PARASITIC INFECTIONS OF THE NERVOUS SYSTEM, WITH AN UPDATE ON FREE-LIVING AMOEBCIC INFECTIONS

NR de Silva, MK de S Wijesundera

1 Professor of Parasitology, Faculty of Medicine, University of Kelaniya, 2 Emeritus Professor of Parasitology, Faculty of Medicine, University of Peradeniya.

Summary
Although the central nervous system (CNS) of humans is not a frequent site of primary infection by parasites, many different protozoa and helminths can affect the brain and spinal cord. This article first briefly reviews these different parasites and their principal clinical manifestations when the CNS is involved. The second part of this article deals at greater length with the amoebae that are known to cause primary infections of the central nervous system, and the disease entities associated with these infections.

Parasitic infections of the CNS: causative agents and clinical presentations
The protozoan infections that affect the nervous system include those caused by amoebae, flagellates and coccidia (Table 1), while a range of nematodes, cestodes and trematodes are also known to cause neurological disorders (Table 2).

Table 1. Protozoan parasites that cause diseases of the nervous system

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Clinical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amoebae</strong></td>
<td></td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>Amoebic brain abscess</td>
</tr>
<tr>
<td>Naegleria fowleri</td>
<td>Primary amoebic meningo-encephalitis</td>
</tr>
<tr>
<td>Acanthamoeba spp</td>
<td>Granulomatous amoebic encephalitis, keratitis</td>
</tr>
<tr>
<td>Balamuthia mandrillaris</td>
<td>Granulomatous amoebic encephalitis</td>
</tr>
<tr>
<td><strong>Flagellates</strong></td>
<td></td>
</tr>
<tr>
<td>Trypanosoma brucei</td>
<td>Encephalitis (in Human African Trypanosomiasis or sleeping sickness)</td>
</tr>
<tr>
<td>rhodesiensis and T. b.</td>
<td></td>
</tr>
<tr>
<td>gambiense</td>
<td></td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td>Encephalitis</td>
</tr>
<tr>
<td><strong>Coccidia</strong></td>
<td></td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>Encephalitis</td>
</tr>
<tr>
<td>Plasmodium falciparum</td>
<td>Cerebral malaria</td>
</tr>
</tbody>
</table>

Table 2. Helminth parasites that cause diseases of the nervous system

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Clinical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nematodes</strong></td>
<td></td>
</tr>
<tr>
<td>Parastrongylus (Angiostrongylus)</td>
<td>Eosinophilic meningo-encephalitis</td>
</tr>
<tr>
<td>cantonensis</td>
<td></td>
</tr>
<tr>
<td>Strongyloides stercolaris</td>
<td>Meningitis in disseminated strongyloidiasis</td>
</tr>
<tr>
<td>Toxocara spp</td>
<td>Visceral larva migrans</td>
</tr>
<tr>
<td>Gnathostoma spinigerum</td>
<td></td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>Eosinophilic meningo-encephalitis</td>
</tr>
<tr>
<td>Onchocerca volvulus</td>
<td>Epilepsy or encephalitis</td>
</tr>
<tr>
<td>Loa loa</td>
<td>Encephalopathy</td>
</tr>
<tr>
<td><strong>Cestodes</strong></td>
<td></td>
</tr>
<tr>
<td><em>Taenia solium</em></td>
<td>Neurocysticercosis gives rise to epilepsy, hydrocephalus or organic dementia</td>
</tr>
<tr>
<td>Echinococcus granulosus</td>
<td></td>
</tr>
<tr>
<td>Echinococcus multilocularis</td>
<td></td>
</tr>
<tr>
<td>Spirometra</td>
<td></td>
</tr>
<tr>
<td><strong>Trematodes</strong></td>
<td></td>
</tr>
<tr>
<td>Schistosoma japonicum</td>
<td></td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td></td>
</tr>
<tr>
<td>Paragonimus westermani</td>
<td></td>
</tr>
</tbody>
</table>

49
Clinical presentations

The clinical manifestations may be of relatively acute onset or more frequently, insidious and slow. On a global scale, the best-known of the parasitic infections that present acutely is cerebral malaria, a complication of *P. falciparum* infections. Human African Trypanosomiasis (sleeping sickness), caused by *T. brucei rhodesiense* and *T. brucei gambiens*, also causes encephalopathy within a few weeks of disease onset in the former and in the chronic stages of the disease in the latter. In American trypanosomiasis caused by *T. cruzi*, nervous system involvement could occur in the acute stage.

