

Naegleria fowleri: 'Brain-eating' amoeba: a review

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Naegleria fowleri is a thermophilic, free-living amoebic organism that causes primary amoebic meningoencephalitis (PAM), a rare but usually fatal infection of the brain. The organism is widely distributed in warm water and soil environments (1), and humans become infected when water containing amoebae enters the nose. The organisms attach to the nasal mucosa, cross the cribriform plate into the olfactory bulbs and then into the brain tissue (2). The infection is acute and rapidly fatal, with diagnosis often made at autopsy (3). Early symptoms are indistinguishable from bacterial meningitis. Crucial to patient survival is an early diagnosis and establishment of an appropriate anti-amoebic drug regimen combined with physical management including management of intracranial pressure.

Globally there have been about 380 cases of PAM identified since the disease was first described in 1965, with a case-fatality rate of about 95% (3). In New Zealand there have been nine reported cases since 1968, the most recent in 2000, all were from the Waikato area, and all were fatal. Since the disease is rare very few, if any, working scientists in New Zealand will have encountered a real case. This report provides a general review of the disease, with the aim of reminding scientists and technicians to be vigilant for the amoebae in purulent CSF samples.

There are over 40 species of the genus *Naegleria*, with *N. fowleri* the only known human pathogen. The genus is named after Kurt Nagler, a German biologist. The species *fowleri* was named after Australian pathologist Malcolm Fowler, who first described the disease in 1965, although he tentatively attributed it to *Acanthamoeba* species (4). *Naegleria* are ubiquitous in the environment, inhabiting soil and bodies of warm freshwater throughout the world both in the tropics and temperate regions. It is found in naturally warm freshwater bodies including lakes, rivers, and reservoirs, as well as geothermally heated and industrially heated water sources (5).

Naegleria are unicellular, eukaryotic organisms which exist in amoeboid, flagellate and cyst forms. The amoeboid form (the 'trophozoite') is the feeding and dividing stage whereas the flagellate stage is 'fleeting' (non-dividing and non-feeding) and thought to be involved in migration when food becomes scarce. The flagellate form is able to quickly lose its flagella and revert to the trophozoite form. It is the trophozoite form which is infective to humans. The dormant cyst is formed when environmental conditions become adverse (6). Amoeboid trophozoites measure 10-25 µm, whereas flagellated forms and cysts are both smaller - up to 16 µm. As a comparison for microscopists, neutrophils measure approximately 10-12 µm.

Naegleria trophozoites feed on bacteria in their environment, or host cells when it has invaded host tissue.

The organism is thermophilic, with trophozoites beginning to proliferate at 30°C and thriving especially well at 35-45°C. For perspective, the sulphur pool at a local commercial facility is routinely 40-42°C, and a recommended safe bath temperature for children and babies is 37-38°C. Both trophozoites and cysts can survive for minutes to hours at 50-65°C, with the cyst being more resistant. The cyst is susceptible to freezing but can survive weeks to months at cold temperatures above freezing, thereby overwintering and able to recommence proliferation during the warmer months (7).

Recreational freshwater usage is responsible for most of the documented cases of PAM. Swimming and diving, bathing, and water sports, including water skiing, wake boarding and jet skiing, are the most common. Warm lakes, ponds and reservoirs are the most common source of infection, as well as underchlorinated swimming pools, tap water, rivers/streams and geothermal water (3).

Epidemiologically, in the USA, most cases occur during the warm summer months in previously healthy individuals (predominantly young males, median age 14 years (3)). In Pakistan, the picture differs with a higher incidence in young men practising ritual nasal ablution as part of the cleansing process before Muslim prayer (8) Nasal irrigation, whether for religious (Islamic, Ayurvedic and Yogic traditions) or health purposes, contributes also to a small number of cases in the USA (3).

Infection is thought to occur when water containing *N. fowleri* amoebae is splashed or forced into the nasal cavity. The infectious dose is unknown. The trophozoites attach to the mucosa and reach the brain via the olfactory nerve, which penetrates through the skull via the 'bony sieve' of the cribriform plate. Proliferation of *Naegleria* ensues, the organism utilising the host tissue as a food source. The release of cytolytic molecules and enzymes contribute to host cell and nerve destruction (9). The site of infection in the brain is most often the frontal lobe, followed by the parietal lobe (10). The resulting immune response is intense and together with organism pathogenicity results in cerebral swelling, tissue destruction, herniation and in most cases death (11).

Malcolm Fowler describes the post mortem results in his preliminary report of amoebic meningitis in 1965 thus: "*The brain was swollen only moderately, having flattened surface convolutions and partially obliterated sulci ... meningeal veins were collapsed, superficial capillaries over the vertex engorged ... a thin, creamy exudate was obvious ... the olfactory bulbs were very reddened, soft and adherent by a mass of sticky exudate to the adjacent frontal cortex... (which was) invaded to considerable depth by amoebae ...*" (4).

