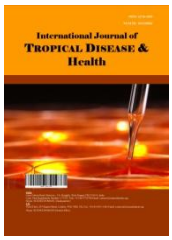

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Epidemiology of Pathogenic Free-living Amoeba

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Authors' contributions

This work was carried out in collaboration between both authors. Author EM designed the literature review and wrote the first draft of the manuscript, Author LH performed the literature searches and conducted the analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Free-living amoeba (FLA) is a free living parasitic organism which have the capability to infect human and attack various vital organs *e.q.*, the central nervous system, eyes, skin, nose, lungs and kidneys; depending on which organ is reached. When it comes to the central nervous system, this disease is very fatal and potentially lethal because the diagnosis of FLA infection is often overlooked. The genera that can infect humans are *Acanthamoeba* spp., *Naegleria* spp. (*Naegleria fowleri*) and *Balamuthia* (*Balamuthia mandrillaris*). This amoeba is widespread in nature and can be found in environmental abandoned waters such as water in swimming pools, rivers, lakes, baths, tubs and water tanks. The mode of transmission or transmission by this free-living amoeba can be accidentally by way of inhalation while swimming, or entering through the mouth or entering through broken skin. Children and young adults are at risk of infection. Diagnosis is made by microscopic examination, namely direct examination to find trophozoites and cysts in specimens such as cerebrospinal fluid and brain tissue or by culture. In addition, serological and molecular tests such as PCR can also be performed.

Keywords: Acanthamoeba spp; Naegleria fowleri; protozoa; inhaled; lethal.

1. INTRODUCTION

Free living amoeba (FLA) is a free living parasite that can infect humans. This parasite can attack several crucial organs from the central nervous system, eyes, skin, nose, lungs and kidneys. When it infects the central nervous system, it can caused a fatal and potentially lethal conditions for

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the patients. The first cases were reported in 1965 in Australia and Florida, United States of America and until now new cases continue to be reported. Even though this amoebic infection has a low morbidity rate but it has high mortality rate because there are still challenges in making correct and proper diagnosis and treating it appropriately [1-4].

These FLA are a bunch of microorganism that can complete its life cycle in nature without the need for a host. FLA is considered as pathogenic and in the same time also opportunistic protozoan that has aerobic properties. Its existence is widespread in nature and around the human environment. This free-living amoeba has many genera but those that reported infect humans and animals are come from the genera *Acanthamoeba, Balamuthia* (*Balamuthia mandrillaris*), *Naegleria* (*Naegleria fowleri*) *and Sapinia* (*Sapinia pedata*). This amoeba can be found in water, soil and air such as water in abandoned swimming pools, rivers, lakes, water tanks, flower pots and dust in air conditioners. The mode of transmission or transmission by this FLA can be transmitted via inhalation or accidentally drank when swimming, entering through the mouth or entering through broken skin [2,5-7].

Diseases caused by free-living amoebas can occur variably in wide range of individuals, from immunocompetent individuals, especially children and young adults, to immunocompromised person. The risk factor for being infected by FLA is very high especially if someone regularly exposed to abandoned water containing the amoeba. The existence of FLA in the environment also depends on the mineral content of the water and the temperature of the environment. Early diagnosis is very important in curing this disease; but it is often very difficult to accomplish because there are no specific clinical symptoms, *e.g.*, primary amebic meningoencephalitis (PAM) infection caused by FLA infection.

Virulence factors that these amoebas have and that allow efficient and successful pathogenesis, for example proteases such as collagenases and some other cytolytic molecules, poorly described in the literature.

Diagnosis of FLA infections such as primary amebic meningoencephalitis caused by *Naegleria fowleri*, granulomatous amebic encephalitis (GAE) caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris* can be established by microscopic examination, namely direct microscopic examination, to find trophozoites and cysts in clinical specimens such as cerebrospinal fluid and brain tissue or by conducting culture specimens. In addition, serological and molecular tests such as PCR can also be performed. In Indonesia, there have not been many reported cases of amoebic infection, this may be due to missed diagnosis by FLA infection and the patient has already died due to a very short incubation period and severe symptoms of CNS infection. [3,4,6,8] The aim of this review is to reveal the epidemiology of free-living amoeba which includes morphology, life cycle, clinical symptoms and establishing the diagnosis of FLA infection..

