

# Amebic Meningoencephalitis & Keratitis

Govinda S. Visvesvara, A. Julio Martinez,  
Mary K. Klassen-Fischer, and  
Ronald C. Neafie

## Introduction

### Definition

Free-living amebae of the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* cause fatal diseases of the central nervous system (CNS) of humans.<sup>1-33</sup> *Naegleria fowleri* causes an acute and fulminant primary amebic meningoencephalitis (PAM) in children and young adults with a history of exposure to fresh water leading to death within 5 to 10 days after the onset of symptoms.<sup>4-6,9,10,16,17,19,25,27,30,31,33,34</sup> *Balamuthia mandrillaris*,<sup>2,3,7,8,19-22,24,26,28-31,35,36</sup> and several species of *Acanthamoeba* (*Acanthamoeba castellanii*, *Acanthamoeba culbertsoni*, *Acanthamoeba rhyodes*, *Acanthamoeba polyphaga*, *Acanthamoeba divionensis*, *Acanthamoeba healyi*, and *Acanthamoeba lenticulata*) cause a chronic, and usually fatal, granulomatous amebic encephalitis (GAE) that may last for several weeks or months.<sup>1,11,18,19,23,30,31,37-42</sup> *Acanthamoeba* sp also cause an eyesight threatening infection, acanthamoeba keratitis, in humans, especially in persons wearing contact lenses.<sup>12,15,17,19,30,31,40,43-52</sup> Additionally, *N. fowleri*, *Acanthamoeba* sp, and *B. mandrillaris* also infect animals.<sup>12-17, 19,28-31, 53-58</sup> *Sappinia diploidea*, another free-living ameba identified in 2001<sup>59</sup> as an agent of meningitis, was reidentified recently as *Sappinia pedata* based on molecular analysis.<sup>60</sup> So far there is only one case reported due to this ameba.

### Synonyms

*Naegleria aerobia* and *Naegleria invadens* are not valid synonyms for *N. fowleri*. *Balamuthia mandrillaris* was previously known as leptomyxid ameba.

### General Considerations

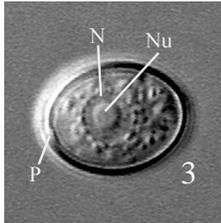
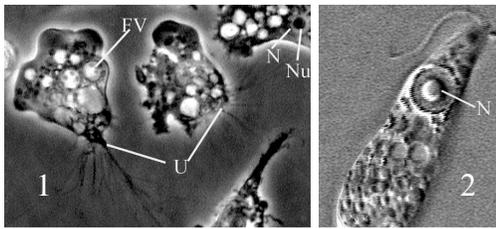
In 1958, Culbertson et al isolated an A-1 strain of *Acanthamoeba* identified as a contaminant in a primary monkey kidney cell culture during production of poliomyelitis vaccine.<sup>61</sup> Immunosuppressed animals inoculated with the ameba developed fatal brain lesions containing amebic trophozoites. Culbertson hypothesized that similar strains might exist in nature and may infect humans.<sup>61</sup> Because of taxonomic uncertainties at that time regarding the genera *Hartmannella* and *Acanthamoeba*, Culbertson designated the A-1 strain as *Hartmannella-Acanthamoeba*, or H-A, amebae. It is now well-established that *Hartmannella* and *Acanthamoeba* are distinct genera and that no true hartmannellid ameba causes CNS infection in humans. Culbertson's A-1 strain is now known as *A. culbertsoni*.<sup>11,15,19,30,47</sup> In 1965, Fowler and Carter described a fatal human infection caused by free-living amebae, which they presumed to be *Acanthamoeba* sp, in the brain of an Australian boy.<sup>5</sup> The infection was later attributed to *N. fowleri*.<sup>5,16,19,30</sup> A year later, Butt et al described the first case of *N. fowleri*

# Report Documentation Page

Form Approved  
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE <b>JUN 2011</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2011 to 00-00-2011</b>	
4. TITLE AND SUBTITLE <b>Amebic Meningoencephalitis and Keratitis</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Roybal Campus, 1600 Clifton Road, Atlanta, GA, 30333</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES <b>See also ADA545141. Chapter 9 from e-book, Topics on the Pathology of Protozoan and Invasive Arthropod Diseases.</b>					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			



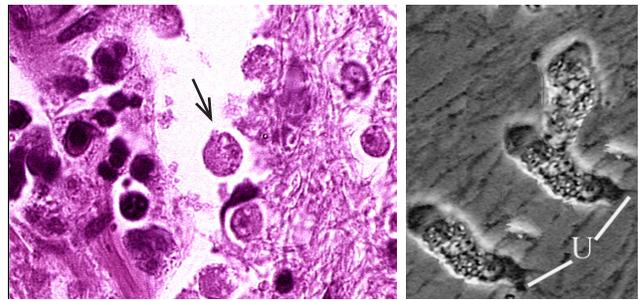
**Figures 9.1 - 9.3**  
*Naegleria fowleri* has a 3-stage life cycle: trophozoite, flagellate, and cyst. Note nucleus (N), nucleolus (Nu), uroid process (U), contractile vacuole (FV), cyst wall pore (P). all magnifications x1000

infection in the United States and coined the term “primary amebic meningoencephalitis.”<sup>4</sup> Later studies showed that PAM was a recently discovered, rather than a new disease. A study of autopsy records and specimens at the Medical College of Virginia revealed cases of PAM dating back to 1937.<sup>10</sup> Studying museum specimens in England, Symmers discovered a case of human PAM dating from 1909.<sup>25</sup> Of the more than 200 cases of PAM reported worldwide, only a few patients have survived.

Granulomatous amebic encephalitis (GAE) caused by *Acanthamoeba* sp usually occurs in persons with HIV/AIDS or who are otherwise immunocompromised, debilitated, or malnourished. At one time, all cases of GAE were attributed to *Acanthamoeba* sp.<sup>28,29</sup> Although in most cases *Acanthamoeba* sp was identified as the causative agent by immunofluorescence or immunoperoxidase techniques, a few cases could not be confirmed in this way. However, with the isolation of *B. mandrillaris* amebae in the brain of a mandrill (a large baboon) in 1986 and subsequent development of an antiserum and an immunofluorescence test for this organism, *B. mandrillaris* has been definitively identified as the etiologic agent of GAE in a number of human and animal cases.<sup>28,29</sup> Fewer than 100 cases of balamuthia GAE have occurred worldwide, approximately 70 in the United States.<sup>7</sup>

## Epidemiology

*Naegleria* sp and *Acanthamoeba* sp are distributed worldwide and are commonly found in soil, dust, fresh water, household water, sewage, thermal effluents, swimming pools, and hot springs.<sup>12,16,19,30,31,47,62</sup> Recently, *N. fowleri* has also been isolated from drinking water.<sup>16,63</sup> *Acanthamoeba* sp have also been isolated from salt water, ocean sediments, heating and air conditioning units, bacterial, mycotic, and mammalian cell cultures, vegetable matter, contact lens paraphernalia, and



**Figures 9.4, 9.5**

Trophozoites are spherical and 10  $\mu\text{m}$  to 18  $\mu\text{m}$  in diameter (arrow). Their slug-like, eruptive movement is produced by smooth hemispheric bulges. The single nucleus has a large, centrally located nucleolus that stains densely with aniline dyes. Note uroid process (U). H&E (left) x400, phase contrast (right). x600

human tissue.<sup>12,15,19,30,47</sup> *Balamuthia mandrillaris* has only recently been isolated from nature.<sup>64-66</sup>

