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Emerging Trends in Free-living Amoebic infections of the Brain

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Free-living amoebic infections; primary amoebic meningoencephalitis, granulomatous amoebic encephalitis; organ transplantation, contraindications.

Additional Key Words:
Naegleria fowleri, Acanthamoeba species, acanthamoebi-asis, *Balamuthia mandrillaris,* leptomyxid amoeba, balamuthiasis, Sappinia species, *Sappinia* diploidea, Sappinia pedata.

Abstract
With the exception of *Acanthamoeba* keratitis in contact lens wearers, brain infections caused by free-living amebae are rare, but increasing today. This review analyzed cases of *Naegleria fowleri* primary amoebic meningoencephalitis and Balamuthia mandrillaris granulomatous amoebic encephalitis for behavioural and demographic risk factors for pathogen exposures, new mechanisms of transmission, clinical outcomes, and management and prevention strategies. Fatal free-living amoebic infections of the brain are increasing today due to more frequent environmental and occupational exposures to opportunistic pathogens by both genetically susceptible and immunosuppressed human hosts in a warming ecosystem that supports disease transmission.

Emerging Trends in Free-living Amoebic Infections of the Brain
Free-living amoebae of the genera *Acanthamoeba, Balamuthia, Naegleria,* and Sappinia are rare causes of infectious diseases in humans, with the exception of *Acanthamoeba* keratitis (AK) which is reported in over 1-2 cases per million contact lens wearers in the United States (US) annually.[1,7] Unlike several *Acanthamoeba* species, only one species of *Naegleria, N. fowleri,* is known to infect humans by causing an acute, fulminant, usually lethal, brain infection, known as primary amoebic meningoencephalitis (PAM).[1,5]

Balamuthia mandrillaris, formerly known as leptomyxid amoeba, is another opportunistic, free-living ameba. Like *Acanthamoeba* spp., B*alamuthia mandrillaris* is capable of causing skin lesions and granulo-matous amebic encephalitis (GAE) in individuals with compromised or competent immune systems. Both Acanthamoeba and Balamuthia-caused GAE are often characterized by nodular or ulcerating granulomatous skin lesions in soil or stagnant freshwater-contaminat-ed wounds or in similar lesions that may appear spontaneously following organ transplantation. Lastly, *Sappinia pedata,* a recently identified free-living amoeba that lives in soil and domestic animal faeces, has caused a single case of non-granulomatous amebic encephalitis in an immunocompetent Texas farmer. Brain infections caused by these ubiquitous, free-living amebae remain rare despite expanding world populations; but are, nevertheless, increasing today due to a combination of unexplained factors that may include increased recreational and occupational exposures in a warming environment and more immunocompromised patients susceptible to opportunistic pathogens.[6,8]

The objectives of this review will be to describe the epidemiology, diagnosis, management, and outcomes of the 4 known free-living amoebic infections of the brain ***(Table 1).*** In addition, US Centers for Disease Control and Prevention (CDC)-laboratory confirmed cases of PAM and *Balamuthia mandrillaris* GAE will be described and analysed for presenting characteristics of environmental exposures, initial infections, and potential for transmission by organ and tissue trans-plantation.

Patients and Methods
Initially, Medline, Pub Med, and Google® search engines were queried for references using all key words as search terms. The only cases of free-living amebic meningoencephalitis included in the analytical case reviews were cases with CDC laboratory-confirmed detection of *N. fowleri* or *Balamuthia mandrillaris* life forms by immunohistopathological techniques, isolation by culture, or by species-specific DNA as detected by polymerase chain reaction (PCR) in cerebrospinal fluid (CSF), brain biopsy, or brain necropsy tissue. Sources of US cases of PAM came from the registry of the CDC's *Naegleria* Workgroup, which confirmed 121 cases of PAM in the US during the period, 1937-2007.[2] Sources of US cases of *Balmuthia* GAE, or balamuthiasis, came from the CDC or its

