

# Free living amoebae: a review

## Abstract

Free living amoebae can be found ubiquitously in soil and water. They belong to different genera like *Naegleria* spp., *Acanthamoeba* spp. and *Balamuthia* spp. They cause infections like encephalitis, keratitis and other infections. Some of them may be non-pathogenic, too. Many virulence factors can be responsible for infections caused by free living amoebae. Mortality may be quite high and rapid, and hence diagnosis needs to be fast, crisp and accurate. In this article the authors try to gather and present available information regarding the pathogenic free living amoebae, including emerging trends in diagnosis and treatment.

**Keywords:** pathogen, free living, diagnosis

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## Introduction

Free living amoebae are found abundantly in soil and water in tropical regions. They are commonplace in warm, dirty fresh water of swimming pools and ponds. They are also traceable in coastal water, sewage water, swimming pools and artificial lakes. Important genera include *Naegleria*, *Acanthamoeba*, *Sappinia* spp. and *Balamuthia* spp. They mainly affect the central nervous system, the skin and cornea. Infections are deadly and mainly reported from children and young adults. Hence they are of tremendous public health concern. They are known to be closely associated with endocytobiont bacteria like *Legionella* spp. and *Pseudomonas aeruginosa*. Hence if these free living amoebae are detectable in hospital water supply systems, these bacteria may well be able to produce nosocomial infections via taps and air-coolers. Free living amoebae lead to fatal meningoencephalitis that is seen mostly in children since they engage more in water-related recreational activities. Diagnosis is often challenging due to non-specific symptoms. Other than human infections, pathogenic free living amoebae can infect animals, too. Due to their tremendous morbidity and mortality, they obviously are of great public health concern. They present with non-specific features of meningitis and encephalitis and thus often confuse the clinicians.

## Materials and methods

Thorough and scientific literature search was carried out using MeSH (Medical Subject Headings) terms using datanases like PubMed and Google Scholar. Inclusion criteria: Articles mentioning free living amoebae, their epidemiology, importance and management. Exclusion criteria: Articles mentioning other amoebae.

## Epidemiology and public health importance

Free living amoebae are increasingly becoming important for causing infections like meningitis and encephalitis in some specified settings. They also cause lesions like keratitis and can harbor pathogenic bacteria like *Legionella* spp. and Non-tubercular Mycobacteria (NTM). They are really commonplace in soil and water. Though found most commonly in stagnant water, they have also been encountered in water supply systems, even in developing countries like The USA. For example, *V. vermiformis* have been isolated from the hospital water supply systems. Additionally, many free living amoebae, like *Vermamoeba* spp., can also be refractory to disinfection by Chlorine.

## *Naegleria fowleri*

This is the free living amoebic protozoan parasite that is known to produce the highest fatality. It causes PAM or primary amoebic meningoencephalitis.<sup>1</sup> Cases have mostly been seen in The USA, India, Pakistan and Australia. *Naegleria fowleri*, also commonly known as the “brain-eating amoeba,” is indeed a free-living, thermophilic protozoan parasite which belongs to the phylum Percolozoa. It is interesting to note that of more than 30 reported species of *Naegleria*, only one, namely *N. fowleri*, is incriminated in an acute and fulminating variant of meningoencephalitis seen in immunocompetent children as well as in young people. *Naegleria fowleri* causes an acute, fulminant infection of the CNS that leads to rapid necrosis of the brain tissue and is hence called primary amoebic meningoencephalitis (PAM). The global fatality rate is about 97-99%. Cases are to be found mostly in children and young adults. There is invariably a prior history of recent exposure to warm, fresh but dirty water in these cases. Cases are hence recorded mainly after swimming in the water of unclean ponds. Such cases have recently been reported in Keralam, South India after incidents of people bathing in swimming pools and ponds adjacent to temples. Although *N. fowleri* infection has been sporadically found in tropical and subtropical regions, recent reports do also hint at a resurgence of cases in India, especially in Keralam, where multiple deaths have been recorded in the last few years.