## Primary Amebic Meningoencephalitis (PAM)

### Infectious Agent

*Naegleria fowleri* has a 3-stage life cycle: trophozoite, flagellate, and cyst (Figs 9.1 to 9.3). Trophozoites are spherical and 10  $\mu\text{m}$  to 18  $\mu\text{m}$  in diameter. Their slug-like, eruptive movement is produced by smooth hemispheric bulges. In tissue sections, the single nucleus has a large, centrally located nucleolus that stains densely with aniline dyes (Figs 9.4 & 9.5). A contractile vacuole is often seen. The posterior end, or uroid, appears to be sticky and often has several fine, trailing filaments. Clumps of bacteria or debris may attach to the uroid. *Naegleria* sp divide by promitosis, wherein the nucleolus and nuclear membrane persist during division. Under adverse conditions, such as a change in the ionic concentration of the milieu, trophozoites differentiate into a piriform, transient, non-feeding flagellate stage. Flagellates commonly have 2 flagella but may have 3 or more and they usually revert to the trophozoite stage. Trophozoites also differentiate into smooth-walled, spherical cysts 7  $\mu\text{m}$  to 15  $\mu\text{m}$  in diameter. Cyst walls may have 1 or more pores plugged with a mucoid substance.<sup>16,67</sup>

### Clinical Features and Pathogenesis

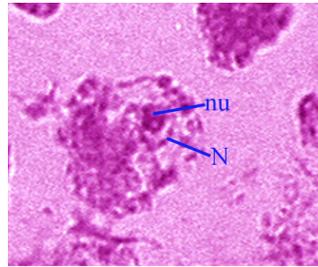
Primary amebic meningoencephalitis (PAM) is an acute, fulminant infection characterized by severe bifrontal or bitemporal headache, spiking fever of 40°C, nausea, vomiting, nuchal rigidity, positive Kernig’s and Brudzinski’s signs, photophobia, confusion, delirium, seizures, and coma. Symptoms appear 1 to 14 days after exposure;

most patients die 3 to 7 days after the onset of symptoms. PAM usually occurs in children and young adults who have recently swum in lakes, heated swimming pools with inadequate chlorination, or other bodies of fresh water that harbor amebae. Trophozoites and/or flagellates enter the swimmer's nostrils and invade the subarachnoid space and CNS parenchyma through the olfactory nerves, the cribriform plate, and the olfactory neuroepithelium. The incubation period is usually 3 to 8 days.<sup>4-6,16,17,19,30,33,34</sup>

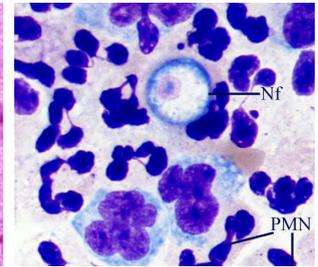
### Pathologic Features

In its early stages, PAM resembles acute bacterial leptomeningitis. However, the purulent exudate is scant compared to that seen in bacterial leptomeningitis, and the inflammatory reaction in the subarachnoid and Virchow-Robin spaces is less intense.<sup>4-6,16,17,19,30,34</sup> There are no clear differences between PAM and acute pyogenic or bacterial meningoencephalitis. The peripheral white blood count is usually elevated, with a predominance of neutrophils. Cerebrospinal fluid (CSF) appears grayish to yellowish-white and may be tinged with red. CSF pressure is elevated, ranging from 300 to 600 mm H<sub>2</sub>O. CSF pleocytosis is usually seen, with a predominance of neutrophils, which may be mistaken for bacterial infection. Early in the infection the CSF white cell count may be low, but as the disease progresses it may increase to as much as 26,000 cells/mm<sup>3</sup>. The CSF glucose level is usually low to normal and CSF protein is high, ranging from 100 to 1,000 mg/dl. Computed tomography (CT) shows obliteration of the cisterns surrounding the midbrain and subarachnoid space. A CT scan of the brain without contrast appears to be normal or may show cerebral edema with obliteration of cisterns surrounding the midbrain and subarachnoid space. However, with intravenous contrast medium marked diffuse enhancement in these regions may be seen.<sup>16,17,19,30</sup>

*Naegleria fowleri* destroys the olfactory neuroepithelium and olfactory bulbs. PAM is characterized by acute hemorrhagic necrosis of both gray and white matter, associated with an acute inflammatory infiltrate consisting mainly of neutrophils, eosinophils, macrophages, and occasional lymphocytes. The cerebral cortex shows evidence of recent superficial hemorrhages in and around the orbitofrontal and temporal lobes, hypothalamus, midbrain, pons, medulla oblongata, cerebellum, and upper portion of the spinal cord. The cortex is most severely affected at the base of the brain, with pockets of amebic trophozoites within edematous and hemorrhagic CNS tissue. Large numbers of amebic trophozoites, some with phagocytosed erythrocytes and myelin fragments, may also be seen deep in the Virchow-Robin spaces and around blood vessels, but with minimal or no inflammatory response. *Naegleria fowleri* generally do not produce cysts in CNS tissue. A focal or diffuse myocarditis has been reported in a few cases of PAM, though microorganisms were not found in myocardial lesions.<sup>16,17,19,30</sup>



**Figure 9.6**  
Trophozoites under reduced bright field light microscopy. Note large, centrally located nucleolus (nu) surrounded by a clear halo within nucleus (N) characteristic of *Naegleria fowleri*. x1250



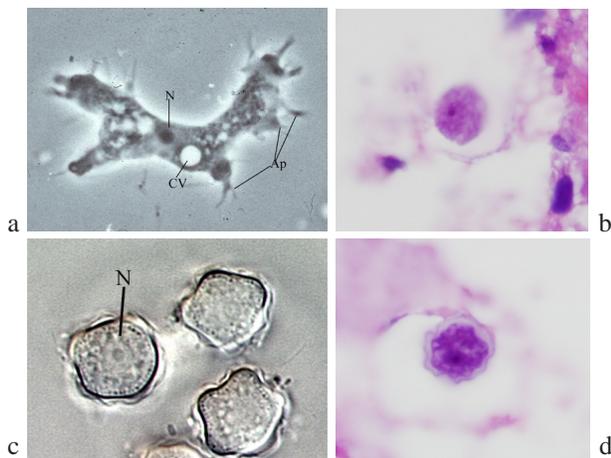
**Fig 9.7**  
*Naegleria fowleri* (NF) in wet mount of CSF smear with accompanying polymorphonuclear (PMN) leucocytes. Wright stain x1000

### Diagnosis

Diagnosis of PAM is confirmed by identifying amebic trophozoites in a wet mount of CSF preparation. A light microscope equipped with phase contrast optics is preferable, but the preparation may also be viewed under brightfield illumination with reduced light. *Naegleria fowleri* trophozoites may be distinguished from host cells by their active directional movement and the large, centrally located nucleolus surrounded by a clear halo (Fig 9.6). CSF smears should be stained with Giemsa, Wright, or trichrome (Fig 9.7). Gram stain is not useful in identifying *N. fowleri*. Specimens of CSF or brain tissue should be kept at 24° to 28°C for no more than 24 hours. CSF should be centrifuged at 1,000 rpm for 5 to 8 minutes. After aspirating all but 0.5 ml of the supernatant, the sediment should be inoculated onto the center of an agar plate that has been precoated with bacteria, and incubated at 37°C. Biopsy or autopsy material should be triturated in a small amount of ameba saline (0.5 ml), placed in the center of an agar plate, and incubated in the same way.<sup>16,17,19,30,31</sup>

