reservoir hosts for many microorganisms (1, 6, 14-16). What is more intriguing is that *Acanthamoeba* and *B. mandrillaris* meningoencephalitis cases present as vascular diseases (masquerading as cerebral vascular occlusion or aneurysm). This is most likely due to ability of amoebae to produce endothelial damage resulting in cytokine release, crossing of the blood-brain barrier, granulomatous inflammation, thromboembolic event, increased vascular permeability and ultimately necrosis.

For chemotherapeutic strategy, current available delivery routes include intravenous, oral and intrathecal administration. However, systemic antimicrobial treatment has its limitations due to its adverse effects and reduced delivery together with delayed diagnosis. Other concerns include, poor pharmacodynamics and pharmacokinetics profiles of available drugs, solubility, CNS penetration, drug-drug interactions, patient's medical conditions, patient's tolerance and *Acanthamoeba* susceptibility to amoebicidal agents (17). In the case of PAM, Amphotericin B deoxycholate preparation is preferable against *N. fowleri* infection compared with its liposomal formulation, albeit it has no effect on *Acanthamoeba* and *B. mandrillaris* (18, 19). More recently, Miltefosine has shown promising results in bio-availability and low drug-drug interactions (18). Of note, the major group of azole and macrolides are amoebistatic rather than amoebicidal. Additionally, nephrotoxic and

hepatotoxic effects due to the use of drugs in patients with compromised renal and liver functions (such as transplant patients) may further complicate the treatment. Potential drug delivery systems which directly target the inoculation sites of amoebae by circumventing the needs for optimal blood-brain barrier penetration should be the focus of future studies, thus increasing the odds of survival in patients with PAM, while minimizing adverse effects and complications from the diseases. Overall, a complete understanding of the pathogenetic mechanisms together with the role of immune system and the development of novel chemotherapeutic approaches in drug delivery (20, 21) is important for the rational development of anti-amoebic therapy.

Concluding remarks

Despite advances in clinical presentation, diagnostic methods and treatment approaches, the mortality associated with CNS infections due amoebae has remained high. Although neuroimaging findings reveal common areas of lesions, they may not be consistent and vary depending on the causative agent. A high level of clinician suspicion is important, especially in refractory cases of meningoencephalitis for rapid diagnosis of the infection, which is a pre-requisite in the successful treatment. Given that only a few individuals among all hosts exposed to these amoebae develop infection suggest the possible presence of underlying predisposing factors. Future research is needed to define genetic, immunological, pathogenic and environmental factors that contribute to deadly ameobic meningoencephalitis. Moreover, the ability of pathogenic amoebae to host other microbial pathogens as reservoirs and act as hyper-parasites has enhanced their capacity as pathogens of increasing importance to human and animal health.

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Figure Legends

Figure 1. The sites of infection of granulomatous amoebic encephalitis due to *Acanthamoeba* spp. The majority of cases were within cerebral cortices with frontal lobe and temporal lobe most affected, followed by parietal and occipital lobe. As for extracortical sites, cerebellum and cortico-medullary junction are most favoured sites. Furthermore, hydrocephalus is observed in few cases, which results from blockage of CSF drainage. Other sites affected include thalamus, caudate nucleus and brainstem. They can also present as normal finding in early neuroimaging.

Figure 2. The sites of infection of granulomatous amoebic encephalitis due to *Balamuthia mandrillaris*. The involvement of temporal lobe is observed in most cases, followed by frontal, parietal, and occipital lobe. In extracortical sites, thalamus was most affected, followed by cortico-medullary junction, cerebellum and basal ganglia.

Figure 3. The sites of infection of primary amoebic meningoencephalitis due to *Naegleria fowleri*. The majority of cases involved the frontal lobe, followed by parietal lobe, and cortico-medullary junction. Furthermore, hydrocephalus is observed in 27% of cases.