affiliated state departments of public health and the California Encephalitis Project [CEP], a joint project launched in 1998 by the California Department of Public Health and the CDC. Similar descriptive analyses were conducted for all CDC laboratory-confirmed cases of GAE caused by *Balamuthia mandrillaris* (N = 28) in the US during the period, 1994-2010.
Significant behavioural, demographic, environmental, ethnic, occupational, and recreational expo-sure characteristics for confirmed cases of PAM and *Balamuthia* GAE were identified over their respective study periods in order to make recommendations for the early diagnosis, critical care management, and prevention of these infections by environmental exposures or by organ transplants. Continuous variables were tested for significant differences by unpaired, two-tailed t-tests; proportions were tested for significant differences by the "ratio test" using the Poisson distribution. A 5% significance level was used for all hypothesis tests. Statistical significance was indicated by p-values less than or equal to 0.05.

Results:

Emerging Trends in Primary Amoebic Meningoencephalitis (PAM)

N. fowleri, the single causative agent of PAM, is a free-living amoeboflagellate that thrives in many types of hot freshwater including shallow inland lakes, geothermal springs, and warm water discharges from electrical power plants.[1,6] The amoeba feeds on bacteria and organic debris in freshwater, and exists in 3 life forms; 2 of which are infective - the environmentally stable, potentially infectious, cyst form and the motile, invasive amoeboid-form, or trophozoite.[9,11] Infective forms typically invade humans via intact or disrupted nasal mucosa; cross the cribriform plate; migrate along the basilar brain from the olfactory bulbs and tracts to the cerebellum; deeply penetrate the cortex to the periventricular system; and incite a purulent meningoencephalitis with rapid cerebral oedema, resulting in early fatal uncal and cerebellar herniation ***(Figure 1)*** [1, 2,10]

An alternative human invasion route in PAM may be through an infection-weakened or traumatically ruptured tympanic membrane (TM), which affords infective forms access to the brain via the middle and inner ear to the acoustic nerve tract and on to the basilar brainstem.[2] The trans-tympanic inoculation of infective *N. fowleri* trophozoites was suspected in 2 PAM deaths in 2 US Gulf Coast states in 2007.[2] In June 2007, a 12-year-old male with access to multiple fresh-water swimming sites in Florida including ditches, drainage canals, and apartment-complex swimming pools, died of confirmed PAM following a 2-day his-tory of ear pain and pressure, possibly *otitis externa* or *media.*[2] In August 2007, a 22-year-old man died of PAM within a week of sustaining a ruptured TM in a fall while wakeboarding in the same Texas freshwater lake as another decedent of PAM earlier in the month.[2] In 2007, 6 cases of PAM in 3 southern-tier US states (Arizona, Florida, and Texas) were reported to the CDC.[2] All 6 patients died within 1 week.[2] In response to these 6 PAM cases, the CDC and its affiliated State Departments of Public Health launched the *Naegleria* Workgroup, whose mission was to educate health authorities about the seasonality and lethality of PAM and its unique behavioral risk factors.2

Initially, a comparative statistical analysis of 121 CDC-confirmed cases of PAM collected by the *Naegleria* Workgroup in the US over the 2 study periods, 1937-1976 vs. 1977-2007, was conducted ***(Figure 2).*** The background frequency of PAM cases in the US was 0-3 cases per year over the entire 60-year study period, 1937-2007; 3 of the 6 cases (50%) in the 2007 cluster investigated by the CDC were males (ages 10, 11, and 22 years) who had been wakeboarding in freshwater lakes.[2] Descriptive analysis of all CDC-documented US cases of PAM during 1937-2007 identified the following presenting clinical characteristics for PAM in the US: (1) male sex; (2) recreational freshwater exposure; (3) summer seasonal exposures; and (4) exposure in a southern-tier US state ***(Table 2).*** There were no differences in the frequency of cases per year per period or in the high case fatality rates per year per period (range = 98%-100%). However, there were more cases in the more recent study period; significantly more deaths in the more recent period ($p < 0.001$); and significantly more clusters of 4 or more cases in the more recent study period ($p = 0.001$) ***(Table 3).***

The presenting epidemiological features, clinical manifestations, initial laboratory and neuroimaging findings of PAM are compared to similar features of the other 3 causes of free-living amoebic infections of the brain in ***Table 1.*** Initial screening laboratory studies are nonspecific, and blood cultures and peripheral blood Gram stains will be negative.