Under a microscope, *N. fowleri* amebae appear as small, motile blotches. After 2 to 3 days incubation, the amebae begin to encyst. After 4 to 5 days incubation, trophozoites and cysts should be visible.<sup>16,17,19,30</sup> Generic identification is based on characteristic patterns of locomotion, morphologic features of the trophic and cyst forms, and enflagellation experiments.<sup>67</sup> However, specific identification of *N. fowleri* is made by using immunohistochemical techniques using *N. fowleri* monoclonal or polyclonal antibodies<sup>16,68</sup> or molecular techniques such as PCR<sup>16,19,30,69</sup> nested PCR<sup>70</sup> and real-time PCR<sup>71</sup> on the cultured amebae or on infected tissue sections. Recently, a multiplex real-time PCR has been developed at CDC that identifies all three amebae (*Acanthamoeba* sp, *B. mandrillaris* and *N. fowleri*). The real-time multiplex PCR is a rapid, sensitive, and specific assay which can be performed and results reported within 4 to 5 hours.<sup>71</sup>

Since PAM has a rapid onset and progresses quickly there is little opportunity for an effective humoral response to



**Figure 9.8 a,b,c,d**

*Acanthamoeba* sp have a 2-stage life cycle consisting of trophozoite (a & b) and cyst (c & d), nucleus (N), contractile vacuole (CV), retractile projections (P). Phase contrast, a and c. x1000 Hematoxylin-eosin b and d. x1000

develop against the amebae. However, in one case with a successful outcome, *N. fowleri* antibody of the IgM class was demonstrated with a titer of 1:4096.<sup>34</sup>

### Treatment

Two patients, one from the United States and the other from Mexico, are known to have survived PAM.<sup>27,34</sup> Other reports of survival are unconfirmed.<sup>16,17,19,30</sup> The American patient was treated aggressively with intrathecal and intravenous amphotericin B (to which *N. fowleri* is highly sensitive) and miconazole, and with oral rifampin.<sup>34</sup> A Mexican boy was treated early with intravenous amphotericin B and fluconazole, and with oral rifampin.<sup>27</sup> Early identification of the etiologic agent and aggressive therapy appear to be key to patient survival.

## *Acanthamoeba* and *Balamuthia* Granulomatous Amebic Encephalitis (GAE)

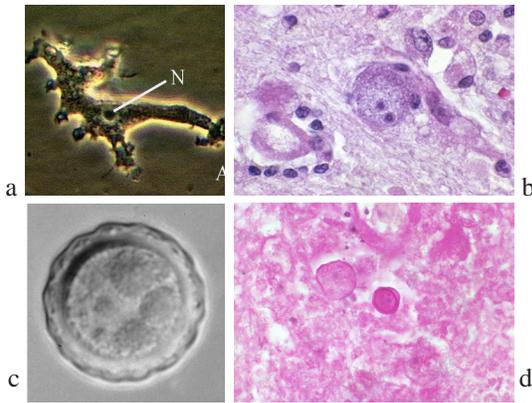
### Infectious Agents

*Acanthamoeba* sp have a 2-stage life cycle consisting of trophozoite and cyst (Figs 9.8a to 9.8d). *Acanthamoeba* sp trophozoites are slightly larger than those of *N. fowleri*, measuring 15  $\mu$ m to 45  $\mu$ m in diameter. A contractile vacuole periodically ruptures on the body surface, which is covered with fine, spine-like, retractile projections (acanthopodia). Trophozoites usually have a single nucleus with a large, dense nucleolus.<sup>67</sup>

*Acanthamoeba* sp divide by conventional mitosis, in which the nucleolus and the nuclear membrane disappear during cell division. The organism does not have a flagellate stage, but it differentiates into a cyst under adverse conditions. Cysts are uninucleate and have a proteinaceous, usually wrinkled, ectocyst and an endocyst made of cellulose. At the point of contact between the ectocyst and endocyst there are pores, or ostioles, usually covered by opercula. The cysts are highly resistant to environmental pressures including desiccation.<sup>72</sup> As many as 24 *Acanthamoeba* sp have been described. These species can be divided into 3 groups based on cyst size and morphology as follows: 1) species that produce large cysts (18  $\mu$ m to 28  $\mu$ m); 2) species that produce cysts smaller than 18  $\mu$ m with prominently wrinkled ectocysts and stellate, polygonal, triangular, oval, or round endocysts; and 3) species that produce cysts smaller than 18  $\mu$ m with slightly wrinkled ectocysts and round or oval endocysts<sup>67</sup> (Table 9.1). However, recent developments in molecular analysis has enabled improvements in the taxonomy of this genus and its phylogenetic relationships. Further, based on the sequencing of the 18S rRNA gene,

Table 9.1 Morphologic features of *Acanthamoeba* sp that cause meningoencephalitis.

Group	Species	Cyst size	Ectocysts	Endocysts
I	<i>A. astronyxis</i>	18 to 28 $\mu$ m	Nearly circular, gently rippled	Rays if stellate endocyst contact ectocyst in the same plane
II	<i>A. castellani</i> , <i>A. divionensis</i> , <i>A. polyphaga</i> , <i>A. rhyssodes</i>	<18 $\mu$ m	Prominently wrinkled	Stellate, polygonal, triangular, oval or round
III	<i>A. culbertsoni</i> , <i>A. healyi</i>	<18 $\mu$ m	Slightly wrinkled	Round or oval



**Figure 9.9a-d**  
*Balamuthia mandrillaris* also has a 2-stage life cycle of trophozoite (a & b) and cyst (c & d). In vitro culture under phase contrast, a and c x1000; tissue sections stained with H&E, b and d. x250

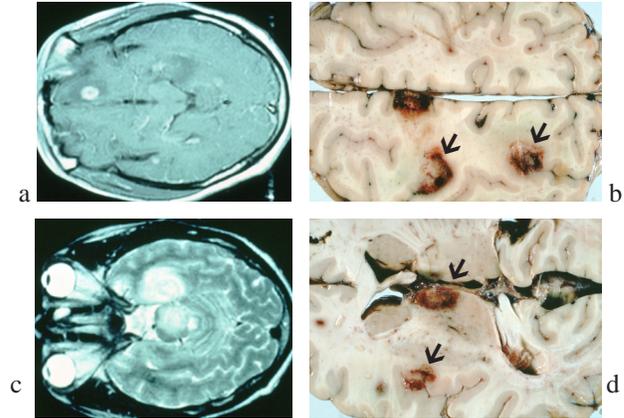
16 genotypes (T1 to T16) have been identified.<sup>12,15,19,30,31,47,73</sup>

*Balamuthia mandrillaris* also has a 2-stage life cycle of trophozoite and cyst (Figs 9.9a to 9.9d). Actively feeding *Balamuthia* trophozoites growing in culture range in size from 12 µm to 60 µm in diameter (average 30 µm). The ameba has a spider-like movement achieved by producing a broad pseudopodium and, sometimes, finger-like determinate pseudopodia. Trophozoites are normally uninucleate, but binucleate forms are occasionally seen. The nucleus usually has a single large nucleolus, but may have up to 2 or 3.<sup>21,28-30</sup>

