Free living amoebae and human disease

Pathogenic FLA are ubiquitous protozoans and despite frequent human contact remain a rare cause of often devastating infection with poor prognosis. Given changes in climate, human encroachment into the environment, increasing immunosuppression, and improving diagnostic capacity, it is likely we will see increased cases in the future. Early diagnosis is challenging but crucial to achieving a favourable outcome. It is best facilitated by improved awareness of FLA disease, appropriate clinical suspicion and early diagnostic testing.

Free living amoebae (FLA) are a cosmopolitan group of protozoan organisms that do not require a host to survive. Despite their ubiquitous nature, these organisms are uncommon human pathogens. However, four genera contain species known to cause invasive disease in humans: *Acanthamoeba*, *Naegleria*, *Balamuthia* and *Sappinia*. *Acanthamoeba* infections may present as granulomatous amoebic encephalitis (GAE), disseminated disease (e.g. cutaneous, sinus or pulmonary infection) or keratitis, with *Balamuthia mandrillaris* causing similar cutaneous infections and GAE. *Naegleria fowleri* is responsible for the rapidly progressive primary amoebic meningoencephalitis (PAM). In addition, a single case of human central nervous system (CNS) infection with *Sappinia pedata* has been reported, along with isolated cases of corneal infection with *Vahliiella* spp., *Hartmannella* spp. and *Paravahlkampfiella* spp.

This review aims to provide an overview of human disease caused by the three most common genera involved, *Acanthamoeba*, *Naegleria* and *Balamuthia*, including their laboratory diagnosis.

**Epidemiology**

Pathogenic FLA are found worldwide and serological studies suggest human exposure is common. Three cases of *Acanthamoeba* GAE have been described in Australia. *Acanthamoeba* sp. tolerate a wide range of temperature, pH and osmolarity and may be found in air, soil and water samples. They are one of the most commonly isolated FLA in the environment, and the most common in human infection. The strongest risk factor for GAE or disseminated infection is immunodeficiency, while keratitis most commonly affects immunocompetent contact lens wearers who often have a history of poor lens hygiene. Exposure is thought to occur by inhalation, mucosal contact or direct inoculation. *Acanthamoeba* species were previously classified based on morphology, but are now grouped into 17 genotypes based on 18S rRNA sequencing, with the majority of pathogenic species belonging to the T4 genotype.

*Naegleria fowleri* may be found in rivers, lakes and soil but does not survive in sea water. As thermophiles, their presence in fresh water is related to temperature and they may even be recovered from thermally polluted waters at high latitudes. Human exposure occurs through contact with intact or disrupted nasal mucosa, commonly through recreational or nasal ablution practices and contaminated drinking water has been implicated as a source of infection in some cases. Australia has featured prominently in the history of *Naegleria fowleri* infection, with cases described in Queensland, New South Wales and Western Australia following the first description of the disease in 1965 by two South Australian pathologists, Fowler and Carter. This discovery was related to an outbreak of 20 cases, attributed to a contaminated overland water pipeline which reached optimal temperatures for *Naegleria* proliferation during the summer months. Cases continued from 1947 to 1972 when public health measures including adequate chlorination were applied. Several cases in Western Australia have also been associated with overland water pipelines, and Australian drinking water guidelines suggest a *Naegleria* monitoring and response protocol for water supplies that seasonally exceed 30°C, or 25°C continually. Today, despite significant advancement in
knowledge regarding risk reduction measures, the incidence of infection with *Naegleria fowleri* appears to be increasing worldwide, with factors such as global warming, substandard water management and sanitation, and changing recreational practices likely involved.

*Balamuthia mandrillaris* is most commonly found in soil, though it may also be recovered from water samples. Infection tends to occur in immunocompetent individuals, most commonly children, probably through inhalation or nasal or cutaneous inoculation. Transmission via organ transplantation has also been described. Six human cases have been described in Australia (Western Australia, Tasmania, Victoria and Queensland), with another being described in a Victorian dog.

**Clinical manifestations**

*Acanthamoeba* sp.

The predominant clinical manifestations of *Acanthamoeba* infection are disseminated disease (e.g. cutaneous, nasopharyngeal or pulmonary infection), GAE and keratitis. With the exception of rare case reports, GAE and disseminated disease occur in the immunocompromised or debilitated. The incubation period is unknown, but thought to be weeks to months in duration. Cutaneous infection usually begins as fibronodular lesions, which progress to non-healing ulcerated lesions over time. While GAE may be fatal within days of symptom onset, it generally assumes a more chronic course, with slow progression over weeks to months. Clinical features of GAE are myriad and include fever, symptoms of meningism, personality and mental status change and, later, focal neurological deficits, coma and death. Imaging of the brain may show single or multiple space occupying lesions which can be ring enhancing.