Neuroimaging studies in PAM are also nonspecific and may be normal on initial cranial CT and MRI scans.[15,16] Subsequent neuroimaging findings may include basilar leptomeningeal enhancement, massive cerebral edema, evidence of elevated intracranial pressure (midline shift, compressed ventricles, com-pressed brainstem and basilar cisterns, absence of sub-arachnoid spaces), and multifocal parenchymal lesions, often with evidence of

hemorrhagic infarction or necrosis.[15,16] In 1998, Kidney and Kim compared the neuroimaging findings by CT and MRI in a case of *N. fowleri*-confirmed PAM and a case of *B. mandrillaris*-confirmed GAE.[16] As contrasted with non-specific, diffuse cerebral edema in PAM, neuroimaging findings in GAE were more localized and included multiple, focal, punctuate, ring-enhancing lesions in the posterior fossa.[16]

Although usually futile, successful management strategies for PAM have included combinations of cerebral edema-reducing therapies (corticosteroids, moderate hyperventilation, diuresis, hypertonic saline) and specific pharmacotherapy with antifungals (amphotericin B, miconazole, voriconazole) and synergistic antibiotics (rifampin, azithromycin) ***(Table 1).***[16,19] Several experimental therapies have shown some promise in treating PAM including the phenothiazines, chlorpromazine and thioridazine, and miltefosine, a phosphocholine analog, more commonly used to treat visceral leishmaniasis.[21,23] The optimal duration of therapy is unknown, but most survivors have been treated for 10 or more days.[17,23]

Today, PAM may be prevented by a combination of untested and not evidence-based educational and behavioral modification strategies including the following.[2,10] (1) Avoid water-related activities, such as swimming, diving, water skiing, and wakeboarding in bodies of warm freshwater, hot springs, and thermally polluted water, such as around coal-burning and nuclear electrical power plants. (2) Avoid similar water-related activities in warm freshwater during prolonged periods of high water temperatures and low water levels. (3) Hold the nose shut or use nose clips to avoid any traumatic disruptions in the nasal mucosal linings during water-related activities in warm freshwater, such as lakes, rivers, reservoirs, ponds, bayous, and hot springs. (4) Avoid similar water-related activities in drainage ditches, wells, retention or oxidation ponds, and irrigation canals. (5) Avoid digging or stirring up sediment during all water-related activities in shallow, warm freshwater areas.

Results:

Emerging Trends in Granulomatous amoebic encephalitis (GAE)

Granulomatous amoebic encephalitis (GAE) is a chronic infection of the brain that may disseminate to other organs hematogenously and usually occurs in immunosuppressed patients with the acquired immunodeficiency syndrome (AIDS) or organ trans-plants, or in patients receiving chemotherapy for cancer or tuberculosis.[6,24,28] GAE may be caused by several species of *Acanthamoeba* or by another, phylogenetically related, free-living ameba, *Balamuthia mandrillaris. Acanthamoeba* species and *Balamuthia mandrillaris* are distributed worldwide in freshwater and soil, and can cause GAE year round. The portal of entry for these opportunistic pathogens is through the respiratory tract or ulcerating skin wounds with hematogenous spread to the CNS and, less commonly, with dissemination to other organs in the severely immunocom-promised ***(Figures 3 and 4).***