Symptoms begin 1-7 days post exposure (average 5 days) and in the early stages of the disease are non-specific and flu-like, most commonly severe headache, photophobia, generalised malaise, fatigue, fever and chills and neck stiffness. As the disease progresses, neurological symptoms, such as confusion, seizures and coma ensue, and patients usually succumb within about 5-10 days (12).

Unless risk factors indicating water activity or hot pool exposure are provided, treatment generally commences for bacterial meningitis (which is ineffective against *N. fowleri*) until lack of positive response initiates further investigation.

KEY CSF FEATURES

- Increased white blood cell count 300 to >1000/µl with a predominance of polymorphs.
- Increase protein and decreased glucose.
- Discoloured CSF, white, grey or bloodstained.
- No organisms (bacteria) seen in gram stain.

Scientists and technicians can make the biggest difference to patient outcome by being alert for motile organisms while performing the cell count. Amoebae move by utilising pseudopodia, a process in which the cytoplasm is extruded through extensions of the cell membrane, resulting in a purposeful forward propulsion. This is distinct from the general random vibration of cells by 'Brownian motion', which the experienced microscopist will be familiar with, or the simple tumbling or floating of a cell in an unsettled suspension.

Specimens for investigation of PAM should be kept at room temperature or around 25°C to facilitate the organism recovery and stimulate motility. Motility may be enhanced by performing a wet preparation on a clean slide in addition to the counting chamber (13). The amoebae measure 10-25 µm and possesses a single nucleus with a large central karyosome. In their report on amoebic meningitis in 1965, Fowler and Carter recognised the causative amoeba as morphologically distinct from *E. histolytica*, at the time the almost exclusive amoeba found to invade the central nervous system of humans (4). The amoebae do not stain well with Gram stain and are easy to overlook or misidentify as white cells. A Giemsa-Wright stain, or trichrome stain of CSF or brain tissue will show the organism morphology more clearly (13). Slides should be fixed with methanol, as heat will cause damage to the structural integrity of the organisms.

If a specific camera function is not available on the microscope, laboratory workers should be capable of capturing microscopic images on a smart phone. The resulting photos and/or moving images are very useful for sharing with remote, specialised personnel if the specimen is received out of hours, or if the microscopist requires a second opinion.

A flagellation test may be used to further confirm identification of *Naegleria*, a procedure in which the organism is induced to convert to its flagellated stage by immersion in sterile water, however flagellation may be variable and false negative results are likely to occur (14).

It is possible to culture the amoebae from patient samples on a nutrient-free agar plate seeded with a lawn of *E. coli* bacteria. Plates should be incubated at 37°C for up to ten days and any amoebic growth further identified by stain, induced flagellation or molecular methods (15).



Figure 1. Four amoeboid *N. fowleri* trophozoites showing typical pseudopodia extrusion. These organisms were grown on a lawn of *E. coli* (visible in the background). Note the large karyosome of the nucleus, particularly visible in the top organism.

Though the presence of amoebae in the wet prep or by stain should be notified without delay to the clinical team, this 'presumptive' diagnosis ultimately requires confirmation by immunochemistry, immunofluorescence, PCR or Next-Generation Sequencing (13). It is important to note that other species of amoebae (eg *Acanthamoeba* spp, *Balamuthia mandrillaris*, *Sappinia* spp and *E. histolytica*) can also cause CNS infections, although their clinical presentations differ. PCR is not available routinely in any New Zealand medical laboratories. The lag time to obtain specific primers or to send the sample offshore for testing means results will almost certainly not be available within a clinically useful timeframe, and therefore suitable only for confirmation.

Treatment

Four cases of patient recovery from PAM have been documented in the USA: the first in 1978, two in 2013 and the most recent in 2016. The 12-year-old girl who survived in 2013 was diagnosed with PAM approximately 30 hours after onset of symptoms. The course of her 26-day antimicrobial treatment included amphotericin B (both intravenous and intrathecal), fluconazole, rifampicin, azithromycin and miltefosine. Dexamethasone was added to her regimen to relieve brain swelling. Her intracranial pressure was also managed with CSF drainage, hyperosmolar therapy, moderate hyperventilation and induced hypothermia (32°C-34°C). The patient was hospitalised for a total of 55 days and is considered to have recovered fully (16).

The 16-year-old boy who survived in 2016 was diagnosed within hours of hospitalisation, received a similar treatment as described above, and recovered fully. The second survivor in 2013, an 8-year-old boy, survived but with permanent brain damage. His treatment was similar to that described above, but without induced hypothermia, and his treatment was delayed by a late diagnosis, after several days of symptoms (17).