*Balamuthia mandrillaris* divides by a complex form of mitosis called metamitosis in which the nucleolus and the nuclear membrane are intact during the initial stages. As mitosis progresses, the nuclear membrane breaks down and the nucleolus disappears. Trophozoites differentiate into cysts under adverse conditions. Light microscopy reveals a wrinkled ectocyst and a thick endocyst. However, ultrastructurally there is a third layer consisting of a structureless mesocyst (Fig 9.9b).<sup>29,65</sup>

### Clinical Features and Pathogenesis

*Acanthamoeba* sp GAE primarily affects immunosuppressed, chronically ill, or otherwise debilitated persons with no history of exposure to bodies of fresh water. There have been a few reported infections in otherwise healthy individuals with no known immunodeficiency or risk factors for HIV.<sup>12,15,17,19,30,47</sup> *Balamuthia mandrillaris* GAE affects the very young or very old, and patients with HIV/AIDS.<sup>17,21,28-30</sup> Whether caused by *Acanthamoeba* sp or *B. mandrillaris*, GAE is a slowly progressive disease of the CNS with no clearly defined incubation period. Onset is insidious, lasting from a few days to several weeks or months. Patients present with focal neurologic deficits, signs of increased intracranial pressure, and neurologic and radiographic features suggestive of an expanding mass. Differen-



**Figure 9.10 a-d**  
 Granulomatous amebic encephalitis caused by *Balamuthia mandrillaris*. Cross section of cerebral hemispheres show multiple foci of hemorrhagic encephalomalacia (arrows) involving the cerebral cortex, subcortical white matter, and basal ganglia. CT Scan, a and c; Horizontal sections of brain after fixation, b and d.

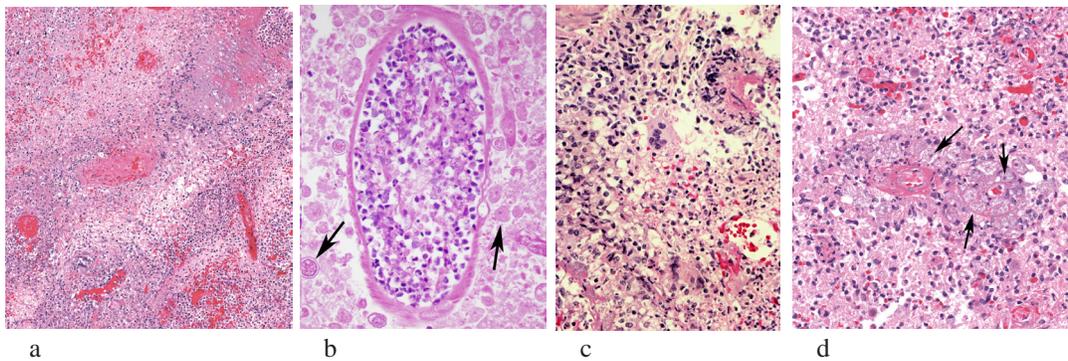
tial diagnosis includes brain tumor, abscess, or intracerebral hematoma. Common clinical symptoms include headache, irritability, confusion, seizures, dizziness, drowsiness, somnolence, and behavioral changes. Diplopia, aphasia, ataxia, altered mental state, lethargy, and hemiparesis are less common symptoms. Some patients, especially those with HIV/AIDS, develop skin lesions, abscesses, or erythematous nodules.<sup>3,17,21,28-30</sup>

CNS disease is a secondary feature of GAE, probably caused by hematogenous dissemination from a primary site such as a skin lesion or the lower respiratory tract.<sup>17,21,28-30</sup> However, there are a few reported cases of granulomatous skin disease caused by *Acanthamoeba* sp that did not spread to the CNS.<sup>42</sup> *Acanthamoeba* sp and *B. mandrillaris* are known to encyst within such lesions (Fig 9.9d). *Acanthamoeba* and *B. mandrillaris* cysts have also been isolated from airborne dust.<sup>17,21,30,65</sup> In an immunocompromised person who inhales cysts or whose damaged skin surface is contaminated with airborne cysts, amebae may excyst into trophozoites and invade the olfactory mucosa or deeper structures of the skin.

Recently, research efforts have focused on the role of free-living amebae as carriers of pathogenic bacteria, such as *Legionella*, in biofilms.<sup>12,15,17,19,30,47</sup>

### Pathologic Features

Granulomatous amebic encephalitis produces moderate edema of the cerebral hemispheres, with focal hemorrhagic softening of the cerebral cortex. Affected areas may produce a mild purulent exudate.<sup>17</sup> On cross section, cerebral hemispheres show multiple foci of hemorrhagic encephalomalacia involving the cerebral cortex, subcortical white matter, and basal ganglia (Figs 9.10a to 9.10d). Structures of the posterior fossa (cerebellum and brain stem) may be



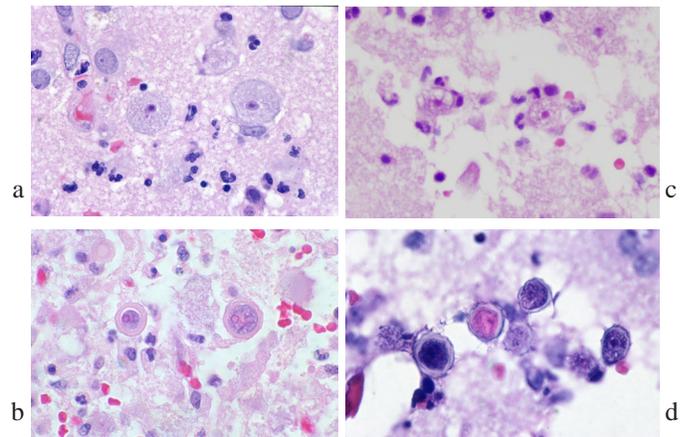
**Figure 9.11 a,b,c,d**  
Microscopically, GAE lesions consist of hemorrhagic necrosis of CNS parenchyma, variable subacute and chronic inflammatory reactions, and amebic trophozoites and cysts around and within blood vessel walls (arrows), magnifications: a x35, b x85, c x75, d x90

more severely affected than the cerebral hemispheres.