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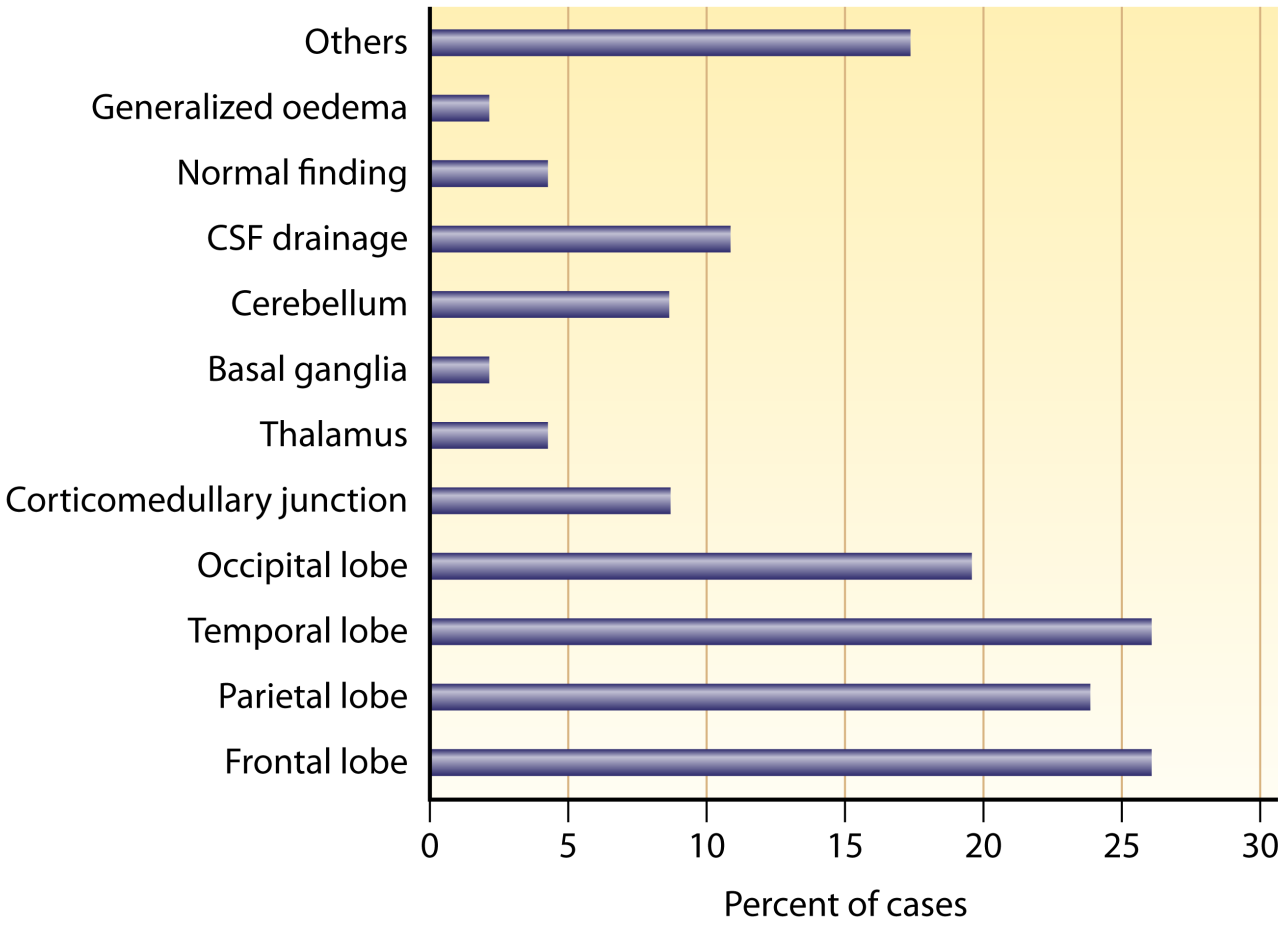