The contributing factors behind this phenomenon are raised temperatures of the environment, inadequate and improper disinfection process of domestic and recreational water, and lack of sufficient clinical suspicion or awareness which could have led to delay in diagnosis. In India, most cases have been reported in children and adolescents after exposure to freshwater at the time of swimming or bathing acts. Cases in India from 2018 till 2025 have mainly been documented in females and less commonly in males. Kolkata and Keralam have witnessed recent clustering of cases. Of note, latest cases in Keralam show a case fatality rate of about 24.5%, which may reflect better and faster diagnosis also. Age range reported was 8 months to 47 years.

## *Acanthamoeba* spp

Culbertson and his team were the first to firmly establish the pathogenic role of *Acanthamoeba* by infecting laboratory mice via intra-nasal inoculation which led to the production of purulent meningoencephalitis. *Acanthamoeba* spp. are also incriminated behind granulomatous amoebic encephalitis (GAE). In GAE, the skin

and the olfactory neuroepithelium serve as possible gateways for entry of the parasite, and sometimes, inflammation can also be observed at these primary sites.<sup>2</sup> There are 3 important species:- *A. culbertsoni*, *A. castellani* and *A. polyphaga*. Till date, 17 genotypes of *Acanthamoeba* have been recognized according to the diagnostic fragment 3 (DF3) analysis of the 18S rRNA gene. Most of these genotypes have been proven to be pathogenic in humans. The predominant genotype that is encountered in human pathogenic *Acanthamoebae* is the T4 genotype. Other genotypes have also been reported to be pathogenic, occasionally.

GAE typically runs in a subacute to chronic course. Till date, about 150 cases of GAE have been reported across the world, and the infection almost always turns out to be fatal owing to the difficulty and delay in clinching diagnosis, compounded by lack of appropriate available chemotherapy.

*Acanthamoeba* spp. are also associated with keratitis following use of contact lenses that are washed in home-made saline.<sup>3</sup> A disseminated form of disease involving organs like lungs, skin, kidneys, and uterus can also be found.

Cases of chronic granulomatous skin infection have also been reportedly caused by *Acanthamoeba* spp. *Acanthamoeba* infection of skin is a rare complication found in immunocompromised hosts like those having acquired immunodeficiency syndrome (AIDS). They may often have concomitant central nervous system involvement, too. *Acanthamoeba* spp. typically affects the skin and central nervous system by disseminating from a primary focus or lesion which is usually detectable in the lungs or sinuses.<sup>4</sup>

### Balamuthia mandrillaris

*Balamuthia mandrillaris* was initially discovered in 1986 by Visvesvara et al., in a necropsy specimen obtained from brain tissue of a mandrill baboon (*Papio sphinx*) who was pregnant. It died of a neurological condition at the San Diego Zoo Wild Animal Park in California, the USA. It is a free living amoebic protozoan parasite with 2 nuclei that closely resembles *Acanthamoeba* spp. *Balamuthia* infections are not common but have been reported in both immunocompetent and immunocompromised persons across all ages. *B. mandrillaris* can enter via wounds present on the skin or the nose and cause skin lesions and the infection is generally fatal. It is termed *Balamuthia* amoebic encephalitis (BAE).<sup>5</sup> GAE due to *Balamuthia* spp. is seen in either very young or very old individuals. Infections are also noted significantly more in the elderly, the immunocompromised and IV drug abusers. *Balamuthia* spp. can also cause a form of disseminated disease which commonly affects the lungs, kidneys, skin and uterus. A case of BAE have recently been documented from Keralam, South India in an immunocompetent host. In 2015 two cases has also been recorded, again from Chandigarh, in North India.<sup>6</sup>