2. FREE-LIVING AMOEBA

Free-living amoeba (FLA) belongs to the class Rhizopoda. These parasites or amoeba can infect humans and also animals. These parasites can live facultatively and actually are pure pathogenic. Some of the species that are often found to infect humans are *Naegleria* spp., *Acanthamoeba* spp. and *Balamuthia* spp.[4,9] In 1965, the first cases of this disease caused by this amoeba were reported in Australia and Florida in the United States and then cases of this disease were reported in the world. This free-living amoeba can be a pathogenic parasite that can infect humans and cause amebic meningitis or better known as primary amoebic meningoencephalitis (PAM).[1,4,6] In 1978, it was reported that a woman who regularly swam in baths in England died of have amebic meningitis. In the same year, there was also a swimming pool epidemic in Czechoslovakia and after investigation it turned out that the cause was free-living amoeba found in swimming pool water, especially in specific site, *e.g.*, pool holes and water pockets that were protected from the influence of chlorine. This FLA is a pathogen that can cause 100% of deaths in infected individuals.

Naegleria fowleri: *Naegleria fowleri* is one of the free-living amoeba that most frequently infects humans among other FLAs and is also the most virulent or highly pathogenic FLA. This parasite is thermophilic and its named previously was *N. gruberi*. In the wild, its source of nutrients come from bacteria, yeast cells and orphaned detritus. *Naegleria fowleri* commonly infects children and young adults. The disease is called primary amebic meningoencephalitis (PAM) which is acute and fatal [1,4,5].

This free-living amoeba lives cosmopolitan in nature, and spread all over the world. This parasite can be found in soil, abandoned stagnant fresh water such as swimming pools, lakes, warm water baths, besides that, this parasite can also be found in air conditioners and water tanks. Many cases of PAM have been reported from many countries such as in the United States, Belgium, Australia, Czechoslovakia, England, Ireland, Venezuela, Panama, Brazil, Papua New Guinea and others [5,9-11].

Naegleria fowleri is a protozoan consisting of ectoplasm and endoplasm. In the endoplasm there is a vesicular nucleus with large karyosome and a nuclear wall filled with chromatin grains, in addition to the nucleus there are also contractile vacuoles and food vacuoles. *Naegleria fowleri* has three forms, namely trophozoite, flagellate and cyst forms, as shown in Fig. 1 [4,9].
The ameboid stage has an irregular shape, oval or rounded with an average size of 29 microns. The single pseudopodium that is expelled extends in one direction called *lobopodia*. Trophozoites eat detritus and bacteria such as *Escherichia coli*. The flagellate stage is a very motile shape, oval like a pear, the core is a vesicular nucleus, there is one contractile vacuole located posteriorly and has two flagella of the same length. This stage is only found for a very short time, e.g., few hours, which will then turn rapidly back into the ameboid stage again. The third form is the cyst form. This stage of the cyst has a round or sometimes oval shape with two-layer walls and has a single nucleus. This stage is 10-14 microns in size. In the cyst wall there are several holes that can be used for excystation [4,12,13].

The way humans are infected is by inhalation or inhalation through the nose when the patient swims or is exposed to water containing *Naegleria fowleri*, the longer humans are exposed to or in contact with water containing this amoeba, the greater the risk of infection and this is a predisposing factor for infection by this parasite. [14] The life cycle of this amoeba can be seen in Fig. 2.