There are several other, less well-known parasitic infections that cause encephalopathy. Toxoplasmosis (primary or re-activated infection) is well known to cause neurological disease in immuno-compromised patients, particularly those with organ transplants and HIV/AIDS. Cases of encephalopathy temporarily related to mass treatment with ivermectin for onchocerciasis have been reported; most of these cases recovered without serious consequence (Boussinesq et al., 2003). Fatalities usually occur in those who are co-infected with *Loa loa* and have a high microfilaraemia. A similar fatal encephalopathy has been described in patients infected with *Loa loa* who have been treated with DEC (Kamgno et al., 2008).

Among the organisms that cause meningo-encephalitis are the pathogenic free-living amoeba, *Naegleria fowleri* and the helminths which cause eosinophilic meningitis. *Angiostrongylus cantonensis* is the most common cause of eosinophilic meningitis worldwide. The rat is the definitive host of the parasite and humans are infected by ingesting third-stage larvae, which develop in a molluscan intermediate host, usually slugs or snails (Bunnag, 1999).

Though neurological manifestations of infection with *Toxocara larvae* (toxocariasis, also known as visceral larva migrans) are rare, it is an important differential diagnosis of various neurological disorders. Manifestations of involvement of the central nervous system include dementia, meningo-encephalitis, myelitis, cerebral vasculitis, epilepsy or optic neuritis (Finsterer & Auer, 2007). A review of the literature from the early 50’s to the present date found 29 cases of brain involvement in toxocariasis (Moreira-Silva et al., 2004). Of these 28 cases, 20 reported different clinical and laboratory manifestations of eosinophilic meningitis, encephalitis, myelitis or radiculopathy.

The presence of encysted larval stages in the brain may also cause patients to present with seizures such as in neurocysticercosis and in trichinellosis. Neurocysticercosis, infection of the brain parenchyma with the larval stage of *Taenia solium*, is a common cause of focal and generalised seizures of late onset, but it less commonly presents as headache, Parkinsonism, or other neurologic abnormalities. Heavy cyst burden in neural tissue can cause encephalopathy with fever, headache, nausea and vomiting, altered mental status, and seizures (Kraft, 2007). Cerebral sparganosis presenting as seizures caused by sparganum, the larval stages of the cestode *Spirometra spp*. is of importance as surgical removal of the larva results in a complete cure (Alibhoy et al., 2007).

Central nervous system involvement may occur in chronic schistosomiasis caused by any schistosome species, but especially by *S. japonicum*. The usual presentation is focal or generalized tonic-clonic seizures and focal deficits. Transverse myelitis is the most common neurologic manifestation of *S. mansoni* infection, which rarely affects the brain. The possible mechanism of central nervous system involvement is the embolization of eggs or ectopic migration of adult worms (Ross et al., 2002).

Among the other trematodes, *Paragonimus westermani* causing paragonimiasis is a major cause of neurological disease in the Far East, due to cyst formation in ectopic sites in the brain and presents as intracranial space occupying lesions. Chronic helminth infections such as hydatidosis, may also present with the features of a slow-growing, space-occupying lesion in the nervous system.

In the current era of international travel, changing lifestyles, eclectic food habits and climate change due to global warming, it is particularly important to be aware of the range of parasitic infections that could present with neurological disease. It is also worthwhile remembering that although very few of the parasites that are natural parasites of humans have a predilection for the CNS, in many zoonotic infections, helminth larvae migrate to brain tissue and persist there.