### Sappinia diploidea

The genus *Sappinia* with the solo species *Sappinia pedata* was proposed for an amoeba with 2 nuclei and pedicellate “cysts”. The proposal was made by Dangeard way back in the year 1896. In 1912, Alexeieff added one more double nucleated, but seemingly sexually reproducing amoeba to this genus and named it *Sappinia diploidea*, that had been described as *Amoeba diploidea* by Hartmann and Nägler in 1908.<sup>7</sup> *Sappinia* is a free-living amoeba which can be isolated from objects like soil, dead plant matter, water supplies, freshwater ponds, surface water and even from the faecal matter of a number of animals like cows, bats, reptiles, King penguins, and even man. It is interesting to note that *Sappinia* has been the reported etiological

agent in 1 case of non-granulomatous amoebic encephalitis, occurring in a male subject with healthy immune system. Since only one case has been reported so far, there is not much available information regarding the pathogenesis of *Sappinia*.<sup>8</sup>

### Vermamoeba vermiformis

This free living parasite was initially termed *Hartmannella vermiformis* by Page in 1967. Later, Smirnov and Page renamed it as *Vermamoeba vermiformis*. Earlier known as *Hartmannella vermiformis*, *Vermamoeba vermiformis* has now been placed in a group under the order Echinamoebida. This is because it differs from all other *Hartmannella* spp. in being worm-shaped and not clavate. This parasite is elongated, shaped a bit like adult nematodes and is indeed closely associated with endocytobiont bacteria like *Legionella* spp. *Pseudomonas aeruginosa* and Non-tubercular mycobacteria (NTM) are also associated with *Vermamoeba* spp. Found freely in natural or man-made aquatic environments, *V. vermiformis* can promote the multiplication of other microorganisms also, and is able to harbor and potentially protect disease-producing bacteria or viruses.<sup>9</sup> As per concern of pathogenicity, it has been reported from painful ulcers near eye.<sup>10</sup> Cases of keratitis due to *Vermamoeba* spp. have also been reported. A couple of years ago, a case of encephalitis caused by *Vermamoeba* spp. has been documented in a 13-year old girl from the state of Keralam in South India in 2024, with somewhat slow course of disease progression.<sup>11</sup>

### Vannella spp

The family Vannellidae has several genera, namely *Vannella*, *Platamoeba* and *Pessonella* spp. *Vannella* spp. is another genus of free living amoebae. It has not yet proven to be pathogenic by itself, but are capable of sheltering pathogenic intracellular microorganisms like Microsporidia. The genus *Vannella* was first proposed by Bovee in the year 1965 and occurs predominantly in water (like marine and fresh water). This genus comprises about 40 described species which have been traced to marine and fresh water sources.

### Other pathogenic free living amoebae

*Hartmannella* spp. is another free-living amoeba which has been found to be non-pathogenic in man. *Paravahlkampfia francinae*, which is a recently described species placed within the genus *Paravahlkampfia*, has been discovered from CSF specimen of a patient having PAM-like symptoms.<sup>12</sup> *Allovaahlkampfia spelae*, which is another recently discovered amoeba, was documented as a cause of long-standing human keratitis in an Egyptian patient in the year 2016.<sup>13</sup>

### Bacteria and viruses that act as endocytobionts within the free living amoebae

Several bacteria and parasites can use FLA as vehicles. Bacteria like *Legionella* spp., *Serratia* spp. and *Stenotrophomonas maltophilia* are carried by free living amoeba. Among these, the effect of free living amoebae (FLA) on *Legionella* spp. is quite well-documented. Co-cultivation with FLA causes regeneration or reactivation of *Legionella* spp. from the so-called ‘viable-but-non culturable’ (or VBNC) state which occurs due to starvation or biocide application. Beside this resuscitating effect of FLA on *Legionella*, such interaction also helps the bacterium withstand the deleterious effects of high levels of biocides.<sup>14</sup> The facultative intracellular pathogen *Listeria monocytogenes* is a foodborne opportunistic bacterium. It can switch promptly from an environmental saprophyte to a potentially fatal human pathogen. *L. monocytogenes* is not digested when present

inside *Acanthamoeba* and bacteria are released from vegetative trophozoites. On the contrary, the bacteria may be killed inside cysts. The non-tubercular mycobacterium *Mycobacterium avium* is able to enter and replicate within *A. castellanii*. Such amoeba-grown mycobacteria reportedly have an enhanced virulence in murine models.<sup>15</sup> *Vannella* spp. also carries microsporidia.