To date, approximately 200 cases of *Acanthamoeba* GAE and 150 cases of *Balamuthia* GAE have been reported with acanthamoebiasis still confined mostly to the immunocompromised; and balamuthiasis affecting both immunocompromised and immuno-competent individuals.[23-26, 28-40] Besides immunocom-promise, other potential risk factors for balamuthiasis may include contact with stagnant freshwater or with contaminated soil, often through agricultural work, desert motorcycling, dirt-biking, or gardening.[32]

There were 28 CDC-confirmed cases of *Balamuthia mandrillaris* GAE in the US during the reporting period, 1994-2010.[28-41] Of the 28 cases, most occurred in immunocompetent patients (n = 25), with an age range of 1.5 to 89 years.[28-41] The mean age of the study population was 25.04 (± 25.85) years with males (n = 20) outnumbering females ***(Table 4).*** There were no statically significant differences in the mean ages of male and female cases, and there were few survivors of *Balamuthia mandrillaris* GAE (n = 7, 4 males, 3 females) ***(Table 4).***[28-41]

A descriptive analysis of all 28 CDC-confirmed US cases of balamuthiasis identified several presenting characteristics of *Balamuthia mandrillaris* GAE cases including male sex, exposure in a southern-tier US state, Hispanic ethnicity in Southern California, con-tact with soil, and recent organ transplant ***(Table 4).***[28-41] A genetic predisposition to *Balamuthia mandrillaris* GAE has now been confirmed in American Hispanics, who appear less able to produce effective antibodies against the free-living amebae, and may be predisposed by more frequent contact with *Balamuthia*-contaminated soils and aerosols in agricultural and landscaping occupations.[47,48]

The incubation period for *Acanthamoeba* GAE could extend for weeks or months after primary inoculation in the skin, sinuses, or lungs, with subsequent draining ulcers, chronic sinusitis, or pneumonia.[27-33] Although primary inoculation with *Balamuthia mandrillaris* is also via the skin or lungs, the incubation period is shorter than in A*canthamoeba* GAE with a mean of 8.5 days and a range of 1-30 days.[27] The clini-cal diagnostic laboratory, and

neuroimaging manifestations of GAE from either causative pathogen are compared to similar clinical features of PAM in ***Table 1.***[23-41]

Recently, immunodiagnostic tests, such as indirect immunofluorescent ultraviolet microscopy and indirect immunofluorescent antibody ultraviolet microscopy with specific anti-pathogen antibodies, and new PCR assays for identification of pathogen DNA have been developed for diagnostic specimens.[42] In 2006, Qvarnstrom and co-investigators at the CDC described a new multiplex real-time PCR assay for the simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris,* and *Naegleria fowleri,* which will permit rapid and specific detection of a single free-living ameba in clinical specimens within 5 hours.[42]

Neuroimaging studies by axial computerized tomography (CT) and/or magnetic resonance imaging (MRI) in GAE are nonspecific and often include single to multiple space-occupying lesions in the brain from the frontal cortex to the cerebellum with ring-enhancing and other focal effects slightly more com-mon in balamuthiasis than acanthamoebiasis.[15,16,35] Evidence of cerebral edema with increased intracranial pressure will often be present and may include midline shifts, cisternal and ventricular compression, and hydrocephalus.[15,16,35]

Treatment strategies for GAE will include techniques to reduce increased intracranial pressure, craniotomy for biopsy or excision of mass lesions, and combination pharmacotherapy with antifungals, anti-protozoal agents, synergistic antibiotics, and several experimental therapies that have shown promise in *vitro,* such as the phenothiazines and miltefosine ***(Table 1).*** In 2004, Schuster and Visvesvara demonstrated that the phenothiazines demonstrated in vitro efficacy against *Balamuthia mandrillaris* in clinical spec-imens.[43] In 2008, Aichelburg and colleagues in Vienna reported treating a patient successfully with disseminated tuberculosis and acanthamoebiasis with topical and oral miltefosine, and a combination of intra-venous fluconazole, trimethoprim-sulfamethoxazole (TMP/SMX), a synergistic antibiotic (amikacin), and 4 tuberculostatic drugs.[23] The optimum duration of drug therapy for GAE is unknown, but most survivors have been treated for weeks to months.[23,30-46] Although case fatality rates in GAE are very high (90-94% in acanthamoebiasis and > 90% in balamuthiasis), successful drug treatment combinations in acanthamoebiasis and balamuthiasis are compared in ***Table* 1.**[22,23,27, 30-33]