Pathogenic free-living amoebae: the parasites

The pathogenic free-living amoebae are rare causes of human disease. *Acanthamoeba* meningo-encephalitis and *Naegleria* primary meningo-encephalitis were first reported in 1965 and 1966 in southern Australia and Florida (Intalapaporn et al., 2004). There are now four genera of these amoebae, which have been implicated as aetiological agents of human disease. These are:

- *Naegleria fowleri*
- *Acanthamoeba* spp.
- *Balamuthia mandrillaris*
- *Sappinia diploidea*

Together, they are known to cause three distinct clinical syndromes:

- Primary amoebic meningo-encephalitis (PAM)
- Granulomatous amoebic encephalitis (GAE)
- Amoebic keratitis (AK)

While *N. fowleri* causes PAM, *Acanthamoeba* spp are known to cause both GAE and AK. *B. mandrillaris* and *S. diploidea* also cause GAE.
**Naegleria** spp. are primarily soil amoebae that are washed off into surface waters and as such are found worldwide in a wide variety of warm stagnant or slow flowing surface water bodies. Recently these amoebae have also been detected in ground water such as wells (Rain et al., 2008). In Sri Lanka, *Naegleriasspp.* have been isolated from river water in the Kandy area and irrigation tanks in the Dry Zone (Wijesundera et al 1996, Gunaratna, 2009). Of the 47 species identified on molecular basis to date, only *N. fowleri* is known as a human pathogen while two other species *N. italica* and *N. australis* are now recognized as potential pathogens (De Jonckee, 2002). The *Naegleria* isolated in Sri Lankan waters is yet to be speicated.

*N. fowleri* is thermophilic, growing at temperatures up to 45°C. The trophozoite stages thrive in large bodies of warm water such as found in heated swimming pools and thermal springs. Chlorination does not affect its growth. It is a facultative pathogen capable of living many generations without infecting a host. A wide range of animals in addition to humans can be hosts to these amoebae.

The life cycle includes three morphological forms: the amoebic trophozoite, which is the feeding, growing, multiplying form; the flagellate trophozoite, which is rapidly motile and the dormant cyst stage. The amoebic trophozoite, which is about 10-30 μm in diameter, has a single nucleus with a large nucleolus. It is usually found on surfaces of vegetation and mud; it feeds on bacteria. The flagellate form, which typically has 2 flagella, is a single cell. It is found in the surface layers of warm bodies of water. The cyst is spherical with a smooth, single wall; it is about 10 μm in diameter and is also found in the same locations as the amoebic trophozoite. Human infection usually occurs while bathing or swimming, when the amoebe in the infected waters enter through the olfactory epithelium in the nose. They penetrate the epithelium, pass along the olfactory nerve branches and enter the cribiform plate to enter the meninges and multiply in the perivascular spaces. The amoebic trophozoites are found in the brain tissue and CSF. The flagellate forms are also occasionally seen in the CSF. When amoebae are present in the CSF, transformation into the flagellate form may be precipitated and visualised by the addition of distilled water to a wet smear.

The symptoms and signs, and changes in the cerebrospinal fluid are like those of purulent bacterial meningitis, but there is no response to anti-bacterials. Infection is almost invariably fatal.

Like *N. fowleri,* the *Acanthamoeba spp.* that infect humans are also found worldwide, feeding on bacteria. However, they are not necessarily associated with warm fresh water, and can also multiply in brackish conditions (Warhurst, 2008). *Acanthamoeba spp.* also have trophozoite and cyst forms, but the trophozoite has only the amoeba stage. It is large (20 - 40 μm in diameter), with a single nucleus and its surface is covered with tiny projections that look like spines (acanthopodia). It is sluggishly motile. The cysts have a polyhedral shape and a double wall. They are about 15 μm in diameter. The widespread, ubiquitous presence of these organisms means that humans are frequently exposed to them, but the parasite usually finds it difficult to colonise humans. Organisms may enter through the nasal passages into the lower respiratory tract, through ulcerated or broken skin or the eye. Infections usually occur in the patients who are immuno-compromised or debilitated in some other way. They are of a chronic type, with a marked granulomatous reaction.

*Balamuthia mandrillaris* is a leptomyxid free-living amoeba, first described as causing disease in 1990. This species is also found worldwide, in soil and water, but the particular ecological niche occupied by the parasite is as yet unknown. It was first isolated from the brain of a mandrill baboon at the San Diego Wild Animal Park. Several reported cases of *B. mandrillaris* had initially been diagnosed as being due to *Acanthamoeba.* Following the development of an immunofluorescence assay, a number of human cases of *B. mandrillaris* meningoencephalitis were diagnosed retrospectively (Intalapaporn et al, 2004).