Amoeba enters the human host through the nose, and it will penetrate the brain tissue and multiply in the brain tissue. The olfactory neuroepithelium of the upper nasal mucosa is the main site of entry for the parasites into the central nervous system. The trophozoites penetrate the cribriform plate of the sphenoid bone and enter the subarachnoid space, then to the brain parenchyma. With the aid of the olfactory nerve that terminates in the olfactory bulb in the subarachnoid space, which is surrounded by the central nervous system and rich with vascularization, which turned out to be a source of important nutrients/medium for the growth of this amoeba during their brain invasion. The incubation period is 5-7 days. Clinical manifestations vary from severe headache in the frontal area, fever, sore throat, nasal congestion and even bleeding, reduced appetite, rapid but deep breathing and photosensitivity. If the parasite infects the meningeal membranes, then the condition of neck stiffness, convulsions and various disorders of the central nervous system can exist, focal neurologic abnormalities may occur late in the course of the disease. The cerebrospinal fluid may be purulent and may contain large numbers of red blood cells and moving amoeba. If treated late or treated appropriately, the patient will fall into a coma and die [1,4,6]. In this PAM, clinical diagnosis is very difficult to confirm, most cases are only discovered after an autopsy is performed. To make a proper diagnosis, it is necessary to recommend an autopsy, especially in AIDS patients. For efficient clinical management, rapid and specific identification of clinical specimens is necessary. Multiplex Real Time PCR gives fast results and has high specificity. In infection by *N. fowleri* usually bloody or sanguinopurulent cerebral fluid. The amount of protein is increased and glucose is low [1,6].

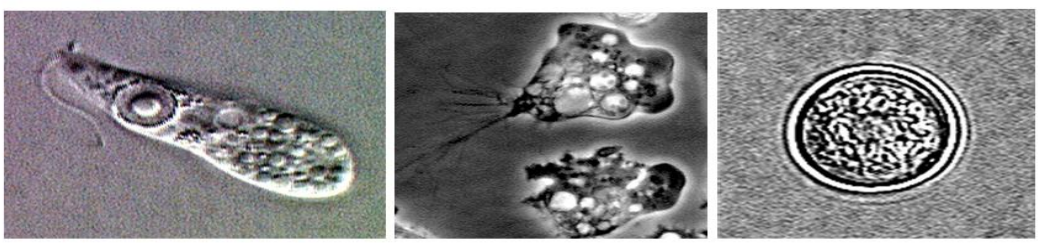

Fig. 1. Morphology *Naegleria fowleri* [12]