World Statistics

The number of cases of PAM that have occurred globally since the disease was first recognised varies depending on the criteria for 'presumptive' and 'definitive' diagnoses. From 1965 to 2018 Gharpure *et al.* (31) performed a literature search and identified 381 cases of PAM caused by *N. fowleri* worldwide: 182 were confirmed by molecular methods, 89 were probable and 110 were suspect (probable and suspect classification if *N. fowleri* was cultured or visualised in the brain tissue but not confirmed by molecular methods). The countries recording the highest incidence were: 156 from the USA, 41 from Pakistan, 26 from India and 22 from Australia. 9 of the cases were from New Zealand. The authors acknowledged that this number is almost certainly an underestimation due to many factors, including the limitations of relying on historical written reports.

In the USA, the annual number of PAM cases remains stable at 0-8 per annum (18) although a retrospective study of unspecified neuroinfectious deaths from 1999-2010 in the USA revealed several more cases which fit the criteria for possible PAM in addition to the laboratory-confirmed cases (10). This study by Matanock *et al.* estimated an additional 16 cases per year in the USA may have been due to PAM. PAM is not a nationally notifiable disease in the USA but the CDC maintains a registry and encourages suspected cases be sent to their Free-Living and Intestinal Amoebas Laboratory for surveillance purposes. This list of national notifiable diseases is revised periodically [per CDC website].

New Zealand

There have been nine cases of PAM reported in New Zealand to date.

Reported cases of PAM in New Zealand

1968: four cases at Waihou River 'Crystal Springs'.
1971: 12yo boy 'Crystal Springs'.
1972: 21yo male 'Golden Springs'.
1976: 15yo male Otumuheke stream by Waikato River above Huka Falls.
1978: adult male 'Crystal Springs'.
2000: 10yo girl Okauia Springs (near Crystal Springs).

The most recent patient (in April 2000) was initially treated with ceftriaxone and vancomycin for suspected pneumococcal meningitis. Motile amoebae were seen in small numbers in subsequent CSF samples taken after the patient's condition deteriorated, and she was switched to IV and intrathecal amphotericin B, and IV rifampicin and cotrimoxazole. Despite this the child died three days after admission (15).

PAM is a notifiable disease in New Zealand. Medical practitioners must notify the local Medical Officer of Health of suspected cases (notification should not await confirmation). [Per NZ Ministry of Health website].

Pool Safety

The NZ Ministry of Health requires all pools to comply with the NZ Quality water standard NZS 5826:2000. This standard covers the operation and maintenance of all fresh and sea water swimming pools, spa pools and geothermal pools. Chlorination (swimming pools containing residual-free chlorine of 1–2 ppm are considered safe) or salination, filtration, exclusion of soil, and high water turnover ensure public pools are at very low risk of harbouring *N. fowleri*. (MOH website). However, the maintenance of the water quality of private swimming pools, which can now easily be heated up to 40°C with the aid of a heat pump, remains the responsibility of the homeowner. Swimmers also remain vulnerable to infection from non-commercial, naturally occurring hot springs, of which New Zealand has an abundance.

Summary

PAM is a very rare disease. Worldwide, large numbers of people are exposed to *N. fowleri* amoebae while participating in recreational water activities, while only a tiny percentage will become infected. Antibody studies support this widespread exposure (20,21).

All New Zealand cases have occurred in the Waikato area of the North Island, in geothermally heated bodies of water, both commercial and natural. Though strict standards are set for commercial pools to lower the risk of *Naegleria* for bathers, there are no such safety measures for the many naturally occurring geothermally heated bodies of water which are found throughout New Zealand, both in the North and South Islands, nor are private swimming pools checked for acceptable sanitation levels.

Global trends have shown that there are risks of amoebic proliferation in any body of fresh water that seasonally exceeds 30°C or continually exceeds 25°C (22,23). Furthermore, it is postulated that global warming will change the environmental distribution of *Naegleria* and cause greater proliferation of the organism potentially leading to more infections (24,25). In the USA, there have already been cases of PAM contracted in the more northern states of Minnesota (2010, 2012), Kansas (2014) and Indiana (2012), where prior to this time the cases had been contracted exclusively from Southern states (13). In New Zealand terms, this could translate as cases of PAM appearing further south than the Waikato, even into the South Island.

Although large bodies of water for recreation are the most common site of exposure, domestic supplies of water should not be overlooked as a source of the amoebae. In the USA in July 2013 a 4 year-old died of PAM after playing on a backyard slip-and-slide fed by home tap water. This was in the same

metropolitan area as a fatal case in 2011 caused by use of non-sterilised tap water in a neti pot for sinus irrigation (26).

New Zealand's last two cases were in 1978 and 2000. It has been over twenty years since the last case. It is imperative that scientists and technicians are alert for the next case in order to give the patient the only chance of a positive outcome.

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