Microscopically, GAE lesions consist of hemorrhagic necrosis of CNS parenchyma, variable subacute and chronic inflammatory reactions, and amebic trophozoites and cysts around and within blood vessel walls (Figs 9.11a to 9.11d). Multinucleated giant cells may be present, particularly in patients with only mild immunosuppression. Astrocytic gliosis may appear around necrotic areas. There may be panarteritis with perivascular cuffing by lymphocytes, some plasma cells and macrophages, and few eosinophils. Fibrinoid necrosis and thrombosis in arterioles may be present. Trophozoites and cysts may be found in liver, lung, kidney, prostate, lymph nodes, skin, and other organs, suggesting hematogenous dissemination of trophozoites and cysts.<sup>17</sup>

## Diagnosis

*Acanthamoeba* sp and *B. mandrillaris* have been isolated from skin and brain biopsy material. *Acanthamoeba* sp have also been isolated from CSF and lung biopsy material. Until recently, *B. mandrillaris* had never been isolated from CSF. However, *B. mandrillaris* was isolated from the CSF of a patient who developed infection after receiving a kidney transplant.<sup>7</sup> To isolate *Acanthamoeba* sp, specimens should be processed the same as for *N. fowleri*. If amebae are present, trophozoites and cysts will appear on agar plates after 1 or 2 weeks of incubation.<sup>12,15,17,19,30,47</sup> *B. mandrillaris* will not grow on agar plates coated with bacteria.<sup>17,30</sup> However, mammalian cell cultures such as monkey kidney or human lung fibroblasts will support the growth of *B. mandrillaris*. *Balamuthia* sp trophozoites and cysts found in CNS tissue are morphologically similar to those of *Acanthamoeba* sp (Figs 9.12a to 9.12d), making it difficult to differentiate them with light microscopy alone.<sup>17,30</sup> It is sometimes possible to distinguish *Balamuthia* sp from *Acanthamoeba* sp based on nuclear morphology; in tissue sections, *B. mandrillaris* trophozoites may have more than 1 nucleolus. In most cases, electron microscopy and/or immunofluorescence assay are necessary to identify *B. mandrillaris* organisms.<sup>2,3,7,17,30,35,36,68</sup> Recent developments in molecular analysis, especially the real-time multiplex PCR has enabled the specific identification of *Acanthamoeba* sp and *B. mandrillaris*



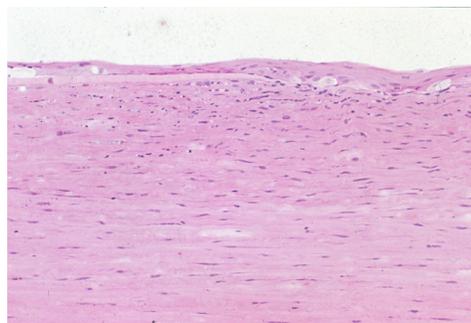
**Figure 9.12 a,b,c,d**

*Balamuthia* trophozoites (a) x250 and cysts (b) x250 found in CNS tissue are morphologically similar to those of *Acanthamoeba* sp trophozoites (c) x250 and cysts (d) x800, making it difficult to differentiate them with light microscopy alone.

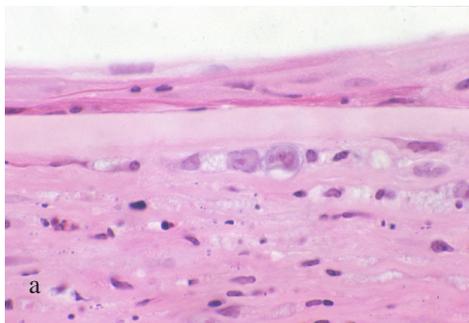
in the CSF, tissue sections and the cultured amebae.<sup>17,30,74-76</sup> Since *Acanthamoeba* sp are ubiquitous in nature, humans are exposed to them and may produce antibodies to these amebae. Although, indirect fluorescent antibody (IFA) and enzyme immunoassay (EIA) have been developed to detect antibody to *Acanthamoeba* sp in sera of patients as well as infected individuals, they are not routinely used as diagnostic tests.<sup>17,19,30</sup> Similarly IFA, flow cytometry, and ELISA techniques have been developed to identify antibodies to *B. mandrillaris* in the sera of healthy persons as well as patients with *B. mandrillaris* GAE.<sup>21,30,77</sup>

## Treatment

There is no effective treatment for GAE and the prognosis is poor. Although several patients are known to have survived because of diagnosis antemortem, GAE is most often identified only at death.<sup>7,17,30,76</sup> Several patients with *Acanthamoeba* sp GAE and *Acanthamoeba* sp cutaneous infection have been treated with a combination of several drugs including sulfadiazine, trimethoprim-sulfamethoxazole,<sup>5</sup> fluorocytosine, fluconazole or itraconazole, and topical application of chlorhexidine gluconate on skin ulcers.<sup>15-17,19,21,30,38-42,78</sup> Recently, miltefosine, an analogue of alkyl phosphocholine,

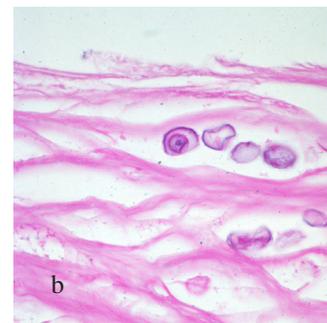


**Figure 9.13**  
*Acanthamoeba* keratitis, irregular corneal epithelial breakdown, stromal infiltrative keratitis, and edema. H&E x100



**Figure 9.14 a,b**

Corneal biopsy. a. Early stages of *Acanthamoeba* keratitis showing destruction of the anterior corneal epithelium associated with acute inflammatory cells (mainly neutrophils) into the superficial and middle layers of the corneal stroma. x500 b. Trophozoites and cysts interspersed between the corneal lamellae. x500



has been used successfully to cure an HIV negative man with *Acanthamoeba* sp GAE and disseminated disease.<sup>37</sup> Additionally, voriconazole also has been used successfully to treat a lung transplant patient with cutaneous *Acanthamoeba* sp infection.<sup>79</sup> Experimental in vitro studies indicate that both *Acanthamoeba* sp and *B. mandrillaris* are sensitive to pentamidine isethionate, azithromycin/clarithromycin and miltefosine.<sup>19,30,32,80-83</sup> Several cases of *B. mandrillaris* GAE infection have survived after treatment with pentamidine, clarithromycin/azithromycin, fluconazole, sulfadiazine and 5-fluorocytosine.<sup>3,17,19,21,30,35,36,78,84</sup> Recently, miltefosine has also been used successfully to cure *B. mandrillaris* GAE.<sup>7</sup>

## Acanthamoeba Keratitis

*Acanthamoeba* sp keratitis (AK) is a painful, vision-threatening disease of the cornea that, if not treated promptly, can lead to chronic ulceration of the cornea, loss of visual acuity, blindness, and enucleation.<sup>12,17,19,30,43-45,48,50-52,78</sup>

### Infectious Agent

Species reported to cause corneal infections are usually associated with group II and genotype T4 and include, among others, *Acanthamoeba hatchetti*, *A. polyphaga*, *A. castellanii*, *A. culbertsoni*, and *A. rhysodes*, although not all species isolated from the environment are clinically relevant. More than 5,000 cases of *Acanthamoeba* sp keratitis have been reported worldwide, over half of them in the United States. The first case of AK was reported in 1973 in a Texas rancher with a history of trauma to the eye.<sup>46</sup> Trophozoites and cysts of *A. polyphaga* were cultured from corneal scrapings and biopsy specimens. In the 1980s, an in-depth epidemiologic and case-control study revealed that the use of soft contact lenses and homemade saline solution were responsible for an increased incidence of *Acanthamoeba* sp keratitis.<sup>51</sup> A recent outbreak of AK during 2004-2007 occurred in the United States. An in-depth epidemiological study found that it was associated with

a particular brand of multipurpose contact lens solution<sup>52</sup> that did not have sufficient activity to kill *Acanthamoeba* sp cysts.<sup>85</sup> Subsequently, this brand of contact lens solution was withdrawn by the company.<sup>52</sup>