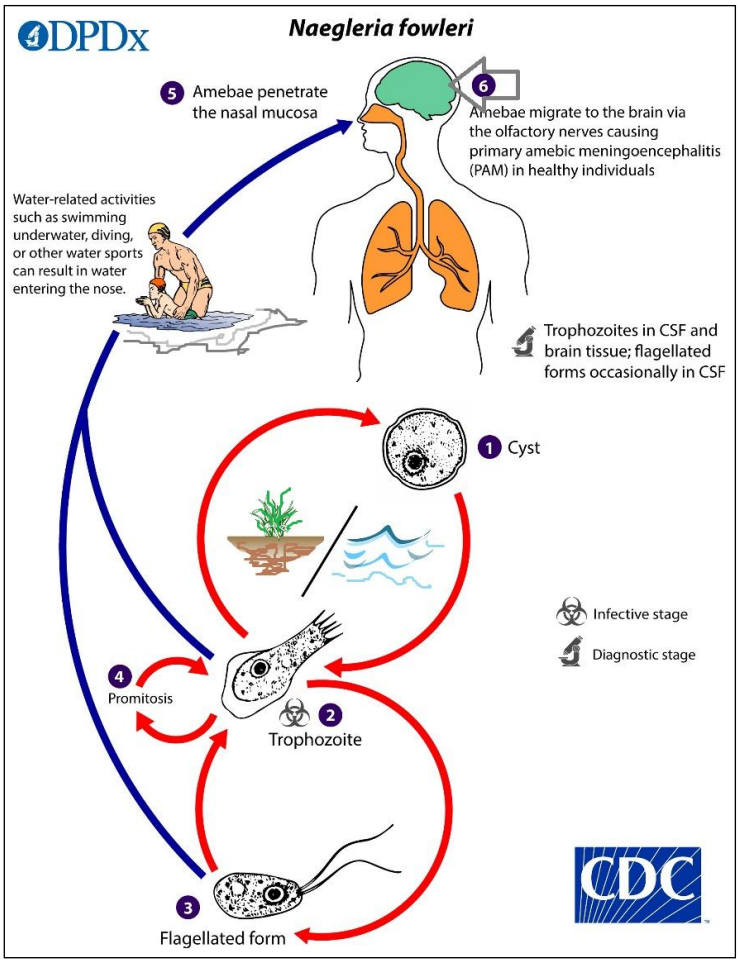

Fig. 2. The life cycle of *Naegleria fowleri* [12, with modification]

Diagnosis can also be made by microscopic examination, by finding amoeba in clinical specimens, *e.g.*, cerebrospinal fluid, in the portion where purulent and exudate exist or and in necrotic tissue conducted at post-mortem examination. For certain circumstances, it can also be examined by culture, immunofluorescent antibody (IFA) and molecular methods, namely by PCR [4,14].

Prognosis in these patients is poor, unfortunately, most patients die before correct and proper diagnosis was made. Only a very few patients can overcome this disease with the administration of high doses of amphotericin B, miconazole, and rifampin [6].

Acanthamoeba spp.: Previously, *Acanthamoeba* had the genus name *Hartmanella*. This *Acanthamoeba* has the same habitat as *Naegleria*. The geographic distribution of this parasite is cosmopolitan in nature. In humans, this parasitic infection can cause a condition of multifocal encephalitis, known as granulomatous amebic encephalitis (GAE) and amebic keratitis. The morphology of *Acanthamoeba* is almost the same as that of *Naegleria* but there is no flagellate stage in *Acanthamoeba*. Only the trophozoite stage was found, namely the amoeboid form and the cyst stage, as shown in Fig. 3 [1,4,12].

The trophozoite stage has various shapes, measuring approximately 30 microns. Several pseudopods are expelled simultaneously from the body surface in several directions. The pseudopodium is actually wider than the pseudopodium secreted by *Naegleria*. The cyst stage is about 20 microns in size, has a circular or ovoid shape. The cyst has a double wall, which is look wrinkled on the outer part [12]. The life cycle is the same as for *N. fowleri*, as shown in Fig. 4.

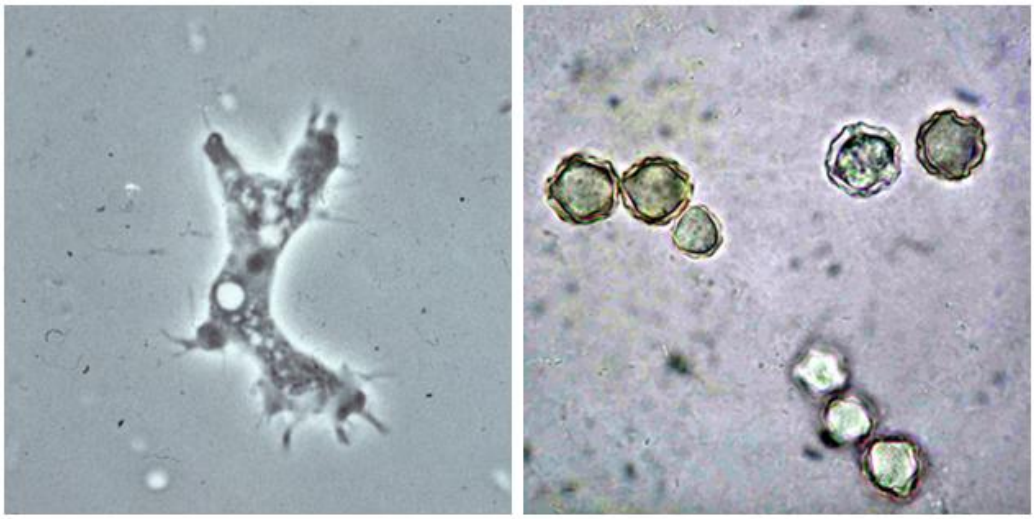

Fig 3. Trophozoites and cyst of *Acanthamoeba spp* [12]