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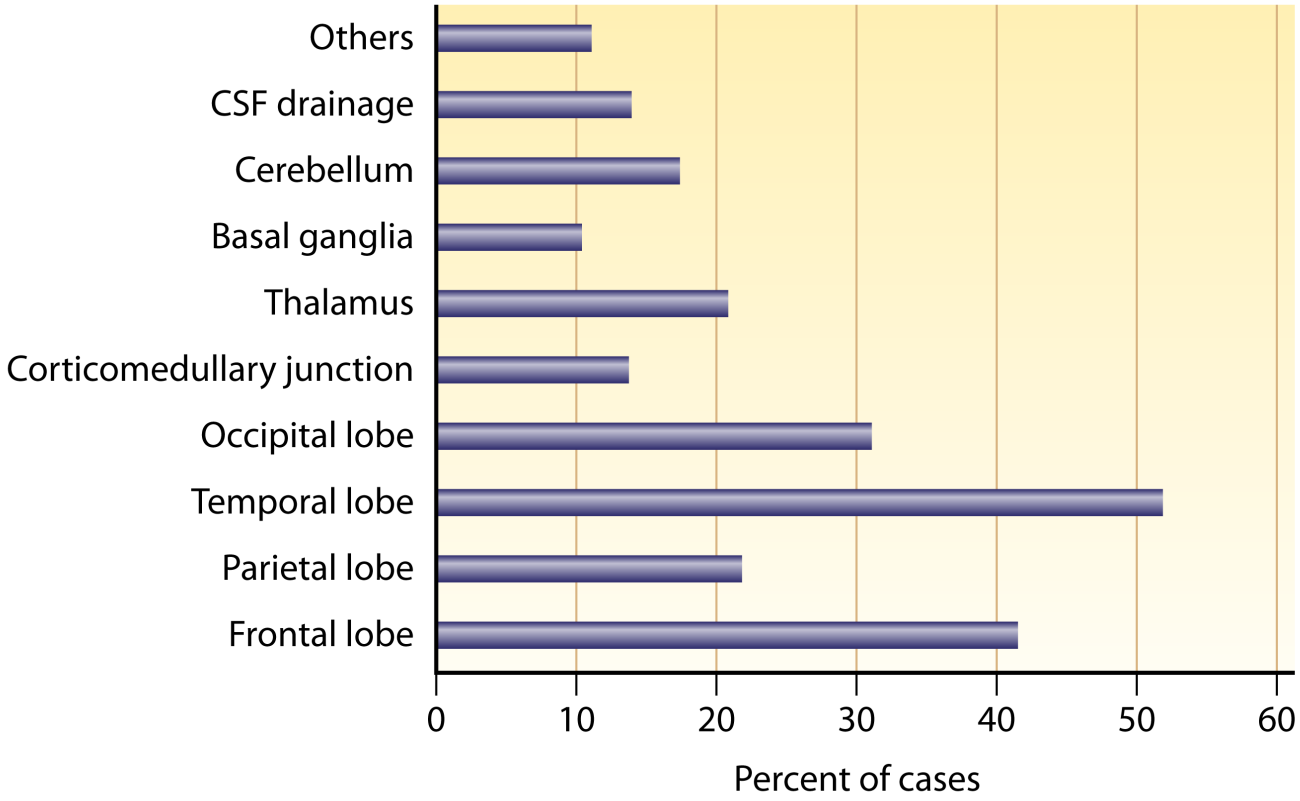

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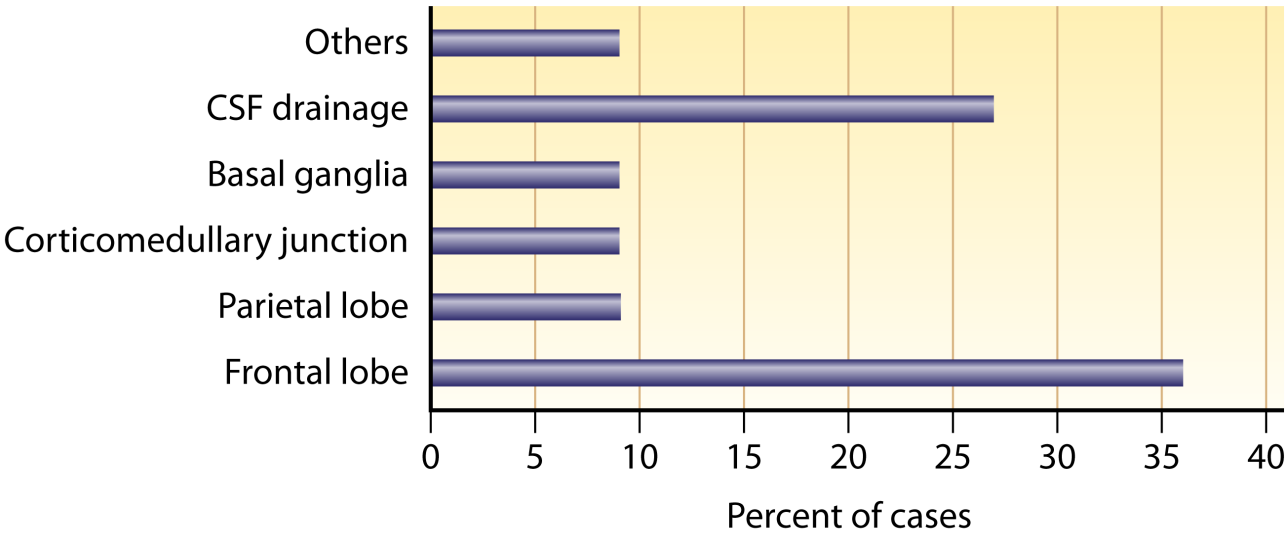