AK causes severe ocular pain, a partial or total paracentral stromal ring infiltrate (Fig 9.13), recurrent corneal epithelial breakdown, and a corneal lesion resistant to common ophthalmic antibacterial medications. In its early stages, AK is frequently misdiagnosed as herpetic keratitis because of the irregular epithelial lesions, stromal infiltrative keratitis, and edema common to both forms of the disease. If left untreated, there is a greater risk of corneal perforation and GAE and enucleation.<sup>17,19,30,44,45, 48</sup>

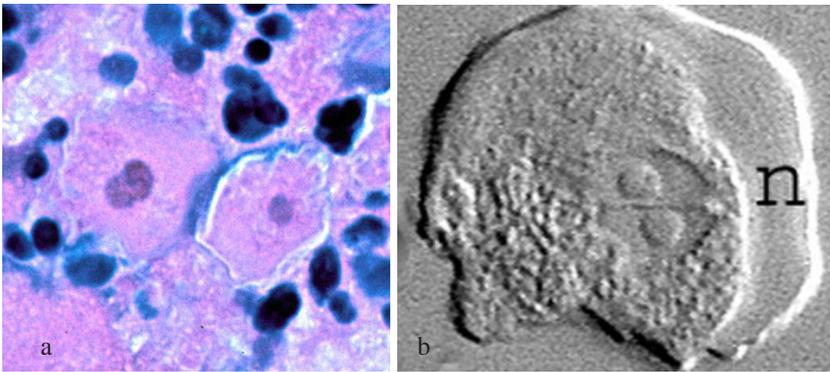
Corneal biopsy reveals that in the early stages of AK there is destruction of the anterior corneal epithelium associated with infiltration of acute inflammatory cells (mainly neutrophils) into the superficial and middle layers of the corneal stroma (Figs 9.14a & 9.14b). As the disease progresses, there is considerable erosion of the corneal stroma, herniation of Descemet's membrane, and perforation of the cornea. Trophozoites and cysts are interspersed between the corneal lamellae.<sup>17, 19,30,44,45,48</sup>

### Diagnosis

Diagnosis is confirmed by finding *Acanthamoeba* sp in corneal scrapings fixed with methanol and stained with Giemsa, Hemacolor®, or trichrome.<sup>17,19,30,44,45,48</sup> *Acanthamoeba* sp can also be isolated into culture on non-nutrient agar plates, as described above. Diagnosis has been established by direct confocal microscopy on the corneal surface.<sup>49</sup>

### Treatment

The current treatment of choice for AK is topical application of a diamide (propamide isethionate or hexamide) and a cationic antiseptic (polyhexamethyl biguanide or chlorhexidine gluconate) for several weeks.<sup>50,53</sup>



**Figure 9.15 a,b**  
*Sappinia* sp, Brain. Amebae are large, usually 40  $\mu\text{m}$  or greater characterized by two nuclei (n) tightly apposed to one another. a. Giemsa. x1250 b. Phase contrast. x1250



**Figure 9.16**  
*Sappinia* sp, Brain. Cysts are also binucleate. Two nuclei (N). Phase contrast. x1000

## Sappinia

*Sappinia* sp have been previously isolated from feces of humans, elk, bison and cattle. A single case of infection with *Sappinia pedata*, previously described as *Sappinia diploidea*, causing encephalitis in an immunocompetent male occurred in 2001.<sup>59</sup> The patient developed headache, seizure, blurred vision, photophobia and vomiting; an MRI revealed a single space-occupying lesion. On examination of the excised lesion amebic trophozoites but not cysts were seen in the brain tissue. The patient was treated empirically with azithromycin, pentamidine isethionate, itraconazole and flucytosine and recovered with no neurological sequelae. *Sappinia* amebae are large and measure 40  $\mu\text{m}$  or greater and are characterized by the presence of two nuclei tightly apposed to one another (Figs 9.15a & 9.15b). Cysts are also binucleate (Fig 9.16) and can survive adverse conditions in the environment. A recently developed real-time PCR detects both species of *Sappinia* but not other free-living amebae including *Acanthamoeba* sp, *B. mandrillaris* and *N. fowleri* or human DNA.<sup>60</sup>