Among the viruses, large viruses, also called Mimiviruses or Bradford coccus, can be found within *Acanthamoeba polyphaga*. Other viruses like the human pathogenic Coxsackievirus B3 (or CVB-3) can also live inside *Acanthamoeba* trophozoites with no particular alteration in the infectivity of the virion. Among the fungi, the true yeast *Cryptococcus* spp. can be found within *Acanthamoeba castellanii*. *Vermamoeba vermiformis* can function as a perfect “Trojan Horse” for pathogenic bacteria like *Bacillus anthracis*, *Legionella* spp., *Paeruginosa*, *Neochlamydia hartmannellae*, *Waddlia* and other *Chlamydia*-like endosymbionts. *V. vermiformis* sometimes can also shelter *Stenotrophomonas maltophilia*, an opportunistic Gram negative rod found commonly in the hospital water supply systems. All these endocytobionts can thus enter the human body via these free living amoeba and hence affect multiple organs like lungs and gut.

### Clinical features

Clinical features of encephalitis caused by free living amoebae are generally atypical. Symptoms of PAM resemble bacterial meningoencephalitis with sudden onset of a cluster of symptoms like fever, headache, altered mentation, seizures and coma. PAM is almost always life-threatening. Only 3 people in the USA out of 138 affected, have been able to survive PAM. GAE follows a more sub-acute course with predominant symptoms like headache, low grade pyrexia, focal neurological deficits and behavioural changes, which typically evolve over a period of weeks and even months.

### Pathogenesis

As *N. fowleri* reaches the olfactory bulbs after the host has swam in dirty warm water, it evokes a brisk immune response via activating the innate immune system, including macrophages and neutrophils. *N. fowleri* enters human body in the trophozoite form. Structures present on the surface of trophozoites, termed as food cups enable the microorganism to ingest bacteria, fungi, and human tissue. In addition to tissue destruction by the food cup, the pathogenicity of *N. fowleri* is also dependent upon the release of cytolytic molecules, in the form of acid hydrolases, phospholipases, neuraminidases, and phospholipolytic enzymes. These factors play a role in host cell and nerve cell damage.

The pathogenesis of *Acanthamoeba* spp. is also dependent on adhesins and numerous other factors. Adherence is a crucial first step in the pathogenesis of *Acanthamoeba* infections; it is mediated in an adhesin-receptor manner. *Acanthamoeba* uses adhesion to host cells as the first step before moving to the deeper tissue to produce keratitis and also to avoid being washed out during the onset of disease. Its ability to survive outside the mammalian host for variable lengths of time and under diverse environmental conditions, like high osmolarity and varying temperatures on the surface of human cornea, also helps in its virulence.

*Acanthamoebae* express a major virulence protein, termed as the mannose-binding protein (MBP). This mediates the adhesion of amoebae to the surface of human cornea. The MBP is a transmembrane protein having characteristics of a typical cell surface receptor. After MBP-mediated adhesion to the host cells, the amoebae produce a contact-dependent metalloproteinase enzyme and

several contact-independent serine proteinase enzymes. Human tear contains many IgA-independent factors which are able to protect against *Acanthamoeba*-induced CPE by inhibiting the action of cytotoxic proteinase enzymes.

*B. mandrillaris* stimulates the activation of host intracellular signaling pathways, thus culminating in a cascade of inflammatory reactions. The cumulative effects of all these events ultimately end up in increasing blood-brain barrier permeability and facilitating infections.