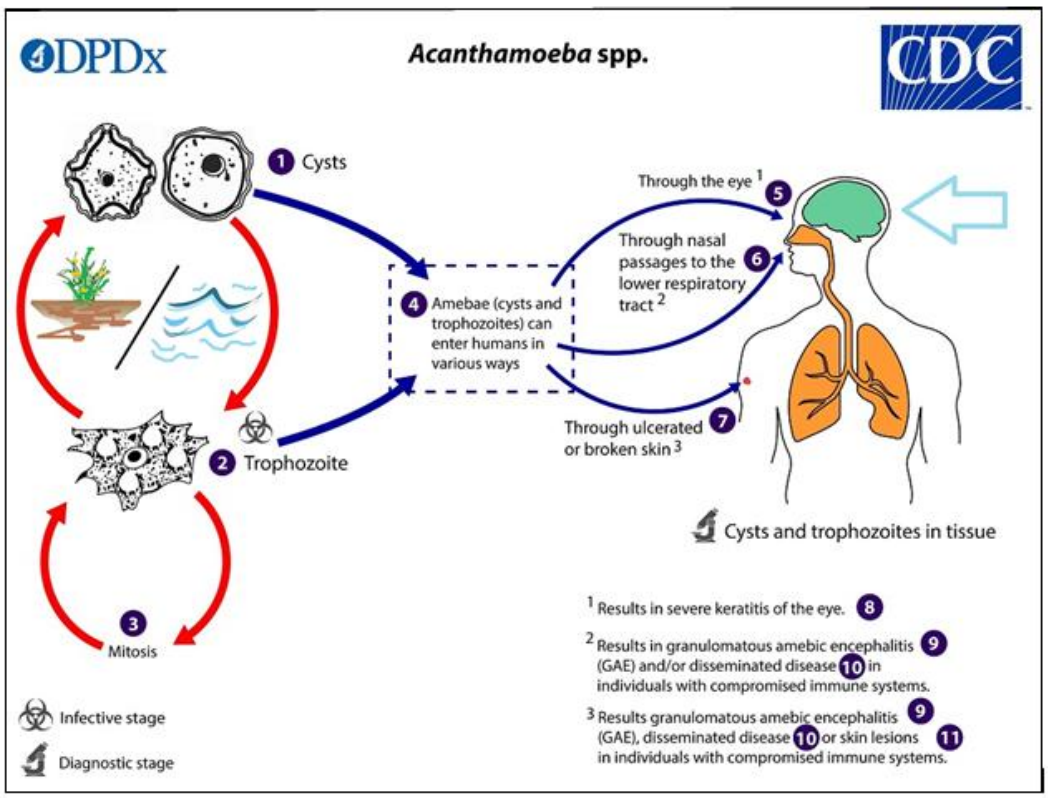

Fig. 4. The life cycle of *Acanthamoeba* spp [12]

Acanthamoeba spp. different from the genus *Naegleria* in terms of their routes when infecting humans. Their trophozoite stage and its cysts of can enter the body through the lower respiratory tract or injured skin and then spread to the brain, presumably haematogenously. *Acanthamoeba* infection in the brain, usually in the brain, usually occurs in immunologically weak individuals such as immunocompromised and or immunosuppressed patients. The infection is usually sub-acute with various central nervous system related clinical symptoms such as headaches, changes in mental status and focal neurologic deficits that can cause death within a

few weeks if not treated properly. In addition to the brain, this parasite can also invade the skin and cause granulomatous like lesions.