As with other amoebae, *B. mandrillaris* has two morphological forms: the trophozoite and the cyst. Trophozoites, which range in size from 12 to 60 μm, are uninucleated with a large, densely staining nucleolus. There may be two or three nuclei in some trophozoites. Cysts may be more readily visualised with either GMS or periodic acid-Schiff stains. They range in size from 6 to 30 μm and appear to be double-walled and three-walled by light and electron microscopy, respectively. Immunofluorescence studies are required for differentiating *B. mandrillaris* and *Acanthamoeba.*

The parasites may enter the human host through the nasal tissue and thence via the lower respiratory tract and circulation or through ulcerated or broken skin. Both forms may be found in the brain tissue of infected individuals.

A single case of amoebic encephalitis thought to be due to *Sappinia diploidea,* a soil-living amoeba, was reported in 2001 (Gelman et al, 2001). More recent work using newly developed real-time polymerase chain reaction assays, has suggested that the organism is most likely to be *S. pedata.* This amoeba had previously been found only in environmental sources, such as soil and tree bark (Gvarnstrom et al, 2009).

**The clinical conditions**

Primary amoebic meningo-encephalitis (PAM) caused by *N. fowleri* has a worldwide distribution.

However, disease is rare and to date only 200 cases have been documented. The majority of cases are from developed countries, probably due to availability of reference diagnostic facilities. A few cases have been
reported in India and Thailand (Shenoy et al, 2002). Although the free-living organism been found in Sri Lanka, no cases of PAM have been reported in Sri Lanka to date.

Classically, the disease occurs in healthy young adults or teenagers with a history of swimming in the type of water-body known to be favoured by N. fowleri. It appears likely that although many people are exposed to infection, for unknown reasons, only a few actually develop disease. Invasion of the brain through the olfactory bulb and cribiform plate results in a rapidly progressive, purulent haemorrhagic necrosis of the frontal cortex, starting with the olfactory bulbs. The pathology is very similar to that of purulent bacterial meningitis. The incubation period in PAM ranges from a few days to about 2 weeks. Patients may complain of distortions in taste and smell at the onset of the illness. They soon develop clinical features of acute meningitis, with fever, headache, vomiting, and neck stiffness. Illness rapidly progresses to deep coma, and convulsions may occur. Patients usually die within a week of onset of symptoms.

The CSF usually has a high white cell count, predominantly with polymorphs. It also has a high protein content and low glucose levels. Trophozoites are not easily seen in a Gram stain; they are better visualized with iron haematoxylin stain. A wet mount of CSF may show motile trophozoites. Definitive diagnosis requires direct demonstration of organism. Formation of the rapidly motile biflagellate form may be precipitated by addition of a drop of distilled water to the CSF; this may be observed in an unstained, wet mount, under a regular microscope.

Although the clinical features and pathological changes resemble those of purulent bacterial meningitis, PAM does not usually respond to anti-bacterial agents. However, systemically intrathecal amphotericin B have been used in patients who have survived. Adjunctive therapies with rifampicin, miconazole, sulfisoxazole have also been tried, but the benefit remains unknown. Most cases described in literature to date, have been fatal.

Granulomatous Amoebic Encephalitis has also been reported worldwide. One hundred and fifty-six human cases of GAE have been reported from 1956 through 1997 at the Centers for Disease Control and Prevention (Atlanta, GA) 63 of them caused by B. mandrillaris (Intalapaporn, 2004). Unlike PAM, GAE due to Acanthamoeba is a disease of immuno-suppressed or otherwise debilitated patients. Balanamthia can also cause GAE in patients who are otherwise healthy. The mode of acquisition of infection is not always clear. Acanthamoebae may be present as a commensal in upper respiratory tract of normal individuals.

When Acanthamoeba spp or B. mandrillaris enters a human host, the primary foci are usually in lungs and skin. The organisms spread haematogenously from there to CNS. The brain involvement is frequently patchy: thalamus, diencephalon, brainstem and posterior fossa. The chronic inflammatory, granulomatous response is characterised by multi-nucleated giant cells. Both trophozoites and cysts present in lesions.