*Acanthamoeba* keratitis is usually a disease of immunocompetent patients, with the strongest risk factor being contact lens use and poor lens hygiene. The disease is usually unilateral, with symptoms including lacrimation, pain, photophobia and foreign body sensation. Signs include a typical corneal ring infiltrate, stromal infiltrates, epitheliopathy and hypopyon. In the absence of effective therapy, it may progress to corneal perforation and loss of vision. The clinical diagnosis of AK can be difficult, as lesions may resemble bacterial or fungal disease, or the dendritic ulcer of HSV infection. Further, more, *Acanthamoeba* trophozoites are not typically inflammatory cells, especially in stained material, usually tissue or CSF. A definitive diagnosis of AK is only possible by positive identification of trophozoites in biopsy, CSF or aqueous humour.

*Naegleria fowleri*

*Naegleria fowleri* causes primary amoebic meningoencephalitis. Symptoms generally occur 2–5 days after exposure and may begin with changes in taste or smell, followed by fever, nausea, vomiting, photophobia and headache. The disease is fulminant, with rapid progression to coma and death.

**Balamuthia mandrillaris**

*Balamuthia mandrillaris* causes GAE in immunocompromised and immunocompetent individuals. The onset of meningoencephalitis is often subacute or chronic, with symptoms developing over a period of 2 weeks to 2 years. It also has the propensity to cause cutaneous lesions that may precede CNS involvement and are similar in appearance to those of *Acanthamoeba* sp. These lesions appear as poorly defined plaques and may be single or with bordering satellite lesions. They often involve the central face and appear to be more common in South America. Cutaneous disease generally progresses to CNS involvement; however, it may resolve with therapy.

**Laboratory diagnosis**

Diagnosis of FLA infection, particularly systemic disease, is challenging: it may masquerade as bacterial or viral infection, exposure events may not be apparent and specialised diagnostic testing availability is limited. Unfortunately as a result, many CNS infections are diagnosed post-mortem. Successful early diagnosis depends on appropriate clinical suspicion and collection of suitable diagnostic material, usually tissue or CSF.

**Microscopy**

CSF samples in cases of PAM appear purulent, with no bacteria evident on gram stain, a polymorphonuclear pleocytosis, elevated protein and decreased glucose. Wet preparations may show motile *Naegleria fowleri* trophozoites (Figure 1a), as there are usually large numbers of organisms in the CSF. These findings contrast with those of GAE caused by *Acanthamoeba or Balamuthia*. While CSF from cases of GAE also demonstrates elevated protein and lowered glucose, these changes are more modest, and a mononuclear, rather than polymorphonuclear inflammatory response is seen. Furthermore, *Acanthamoeba or Balamuthia* trophozoites are not typically seen in CSF preparations.

Trophozoites from all pathogenic FLA species can be difficult to differentiate from host inflammatory cells, especially in stained tissue sections. The nuclear characteristics of amoeba can be helpful in differentiating these parasites from host cells, with *Naegleria fowleri* possessing a nucleus with a large, round, central nucleolus and *Acanthamoeba* and *Balamuthia* (Figure 1b) a rounded nucleus with large, central nucleolus forming a halo. Polyclonal and monoclonal antibodies, with a secondary detecting fluorescent anti-IgG antibody (such as FITC), may be used to identify and
differentiate each of these amoebae in tissue specimens26 (as can molecular methods), though availability is limited to the CDC, Atlanta, USA.

In *Acanthamoeba* keratitis, a diagnosis may be made by demonstrating trophozoites and/or cysts in corneal samples. It is possible to directly identify *Acanthamoeba* trophozoites within the cornea using confocal microscopy and in experienced hands this technique is sensitive and specific29. Trophozoites and cysts may be revealed by staining the smear with H&E or Giemsa, while cysts are also readily identified using PAS and fluorescent stains, such as calcofluor white and acridine orange30. On occasion, non-specific fluorescence or binding to fungi in mixed infections can lead to diagnostic errors31, especially when used by inexperienced microscopists.

**Culture**

Samples intended for amoebic culture should be kept at room temperature and processed as quickly as possible. Freezing should be avoided, particularly for samples where *Naegleria* is suspected (the cyst stage is more fragile), as this compromises organism viability. *Naegleria fowleri* and *Acanthamoeba* sp. (Figure 1c) can be readily cultured using non-nutrient media containing live or killed non-mucoid bacteria (usually *E. coli* or *Enterobacter sp*) as a food source32. *Acanthamoeba* and *Naegleria* will cover the agar surface in 1–2 days when incubated at 37°C and their presence can be confirmed by examination of the plate with a plate microscope, or by performing microscopy of a wet mount from the agar plate. *Balamuthia* do not appear to use bacteria as a food source and therefore cannot be cultivated in the same fashion32. They may be successfully cultured using axenic and tissue culture methods32, however with generation times of around 25 h, culture is a lengthy process and not part of routine diagnostic testing.