Several species of *Acanthamoeb*a are *A. culbertsoni, A. polyphaga, A. castellani, A. astronyxis, A. hatchetti, A. rhysodes, A. divionensis, A. lugdunensis* and *A. lenticulata*. The most important species epidemiologically is *A. culbertsoni*. This parasite can also infect the corneal part of the eye, causing a disease called amebic keratitis or Acanthamoeba keratitis. The incidence of this disease is increasing globally. People who are susceptible to this kind of infection are outdoor worker such as construction workers who already have underlying corneal disorders. In addition, this infection also often occurs in people who wear contact lenses for a longer period of time than recommended; and this is due to the lack of hygiene in caring for their contact lenses, *e.g.*, using washing liquid or soaking contact lenses that are contaminated with this amoeba.

The diagnosis is made by microscopic examination, by finding trophozoites in the cerebrospinal fluid in the portion where purulent and exudative exist. The appearance of cysts is characteristic; i.e. wrinkled inner wall of necrotic tissue. Amebic keratitis can be diagnosed by finding the parasite on a corneal smear with a Giemsa stain or an acid-Schiff stain. In addition, for diagnosis, culture, IFA and PCR examinations can be carried out.[4,5,8]

Balamuthia mandrillaris: *Balamuthia mandrillaris* was first identified in 1986 in clinical specimens from the post-mortem examination out of brains of a dead baboon at the San Diego Zoo. Since then, it is estimated that there are more than 200 cases of infection by *Balamuthia* worldwide. Unfortunately, very little is known on how a person can become infected with *Balamuthia*.

Balamuthia has two stages in common with *Acanthamoeba*, namely the trophozoite stage and the cyst stage. The size of the trophozoite stage is 12-60 microns and moves slowly while the cyst stage is spherical in shape with a size of 6-30 microns [10]. The morphology of *Balamuthia* can be seen in Fig. 5.

Balamuthia enters the body through soil containing the parasite by direct contact with wounds on the skin or open wounds, it can also infect humans through inhalation of dust containing *Balamuthia* or suck into the mouth.

When it enters the body, the amoeba can reach the brain and cause Granulomatous Amebic Encephalitis (GAE) [1,4,5,19,20]. The life cycle of *Balamuthia mandrillaris* can be seen in Fig. 6.

GAE is a very severe disease and more than 95% is fatal which causes death. The time it takes from exposure to *Balamuthia* until the first symptoms appear is GAE, which is from a few weeks to several months. Confirmation of the diagnosis of infection by *B. mandrillaris* conducted by a microscopic examination of cerebrospinal fluid can be performed to detect the trophozoite stage. In addition, it can be examined by culture, IFA and PCR methods [1,3].

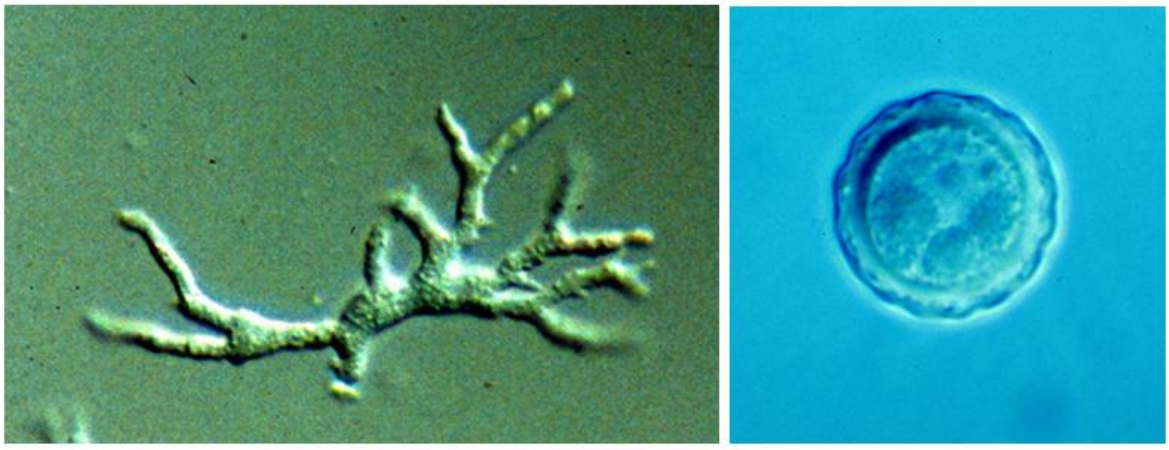

Fig. 5. *Balamuthia mandrillaris*.[12]