The onset of changes in mental status is usually insidious, with focal neurological deficits, seizures, fever, headache and visual abnormalities. The duration of illness varies, but it is usually about a month. Skin lesions may be seen at the original site of infection.

Multiple, non-enhancing lesions may be seen on CT. Lumbar puncture is contra-indicated; in any case, amoebae are not found in CSF. Diagnosis is usually made at autopsy, and brain sections will show granulomatous lesions with amoebae and cysts. Concurrent skin lesions may be biopsied to make an indirect diagnosis. The differentiation between Acanthamoeba and Balamuthia in tissue sections requires immunohistochemistry.

As with PAM, GAE is also nearly uniformly fatal. Diazidines such as pentamidine, propamidine have shown the greatest in vitro activity. Azole antifungals and some aminoglycosides such as neomycin and paromomycin have also been tried, but with little success.

Amoebic keratitis caused by infection with Acanthamoeba spp is usually associated with minor trauma to the eye or use of contact lenses. Especial risk factors include soaking contact lenses in homemade saline solutions, swimming with contact lenses, and use of extended wear contact lenses. The organism has been isolated from a Sri Lankan patient with central corneal ulceration (Wijesundera et al, 2001).

The initial clinical features of amoebic keratitis include the sensation of a foreign body in eye, tearing, photophobia and ocular pain. In full-blown infections, filiform dendiform keratitis, hypopyon, increased intra-ocular pressure, and anterior nodular scirrhus may occur. Laboratory diagnosis may be made by examining corneal scrapings. Wet mounts can show motile trophozoites and cysts, while fixed slides can be stained with Giemsa or periodic Acid Schiff reagent or calcofluor white to visualize the amoebae. If the patient uses contact lenses, the contact lens and storage system should also be examined for the presence of amoebae. Acanthamoeba keratitis is curable if detected early. The affected areas of cornea should be debrided. Specific treatment with topical propamidine, together with neosporin and miconazole is required for about 1 month.

References


18. Wijesundera NLS, Ranaweera RLAR & Wijesundera MdeS. Preliminary studies on pathogenic free living amoebae in water samples in Kandy. 6th Academic Sessions of the Sri Lanka College of Microbiologists 1996.


19. Wijesundera NLS, Ranaweera RLAR & Wijesundera MdeoS. Preliminary studies on pathogenic free living amoebae in water samples in Kandy. 6th Academic Sessions of the Sri Lanka College of Microbiologists 1996.

**ACINETOBACTER BAUMANNII – A SUCCESS STORY OF A NOSOCOMIAL PATHOGEN**

Sujatha Pathirage
Consultant Microbiologist, National Institute of Health Sciences, Kalutara.

The most important representative of the genus *Acinetobacter*, *Acinetobacter baumannii*, has emerged as one of the most troublesome pathogens in healthcare institutions globally. It has a remarkable ability to **up-regulate** or acquire resistant determinants. *Acinetobacter baumannii* resistant to all known antibiotics have now been reported, signifying a sentinel event which need **prompt** action by the international healthcare community. Acting in synergy with this emerging resistant profile is the uncanny ability of *A. baumannii* to survive for **prolonged** periods throughout hospital environments, thus potentiating its ability for nosocomial spread (Peleg AY et al).

**Acinetobacter spp** have been implicated in a variety of nosocomial infections, including bacteraemia, urinary tract infections, nosocomial meningitis. However, its predominant role is as agent of nosocomial pneumonia, particularly ventilator associated pneumonia in patients confined to hospital intensive care units (ICU). In more recent times, infection involving central nervous system, skin and soft tissue, and bone have emerged as highly problematic for certain institutions.

**Microbiology**

The genus *Acinetobacter*, as currently defined, comprises gram-negative, strictly aerobic, non fermenting, non fastidious, non motile, catalase positive, oxidase negative bacteria with a DNA G+C content of 39% to 47%. Acinetobacter species of human origin grow well on solid media that are routinely used in clinical microbiology laboratories. These organisms form