**Nucleic acid testing**

The use of molecular testing to diagnose and confirm infections with FLA has transformed diagnostics in this area. It allows more rapid diagnosis, with greater sensitivity than other methods and reduces the requirement for specialist, experienced staff to discern subtle microscopic features. Most molecular assays use ribosomal genes, such as the 18S rRNA or ITS repeat regions, as targets for PCR. However, as requests are infrequent, these tests are generally only offered by reference or research laboratories. Of particular note is a multiplex PCR, described by Qvarnstrom et al.33. This assay uses 18S rRNA primer/probe sets to accurately identify *Acanthamoeba* to the genus level and *Naegleria fowleri* and *Balamuthia mandrillaris* species. The sensitivity is reported at one amoeba per sample. Sullivan Nicolaides Pathology instituted

![Figure 1](https://example.com/figure1.png)

*Figure 1. (a) Naegleria trophozoite in CSF wet prep (x400). (b) Balamuthia trophozoite in brain tissue (haematoxylin and eosin). (c) Acanthamoeba cysts in culture, wet prep (x400).*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Eye</th>
<th>CNS</th>
<th>Skin</th>
<th>Sinus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acanthamoeba</em> spp</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td>81</td>
</tr>
<tr>
<td><em>Balamuthia mandrillaris</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>63</td>
</tr>
<tr>
<td><em>Naegleria fowleri</em></td>
<td></td>
<td>2</td>
<td></td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>Total tests</td>
<td>52</td>
<td>24</td>
<td>4</td>
<td>1</td>
<td>81</td>
</tr>
</tbody>
</table>

*Duplicates excluded (same patient and site, within 2 months). CNS, central nervous system.*
this test in 2010 and our experience is summarised in Table 1. As a significant number of ocular specimens have been tested against all three targets, a large number of negatives for Naegleria fowleri and Balamuthia mandrillaris are expected. It is also notable that we have recently reported a probable false negative Acanthamoeba result. The referred CSF specimen tested negative at our laboratory, but positive at the CDC (using the same multiplex PCR). It is possible the small volume of CSF received at our laboratory contributed to this result (only 100 μL was received, where 200 μL is the standard volume for extraction).

**Treatment and prognosis**

**Primary amoebic meningitis**

There have been few survivors of primary amoebic meningitis and the factors that have resulted in successful therapy are poorly defined, though early diagnosis and institution of therapy appears critical. The treatment of choice is the antifungal amphotericin B, which is often used both intravenously and intrathecally. Other agents used in survivors include miltefosine, sulfisoxazole, fluconazole, miconazole and rifampicin.

**Granulomatous amoebic encephalitis**

Acanthamoeba

Suboptimal efficacy of antimicrobial agents, the high morbidity of patients affected and tendency to late diagnosis contribute to a poor prognosis in Acanthamoeba GAE, with a mortality rate of >90%. In the few reported survivors of GAE or cutaneous infection, most have received combination therapy. The agents used have included trimethoprim-sulfamethoxazole, flucytosine, sulfadiazine, penicillin G, chloramphenicol, pentamidine, fluconazole and itraconazole. More recently, it appears that the inclusion of miltefosine in combination regimens may result in improved survival.

Balamuthia

Early recognition of cutaneous disease is critical to allow early therapy and prevent progression to CNS infection. Unfortunately, the overall prognosis in Balamuthia CNS infection is extremely poor. However, there are several case reports of survival in the literature, including an Australian case. These cases have received varied combination therapy regimens, with agents including pentamidine, flucytosine, fluconazole, macrolides, sulfadiazine, miltefosine, chloriridazone, amphotericin B, albendazole and trimethoprim-sulfamethoxazole. Miltefosine, in particular, has demonstrated amoebicidal activity *in vitro* and its inclusion in combination regimens may offer a survival advantage.

**Acanthamoeba keratitis**

Like disseminated disease, success in treatment is dependent on early diagnosis and institution of therapy. Fortunately, success rates in treating Acanthamoeba keratitis are more promising, with cure rates in the literature generally greater than 75–85%. Topical chlorhexidine and polyhexamethylenebiguanide (PHMB) are effective against trophozoites and cysts and form the mainstay of therapy. These are usually used in combination with diamidine, propamidine or hexamidine, though other agents including ketoconazole, itraconazole, voriconazole and topical imidazoles have been used. Surgical intervention, including enucleation, is sometimes required in severe cases.

**References**


Biographies
Dr Evan Bursle, BSc, MBBS, is a microbiology and infectious diseases registrar based at Sullivan Nicolaides Pathology. Although his microbiological tastes are broad and still being refined, he has a keen interest in parasitology.

Dr Jenny Robson, MBBS (Hons I); FRACP, FRCPA, FACTM, is an infectious disease physician and microbiologist who has worked for the past 26 years at Sullivan Nicolaides Pathology. She has a broad range of interests, which includes travel and tropical medicine.