## References

- Barete S, Combes A, De Jonckheere JH et al. Fatal disseminated *Acanthamoeba lenticulata* infection in a heart transplant recipient. *Emerg Infect Dis.* 2007; 13:736-738.
- Bakardjiev A, Azimi PH, Ashouri N et al. Amebic encephalitis caused by *Balamuthia mandrillaris*: Report of four cases. *Pediatr Infect Dis.* 2003;22:447-452.
- Bravo FG, Cabreara G, Gottuzo E, Visvesvara GS. Cutaneous manifestations of infections by free-living amoebas. In: *Tropical Dermatology*, Tyring SK, Lupin O, Henggae UR, eds. Philadelphia: Elsevier; 2006, pp 49-55.
- Butt CG. Primary amebic meningoencephalitis. *N Engl J Med.* 1966;274:1473-1476.
- Carter RF. Primary amoebic meningo-encephalitis. An appraisal of present knowledge. *Trans R Soc Trop Med Hyg.* 1972;66:193-213.
- Centers for Disease Control and Prevention (CDC). Primary amebic meningoencephalitis-Arizona, Florida, and Texas, 2007. *Morb Mort Wkly Rep.* 2008;57:573-577.
- Centers for Disease Control and Prevention (CDC). *Balamuthia mandrillaris* transmitted through organ transplantation – Mississippi, 2009. *Morb Mort Wkly Rep.* 2010;59:1165-1170.
- Centers for Disease Control and Prevention (CDC). Notes from the field: transplant transmitted *Balamuthia mandrillaris* – Arizona, 2010. *Morb Mort Wkly Rep.* 2010;59:1182.
- Cogo PE, Scaglia M, Gatti S, et al. Fatal *Naegleria fowleri* meningoencephalitis, Italy. *Emerg Infect Dis.* 2004;10:1835-1837.
- Gustavo dos Santos Neto. Fatal primary amebic meningoencephalitis. A retrospective study in Richmond, Virginia. *Am J Clin Pathol.* 1970;54:737-742.
- Kaul DR, Lowe L, Visvesvara GS, et al. *Acanthamoeba* infection in a patient with chronic graft-vs-host disease occurring during treatment with voriconazole. *Transplant Infect Dis.* 2008; 10:437-441.
- Khan NA. *Acanthamoeba* spp. In: *Emerging Protozoan Pathogens*. Khan NA, ed. New York, NY: Taylor and Francis; 2008, pp 71-118.
- Kinde H, Read DH, Daft BM et al. Infections caused by pathogenic free-living amoebas (*Balamuthia mandrillaris* and *Acanthamoeba* spp) in horses. *J Vet Diagn Invest.* 2007;19:317-322.
- Lozano-Alarcon F, Bradley GA, Houser BS, Visvesvara GS. Primary amebic meningoencephalitis due to *Naegleria fowleri* in a South American tapir. *Vet Pathol.* 1997;34:239-243.
- Marciano-Cabral F, Cabral G. *Acanthamoeba* spp. as agents of disease in humans. *Clin Microbiol Rev.* 2003;16:273-307.
- Marciano-Cabral F, Cabral G. *Naegleria fowleri*. In: *Emerging Protozoan Pathogens*, Khan NA, ed. New York, NY: Taylor and Francis; 2008, pp 119-152.
- Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amoebas. *Brain Pathol.* 1997;7:583-598.
- Mckeller M, Mehta L, Greenlee J et al. Granulomatous *Acanthamoeba* encephalitis mimicking a stroke: correlation with sequential MRI and biopsy, in vitro culture, immunofluorescence and molecular analysis. *J Clin Microbiol.* 2006;44:265-269.
- Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non opportunistic pathogens of humans and animals. *Int J Parasitol.* 2004;34:1001-1027.
- Schuster FL, Glaser C, Honarmand S, et al. *Balamuthia* encephalitis risk, Hispanic Americans. *Emerg Infect Dis.* 2004;10:1510-1512.
- Schuster FL, Visvesvara GS. *Balamuthia mandrillaris*. In: *Emerging Protozoan Pathogens*, Khan NA, ed. New York, NY: Taylor and Francis; 2008, pp 71-118.
- Schuster FL, Yagi S, Gavali S, et al. Under the radar: *Balamuthia* amebic encephalitis. *Clin Infect Dis.* 2009;48:879-887.
- Shirwadkar CG, Samant R, Sankhe M, et al. *Acanthamoeba* encephalitis in a patient with systemic lupus, India. *Emerg Infect Dis.* 2006;12:984-986.
- Silva-Vergara ML, Da Cunha Colombo ER, Vissoto E de F, et al. Disseminated *Balamuthia mandrillaris* amoeba infection in an AIDS patient from Brazil. *Am J Trop Med Hyg.* 2007;77:1096-1098.
- Symmers WC. Primary amoebic meningoencephalitis in Britain. *Br Med J.* 1969;4:449-454.
- Tavares M, Correa da Costa JM, Carpenter S, et al. Diagnosis of first case of *Balamuthia* amebic encephalitis in Portugal by immunofluorescence and PCR. *J Clin Microbiol.* 2006; 44:2660-2663.
- Vargas-Zepeda J, Gomez-Alcala AV, Vasquez-Morales JA, et al. Successful treatment of *Naegleria fowleri* meningoencephalitis by using intravenous amphotericin B, fluconazole and rifampicin. *Arch Med Res.* 2005;36:83-86.
- Visvesvara GS, Martinez AJ, Schuster FL, et al. *Leptomyxid amoeba*, a new agent of amebic meningoencephalitis in humans and other animals. *J Clin Microbiol.* 1990;28:2750-2756.
- Visvesvara GS, Schuster FL, Martinez AJ. *Balamuthia mandrillaris*, n. g., n. sp., agent of amebic meningoencephalitis in humans and other animals. *J Eukaryot Microbiol.* 1993;40:504-514.
- Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri* and *Sappinian diploidea*. *FEMS Immunol Microbiol.* 2007;50:1-26.
- Visvesvara GS. Free-living amoebae as opportunistic agents of human disease. *J Neuroparasitol.* 2010;1:1-13.
- Walochnik J, Obwaller A, Gruber F et al. Anti *Acanthamoeba* efficacy and toxicity of miltefosine in an organotypic skin equivalent. *Antimicrob Agents Chemother.* 2009;64:539-545.
- Yoder JS, Eddy BA, Visvesvara GS, et al. The epidemiology of primary amebic meningoencephalitis, 1962-2008. *Epidemiol Infect.* 2010;138:968-975.
- Seidel JS, Harmatz P, Visvesvara GS, Cohen A, Edwards J, Turner J. Successful treatment of primary amebic meningoencephalitis. *N Engl J Med.* 1982;306:346-348.
- Cary LC, Mauil E, Porter C, et al. *Balamuthia mandrillaris* meningoencephalitis: survival of a pediatric patient. *Pediatrics.* 2010;125:e699-e703.
- Deetz TR, Sawyer MH, Billman G, Schuster FL, Visvesvara GS. Successful treatment of *Balamuthia* amebic encephalitis: presentation of 2 cases. *Clin Infect Dis.* 2003;37:1304-1312.
- Aichelburg ACV, Walochnik J, Assadian O, et al. Successful treatment of disseminated *Acanthamoeba* sp. infection with miltefosine. *Emerg Infect Dis.* 2008;14:1743-1746.
- Fung KT-T, Dhillon AP, McLaughlin J, et al. Cure of *Acanthamoeba* cerebral abscess in a liver transplant patient. *Liver Transplantation.* 2008;14:308-312.
- Martinez MS, Gonzalez-Madiero G, Santiago P, et al. Granulomatous amebic encephalitis in a patient with AIDS. Isolation of *Acanthamoeba* sp Group II from brain and successful treatment with sulfadiazine and fluconazole. *J Clin Microbiol.* 2000;38:3892-3895.
- Petry F, Torzewski M, Bohl J, et al. Early diagnosis of *Acanthamoeba* infection during routine cytological examination of cerebrospinal fluid. *J Clin Microbiol.* 2006;44:1903-1904.
- Saxena A, Mittal S, Burman P, Garg P. *Acanthamoeba* meningitis with successful outcome. *Ind J Pediatr.* 2009;1063-1064.
- Slater CA, Sichel JZ, Visvesvara GS, et al. Brief report: successful treatment of disseminated *Acanthamoeba* infection in an immunocompromised patient. *New Engl J Med.* 1994;331:85-87.
- Bang S, Edell E, Eghrari AO, Gottsch JD. Treatment with voriconazole in 3 eyes with resistant *Acanthamoeba* keratitis. *Am J Ophthalmol.* 2010;149:66-69.
- Clarke DW, Niederkorn JY. The pathophysiology of *Acanthamoeba* keratitis. *Trends Parasitol.* 2006;22:175-180.
- Dart JKG, Saw VPJ, Kilvington S. *Acanthamoeba* keratitis: Diagnosis and Treatment Update 2009. *Am J Ophthalmol.* 2009;148:487-499.
- Jones DB, Visvesvara GS, Robinson NM. *Acanthamoeba polyphaga* keratitis and *Acanthamoeba uveitis* associated with fatal meningoencephalitis. *Trans Ophthalmol Soc U K.* 1975;95:221-232.
- Khan NA. *Acanthamoeba* spp.: In: *Emerging Protozoan Pathogens*, Khan NA, ed. New York, NY: Taylor and Francis; 2008, pp 71-118.
- Panjwani N. Pathogenesis of *Acanthamoeba* keratitis. *Occul Surf.* 2010;8:70-79.
- Pfister DR, Cameron JD, Krachmer JH, Holland EJ. Confocal microscopy findings of *Acanthamoeba* keratitis. *Am J Ophthalmol.* 1996;121:119-128.
- Seal DV. *Acanthamoeba* keratitis update – Incidence, molecular epidemiology and new drugs for treatment. *Eye.* 2003;17:893-905.
- Stehr-Green JK, Bailey TM, Visvesvara GS. The epidemiology of *Acanthamoeba* keratitis in the United States. *Am J Ophthalmol.* 1989;107:331-336.
- Verani JR, Lorick SA, Yoder JS, et al. National outbreak of *Acanthamoeba* keratitis associated with use of contact lens solution, United States. *Emerg Infect Dis.* 2009;15:1236-1242.
- Daft BM, Visvesvara GS, Read DH, Kinde H, Uzal FA, Manzer MD. Seasonal meningoencephalitis in Holstein cattle caused by *Naegleria fowleri*. *J Vet Diagn Invest.* 2005;17:605-609.