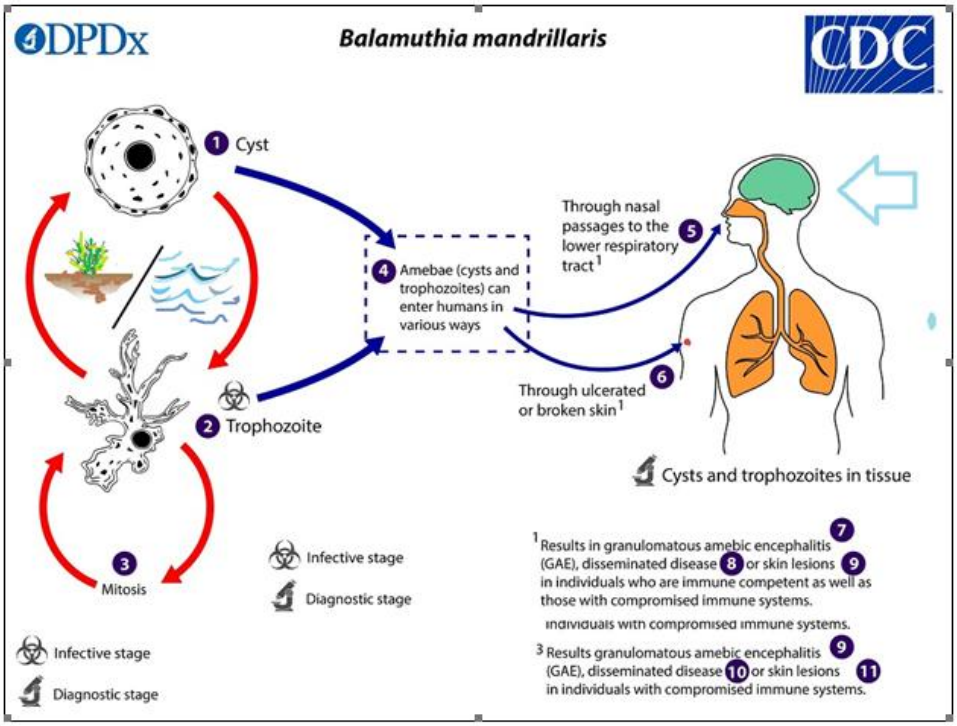

Fig. 6. Complete life cycle of *Balamuthia mandrillaris*. [12]

Unlike approaches to more common parasitic agents, *e.g.*, malaria with no matter how obscure the clinical manifestations, cases of FLA are often missed because they are rarely recognized. [21] FLA is also not part of normal flora or common human microbiome, so their existence in the human body, especially in deep organ is often hidden and hard to detect [22]. A fast and efficient diagnosis is mandatory, however it depends on two variables: (1) the familiarity of practitioner with the symptomatology and treatment of FLA infections, and also (2) the sufficiency and appropriate clinical material (qualitatively and quantitatively) in order to be processed for making a fast and definitive diagnosis [23].

Until this present time, it is not known whether FLA infections are rare because they are underreported or due to misdiagnosis. Most infections have been diagnosed post-mortem. It is not possible to determine the rarity of these infections, since these infections are relatively unknown and in many cases autopsies are not performed [1,23].

3. CONCLUSION

Free-living amoeba is a potentially lethal pathogen that can be found in the environment such as abandoned river water, ponds, lakes, water storage tanks and swimming pool water. FLA can infect humans by attacking multi-organ, including the central nervous system, eyes, skin, lungs, kidneys and other organs. This infection can be fatal and cause death within a few days if it attacks the central nervous system and proper diagnosis by FLA is often missed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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