### Laboratory diagnosis

Specimens for diagnosis of free living amoebae generally include CSF, corneal scraping or brain tissue. *Naegleria fowleri* and *Acanthamoeba* spp. can be diagnosed by microscopy, molecular techniques like PCR and also culture on Non-nutrient agar plate with *E. coli* overlay. Trophozoites of *Naegleria fowleri* are rapidly moving. Being a free-living amoeba, *N. fowleri* can also switch phenotype according to environmental conditions. Under favourable circumstances, it shows a reproductively-active trophozoite stage. The trophozoite stage is regarded as the infective stage. Under non-nutrient conditions but in presence of water, trophozoites do switch to a transient flagellate stage, allowing long distance movement. This happens often for obtaining nourishment.

*Acanthamoeba* spp. can be cultivated at 30 °C on Non-nutrient agar plate with *E. coli* or *Enterobacter aerogenes* overlay. Another medium which supports good growth of *Acanthamoeba* spp. consists of peptone, yeast extract and glucose. However, the Roswell park memorial institute 1640 (RPMI 1640) medium is also an excellent commercially available medium for growing this parasite. In non-nutrient agar with bacterial overlay plates, amoebae devour the bacteria, multiply, and then differentiate into cysts once all the bacteria have been consumed. A unique and typical feature of *Acanthamoeba* is the presence of fine, tapering, thorn-like pseudopodia, called acanthopodia, that arise from its surface. The trophozoites usually range in size from 15 to 45 µm and have a single nucleus with one centrally placed, large, densely staining nucleolus.

Morphologically *Balamuthia mandrillaris* has 2 stages in its life cycle, namely a vegetative trophozoite stage and a dormant cyst stage. The trophozoites show broad pseudopodia and have filamentous structures. *Balamuthia mandrillaris*, unlike *Acanthamoeba* and *N. fowleri*, grows poorly on nonnutrient agar plates with bacteria but can be grown well axenically in a complete chemical medium as well as on mammalian tissue culture. It can be grown easily in mammalian cell culture systems like monkey kidney (E6) or human lung fibroblasts. The trophozoites of *V. vermiformis* show typical worm-shaped or slug-like (elongated, cylindrical) morphology originally described by Page and Smirnov et al.

Even *Vannella* spp. can be identified by this technique (in water, trophozoites may switch to a transient flagellate stage, allowing long-distance movement). Here the incubation temperature is about 30 °C. *Acanthamoeba* devours *E. coli*, grows in culture and then transforms into cysts. Emerging techniques for culturing *Acanthamoeba* spp. include monitoring by respiration analysis from culture, with oxygen-sensitive microplates (OSM).

*Vermamoeba* trophozoites appear like slugs while *V. vermiformis* cysts are of spherical, round shape with a diameter of 6-9µm. The cyst consists of a 50 nm thick endocyst and a 110-140nm thick ectocyst. Trophozoites of *Acanthamoeba* spp. are spiny while cysts seem to be more rounded. The trophozoite form of *Vannella* spp. is fan-shaped

and few are also capable of forming cysts. Both trophozoites and cysts of *Sappinia* spp. are binucleate, with 2 tightly apposed nuclei. Both *S. diploidea* and *S. pedata* can be grown on non-nutrient agar plate which are coated with bacteria.

Among newer assays, metagenomic next-generation sequencing (mNGS) of cerebrospinal fluid can be employed successfully to diagnose infections caused by free living amoebae. PCR has also been successfully used to diagnose BAE.

### Treatment

Keratitis due to free living amoebae is potentially vision-threatening. An approach called theranostics which combines both diagnosis and therapy might be utilized to eradicate and diagnose keratitis cases caused by free living amoebae.