54. Finnin PJ, Visvesvara GS, Campbell BE, Fry DR, Gasser RB. Multifocal *Balamuthia mandrillaris* infection in a dog in Australia. *Parasitol Res.* 2007;100:423-426.
55. Morales JA, Chavez AJ, Visvesvara GS, Dubey JP. *Naegleria fowleri* – associated encephalitis in a cow from Costa Rica. *Vet Parasitol.* 2006;139:221-223.
56. Rideout BA, Gardiner CH, Stalis IH, et al. Fatal infections with *Balamuthia mandrillaris* (a free-living amoeba) in gorillas and other old world primates. *Vet Pathol.* 1997;34:15-22.
57. Visvesvara GS, De Jonckheere JF, Sriram R, et al. Isolation and molecular analysis of *Naegleria fowleri* from a cow brain that died of primary amebic meningoencephalitis. *J Clin Microbiol.* 2005;43:4203-4204.
58. Visvesvara GS, Shoff ME, Sriram R, et al. Isolation, morphologic, serologic and molecular identification of *Acanthamoeba* T4 genotype from the liver of a Temminck's tragopan (*Tragopan temminckii*). *Vet Parasitol.* 2010;170:197-200.
59. Gelman BB, Popov V, Chaljub G, et al. Neuropathological and ultrastructural features of amebic encephalitis caused by *Sappinia diploidea*. *J Neuroopathol Exp Neurol.* 2003;62:990-998.
60. Qvarnstrom Y, da Silva AJ, Schuster FL, Gelman BB, Visvesvara GS. Molecular confirmation of *Sappinia pedata* as causative agent of amebic encephalitis. *J Infect Dis.* 2009;199:1139-1142.
61. Culbertson CG, Smith JW, Minner JR. *Acanthamoeba*: observations on animal pathogenicity. *Science.* 1958;127:1506.
62. Stockman LJ, Wright CJ, Visvesvara GS, et al. Prevalence of *Acanthamoeba* spp. and other free-living amoebae in household water, Ohio, USA-1990-1992. *Parasitol Res.* 2010;108:621-627.
63. Bright KR, Marciano-Cabral F, Gerba CP. Occurrence of *Naegleria fowleri* in Arizona drinking water supply wells. *JAWWA.* 2009;101:43-50.
64. Dunnebacke TH, Schuster FL, Yagi S, et al. *Balamuthia mandrillaris* from soil samples. *Microbiology.* 2004;150:2837-2842.
65. Niyyati M, Lorenzo-Morales J, Rezaeian M, et al. Isolation of *Balamuthia mandrillaris* from urban dust, free of known infectious involvement. *Parasitol Res.* 2009;106:279-281.
66. Schuster FL, Dunnebacke TH, Booton CG, et al. Environmental isolation of *Balamuthia mandrillaris* associated with a case of amebic encephalitis. *J Clin Microbiol.* 2003;41:3175-3180.
67. Page FC. A New Key to Fresh Water and Soil Gymnamoebae. Cumbria, England: Fresh Water Biological Association; 1985:1-122.
68. Guarner J, Bartlett J, Shieh W-J, Paddock CD, Visvesvara GS, Zaki SR. Histopathologic spectrum and immunohistochemical diagnosis of amebic meningoencephalitis. *Modern Pathol.* 2007; 20:1230-1237.
69. Zhou L, Sriram S, Visvesvara GS, Xiao L. Genetic variation in the internal transcribed spacer and mitochondrial small subunit rRNA gene of *Naegleria* spp. *J Eukaryot Microbiol.* 2003;50:522-526.
70. Reveiller FL, Cabanes PA, Marciano-Cabral F. Development of a nested PCR assay to detect the pathogenic free-living amoeba *Naegleria fowleri*. *Parasitol Res.* 2002;88:443-450.
71. Qvarnstrom Y, Visvesvara GS, Sriram R, da Silva AJ. A multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Naegleria fowleri*. *J Clin Microbiol.* 2006;44:3589-3595.
72. Sriram R, Shoff M, Booton CG, et al. Survival of *Acanthamoeba* cysts after desiccation for more than 20 years. *J Clin Microbiol.* 2008;46:4045-4048.
73. Booton GC, Visvesvara GS, Byers TJ, Kelly DJ, Fuerst PA. Identification and distribution of *Acanthamoeba* species genotypes associated with nonkeratitis infections. *J Clin Microbiol.* 2005;43:1689-1693.
74. Booton GC, Carmichael Jr., Visvesvara GS, et al. Genotyping of *Balamuthia mandrillaris* based on nuclear 18S and mitochondrial 16S rRNA genes. *Am J Trop Med Hyg.* 2003;68:65-69.
75. Booton GC, Carmichael JR, Visvesvara GS, Byers TJ, Fuerst PA. Identification of *Balamuthia mandrillaris* by PCR assay using the mitochondrial 16S rRNA gene as a target. *J Clin Microbiol.* 2003;41:453-455.
76. Yagi S, Schuster FL, Visvesvara GS. Demonstration of *Balamuthia* and *Acanthamoeba* mitochondrial DNA in sectioned archival brain and other tissues by the polymerase chain reaction. *Parasitol Res.* 2008;102:211-217.
77. Schuster FL, Yagi S, Wilkins PP, Gavali S, Visvesvara GS, Glaser CA. *Balamuthia mandrillaris*, agent of amebic encephalitis: detection of serum antibodies and antigenic similarity of isolates by enzyme immunoassay. *J Eukaryot Microbiol.* 2008;55:313-320.
78. Visvesvara GS. Amebic meningoencephalitis and keratitis: challenges in diagnosis and treatment. *Curr Op Infect Dis.* 2010; 23:590-594.
79. Walia R, Montoya JG, Visvesvara GS, et al. A case of successful treatment of infection of *Acanthamoeba* in a lung transplant recipient. *Transplant Infect Dis.* 2007; 9:51-54.
80. Schuster FL, Visvesvara GS. Axenic growth and drug sensitivity studies of *Balamuthia mandrillaris*, an agent of amebic meningoencephalitis in humans and other animals. *J Clin Microbiol.* 1996;34:385-388.
81. Schuster FL, Visvesvara GS. Efficacy of novel antimicrobials against clinical isolates of opportunistic amebas. *J Eukaryot Microbiol.* 1998;45:612-618.
82. Schuster FL, Guglielmo J, Visvesvara GS. In vitro activity of miltefosine and voriconazole on clinical isolates of free-living amebas: *Balamuthia mandrillaris*, *Acanthamoeba* spp., and *Naegleria fowleri*. *J Eukaryot Microbiol.* 2006;53:893-905.
83. Walochnik J, Duchene M, Seifert K, et al. Cytotoxic activities of alkylphosphocholines against clinical isolates of *Acanthamoeba* spp. *Antimicrob Agents Chemother.* 2002;46:695-701.
84. Jung S, Schelper RL, Visvesvara GS, Chang HT. *Balamuthia mandrillaris* meningoencephalitis in an immunocompetent patient: an unusual clinical course and a favorable outcome. *Arch Pathol Lab Med.* 2004;128:466-468.
85. Johnston SP, Sriram S, Qvarnstrom Y, et al. Resistance of *Acanthamoeba* cysts to disinfection in multiple contact lens solutions. *J Clin Microbiol.* 2009;47:2040-2045.