Table 1. The use various methods in the diagnosis of granulomatous amoebic encephalitis (GAE) due to *Acanthamoeba* spp., and *Balamuthia mandrillaris* and primary amoebic meningoencephalitis due to *Naegleria fowleri*. The data is presented as percent of cases reviewed in this study. Percentage of cases by diagnostic modalities corresponds with number of cases as indicated in parenthesis. Notably, some cases may involve more than one diagnostic modalities.

Disease (total cases reviewed)	**Diagnostic modality**	**Method**	**Percentage of cases [no. of cases]**
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]
	Post-mortem	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]
	Post-mortem	Microscopy	10.34 [3]
		IFA	20.68 [6]
	Skin biopsy		6.9 [8%]
PAM due to *N. fowleri* **(n=11)**	**Post-mortem**	Microscopy	36.4 [4]
		IFA	18.2 [2]
		PCR	9.1 [1]
	CSF	Microscopy	18.2 [2]
		Culture	18.2 [2]

Table 2. The use various individual drugs in the treatment of granulomatous amoebic encephalitis (GAE) due to *Acanthamoeba* spp., and *Balamuthia mandrillaris* and primary amoebic meningoencephalitis due to *Naegleria fowleri*. Non-specific treatment includes general measures to reduce intracranial pressure and inflammation (mannitol, decompressive craniotomy, corticosteroids) and treatment for differential diagnosis (cephalosporins for bacterial meningitis). In cases of combinations of drugs, the therapeutic agents are calculated independently. The data is presented as percent of cases [no. of cases].

	GAE due to *Acanthamoeba* (total cases reviewed = 46)	GAE due to *B. mandrillaris* (total cases reviewed = 29)	PAM due to *N. fowleri* (total cases reviewed = 11)
Non-specific	19.5 [9]	20.7 [6]	18.2 [2]
Miltefosine	15.2 [7]	13.8 [4]	-
Pentamidine	13 [6]	31 [9]	-
Sulfadiazine	19.5 [9]	34.5 [10]	18.2 [2]
Flucytosine	13 [6]	24.1 [7]	-
Macrolides (Azithromycin, Clarithromycin)	17.4 [8]	31 [9]	-
Azoles	41.3 [19]	48.3 [14]	18.2 [2]
Carbapenems	4.3 [2]	3.4 [1]	-
Sulfonamides (Trimethoprim-Sulfamethaxazole)	34.8 [16]	3.4 [1]	-
Rifampicin	37 [17]	6.9 [2]	18.2 [2]

Chloramphenicol	6.5 [3]	-	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]	-	-
Tetracyclines	-	3.4 [1]	-

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Table 3. Selected cases of ameobic meningo-encephalitis with successful prognosis.