Guanidine hydrochloride can be used with Ribavirin for PAM caused by *Naegleria fowleri*. Experience has demonstrated good results with biguanide (Chlorhexidine 0.02%) along with oral itraconazole or ketoconazole.<sup>16</sup> Topical chlorhexidine is particularly useful for treating keratitis caused by *Vermamoeba* spp. Recently, a novel class of compounds called isobenzofuranones have been documented to kill trophozoites of *N. fowleri* by way of programmed cell death.<sup>17</sup>

### Discussion

Free living amoebae are hence quite deadly. They can be found in drinking water supply systems, that may further compound the problem in hospitals.<sup>18</sup> Hospital environments have been shown to contain *Vermamoeba* isolates tolerating up to 46.5 °C–55.7 °C. FLA are also seen in biofilm sources in the hospital environments. Other than *Legionella* that is associated with free living amoebae in pond water, bacteria that are consistently associated with free living amoebae in drinking water systems are *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Delftia acidovorans*, *Sphingomonas paucimobili* and *Comamonas testosterone*.<sup>19</sup> Many fungi like *Cryptococcus* spp. can also reside inside free living amoeba, like *Acanthamoeba castellanii*. Till 2019, over 600 cases of amoebic encephalitis caused by pathogenic free-living amoebae belonging to the genera *Balamuthia mandrillaris*, *Acanthamoeba* spp., and *Naegleria fowleri* have been reported worldwide.<sup>20</sup> Besides causing a plethora of infections, free living amoeba can serve as excellent vehicles for phylogenetically diverse microorganisms, collectively called endocytobionts.<sup>21</sup> Regarding public health importance, free living amoebae are incriminated in meningitis and encephalitis with considerable mortality and abound in warm, untreated fresh water and also hospital water supplies. They are also quite resilient and can survive harsh conditions easily.

*Vermamoeba* spp., for example, demonstrates appreciable survival in high osmotic pressure, cold temperatures and high altitudes like snow from mountains, and also water samples recovered from hot springs.<sup>22</sup> The clustering of recent cases in India, particularly in Keralam, definitely warrants scientific attention and research. These regions have warm ambient conditions like tropical climate, monsoon-enhanced stagnant freshwater, and increased recreational water usage.<sup>23</sup> So one should always be vigilant about the risk posed by these free living amoebae while engaging in playful recreational activities in water bodies in the tropics. Recent studies indicate the presence of free living amoebae in swimming pools and recreational water in about 44.3% cases.<sup>24</sup> Free living amoebae also abound in hospital water systems in 48-52% cases as evident in a study from South Africa.<sup>25</sup> *Acanthamoeba* spp. have also been found in water systems in critical units in India.<sup>26</sup> Even in store water in rural households, free

living amoeba like *Vermamoeba* spp. and *Acanthamoeba* genotype T4 have been found to occur and produce biofilms.<sup>27</sup>

Hence, free living amoebae should be accorded their due importance, specially with respect to nosocomial infections and public health at large. They are relevant in our everyday life and recreational activities. It is also highly relevant with respect to one health and travel medicine. There are still some research gaps in the field of free living amoeba, like the mode of survival of the endocytobionts in the free living amoeba, and way of survival of FLA in the hospital water supply systems. These interesting aspects of free living amoebae demand special scientific attention and indeed also warrant a closer look into their epidemiology and clinical aspects. More research is awaited in the field of free living amoeba, particularly in their mechanisms of biofilm formation, Chlorine resistance and modalities for successful treatment.

### Conclusion

Free living amoebae can be found profusely in stagnant dirty water and are deadly. They are very important causes of encephalitis, meningitis, keratitis and skin infections in man. They can coexist in the environment with many pathogenic bacteria, viruses and parasites. Thus they are significant from public health and one health viewpoint. They can also percolate into the hospital water systems. Heightened awareness about these pathogens needs to be generated amongst the general public, and attention should also be given to their timely management (Figure 1).

### Picture gallery:



Figure 1 *Sappinia pedata* (image drawn by author)

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## Conflicts of interest

The author declare that there are not conflicts of interest.

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