Patient description	Causative agent	Treatment
2000: a 33 year-old man	*Acanthamoeba* spp.	Sulfazidine, pyrimethamine and fluconazole with left homonymous hemianopia (visual field defects)
2002: a 45 year-old lady	*Acanthamoeba* spp.	Rifampicin, cotrimaxazole, fluconazole and ceftriaxone for 4 weeks, followed up 1 year for facial nerve palsy
2006: a 10 year-old boy	*Acanthamoeba* spp.	Ketoconazole and rifampicin, duration of therapy is unknown.
2008: 25 year-old young man	*Acanthamoeba* spp.	Miltefosine and follow up for 24 months. Seronegative for *Acanthamoeba* after treatment but neurological deficits did not improve.
2009: a 63 year-old man with history of contact with contaminated water	*Acanthamoeba* spp.	Amphotericin B and rifampicin. Patient was discharged after 78 days of hospitalization.
2011 survival case of GAE, the patient was a 2 year-old boy with underlying acute lymphoblastic leukemia	*Acanthamoeba* spp.	Meropenem, teicoplanin, fosfomycin, metronidazole, and liposomal amphotericin B, resulting in symptom resolution.
2012: an immunocompetent 38 year-old man	*Acanthamoeba* spp.	Voriconazole and miltefosine, he achieved radiological and clinical relief after 6 days of initiation of treatment. He was followed up for refractory seizure complication since then
2012: a 2 year-old boy	*Acanthamoeba* spp.	Cotrimoxazole, rifampicin, ketoconazole, improvement after 2 days
2014: a 30 year-old man	*Acanthamoeba* spp.	Rifampicin, sulfamethoxazole and trimethoprim, fluconazole for 2 weeks, asymptomatic after 2 weeks of follow up
2016: a 2 year-old boy	*Acanthamoeba* spp.	Ceftazidime, metronidazole, fluconazole and rifampicin for 3 weeks
2016: an 11 year-old girl	*Acanthamoeba* spp.	Amphotericin B, sulfamethoxazole and trimethoprim, and

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		rifampicin
2016: a 12 year-old boy	*Acanthamoeba* spp.	Amphotericin B, sulfamethoxazole and trimethoprim, and rifampicin
2016: a 9 months old girl	*Acanthamoeba* spp.	Amphotericin B, sulfamethoxazole and trimethoprim, and rifampicin
2003: a 64 year-old man	*Balamuthia mandrillaris*	Amphotericin B, flucytosine, fluconazole, sulfadiazine for 5 years, clarithromycin for 2 years, pentamidine for 18 days
2003: a 5 year-old girl	*Balamuthia mandrillaris*	Flucytosine, fluconazole for 2 years, pentamidine for 34 days and clarithromycin for 2 years
2004: a 72 year-old lady	*Balamuthia mandrillaris*	Pentamidine, sulfadiazine, fluconazole, clarithromycin, hospitalized for 13 days
2004: a 72 year-old man	*Balamuthia mandrillaris*	Fluconazole, sulfadiazine, clarithromycin and pentamidine isethionate, duration of therapy is unknown
2006: a 10 year-old girl	*Balamuthia mandrillaris*	Albendazole, itraconazole, sulfamethoxazole and trimethoprim for 6 months
2006: an 8 year-old boy	*Balamuthia mandrillaris*	Albendazole and itraconazole for 14 months
2010: a 21 year-old lady	*Balamuthia mandrillaris*	Albendazole, fluconazole for 7.5 months and miltefosine for 7 months
2010: a 2 year-old boy	*Balamuthia mandrillaris*	Pentamidine (stopped after 2 months), sulfadiazine, flucytosine, clarithromycin and fluconazole
2010: a 27 year-old man	*Balamuthia mandrillaris*	Sulfadiazine, azithromycin and miltefosine for unspecified duration
2011: a 27 year-old male, organ recipient	*Balamuthia mandrillaris*	Pentamidine, sulfadiazine, flucytosine, fluconazole, azithromycin and miltefosine
2011: an 80 year-old lady	*Balamuthia mandrillaris*	Pentamidine, itraconazole, azithromycin, sulfadiazine,

		flucytosine, liposomal amphotericin
2013: a 5 year-old girl	*Balamuthia mandrillaris*	Flucytosine, fluconazole, azithromycin, pentamidine and sulfadiazine, changed to final regimen azithromycin, fluconazole and miltefosine
2013: 4 year-old immunocompetent girl with history of water contact with floods around her residence	*Balamuthia mandrillaris*	flucytosine, fluconazole, azithromycin, pentamidine and sulfadiazine
2002: a 26 year-old female	*Naegleria fowleri*	Rifampicin, amphotericin B and ornidazole for 2 weeks
2008: an 8 months old male	*Naegleria fowleri*	Amphotericin B, chloramphenicol and rifampicin and achieved afebrile at day 7 of treatment
2013: two survivors, a 12-year-old female and a male	*Naegleria fowleri*	Both were given amphotericin B, fluconazole, rifampin, azithromycin, dexamethasone, miltefosine

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