Untested prevention and control strategies for GAE may include (1) consideration of GAE in organ transplant and immunocompromised patients with encephalitis and skin ulcers not improving with standard therapies; (2) recognition of genetic risk factors for acanthamoebiasis and balamuthiasis in Hispanics less able to produce antibodies against causative free-living amebae; and (3) recognition of other soil or stagnant freshwater risk factors in both immunocompetent and immunosuppressed patients with skin ulcers and unexplained meningoencephalitis.[47,48]

Results:

A Case Report of Sappinia Amoebic Encephalitis (SAE)

A single case of SAE was reported in a 38-year-old immunocompetent male farmer in Texas who had contact with grazing animals and fecal-contaminated aerosols and soil.[51,52] The clinical, diagnostic laboratory, and neuroimaging findings and the successful management strategies employed in this case are com-pared to similar outcomes from other free-living amebic infections in ***Table 1.***[51,52]

Results:

Transmission of Free-living Amoebic Infections by Organ Transplantation

Acanthamoebae have been well recognized as opportunistic pathogens causing fatal GAE in patients with AIDS and following organ and tissue transplants since the 1990s.[53-60] In 1999, Oliva and colleagues successfully treated a patient with widely disseminated acanthamoebiasis following a single lung transplant for sarcoidosis with a combination of pentamidine, 5-flu-orocytosine, itraconazole, and topical chlorhexidine gluconate-ketoconazole cream for skin lesions.[44] The patient presented with over 20 painful nodular skin lesions scattered over the trunk and extremities, elevated liver enzymes, and respiratory failure.[44] *Acanthamoeba castellani* trophozoites were recovered from abscessed skin lesion aspirates, bronchoalveolar lavage fluid, and liver biopsy.[44] In 2007, Barete and co-authors reported a fatal case of acanthamoebiasis, despite treatment with pentamidine, 5-fluorocytosine, and itraconazole, in a heart transplant patient who presented with carbuncles and abscesses scattered over the extremities and trunk, septic shock, and multi-organ failure.[25] *Acanthamoeba* trophozoites were recovered from skin lesion biopsies and molecular analysis of the DNA extracted from isolated amebae cultured from skin lesions on agar plates coated with Escherichia coli confirmed the diagnosis of widely disseminated acanthamoebiasis caused by *Acanthamoeba lenticulata.*[25]

Like *Acanthamoeba* species, disseminated *Balamuthia mandrillaris* infections also cause skin lesions and GAE, and were recently associated with 2 case clusters of organ transplant-transmitted infections in the US with 3 deaths in organ transplant recipients.[59,60] In late 2009, a 4-year-old boy living in Mississippi developed a transient febrile illness, was diagnosed with influenza, and treated with antivirals.[59] Within a week, he developed headache and seizures with cerebral edema and ring-enhancing intracerebral lesions on MRI, consistent with a diagnosis of post-influenza autoimmune disseminated encephalomyelitis.[59] Despite supportive treatment for seizures and cerebral edema, seizures recurred, intracranial lesions progressed, and cerebral hernia-tion and brain death occurred approximately 2 weeks following the onset of CNS symptoms.[59] The child's heart, liver, and kidneys were transplanted into 4 different recipients at 3 transplant centers.[59]

Histopathological examination of donor brain tissue at the CDC was consistent with free-living amebic encephalitis, later PCR-confirmed as *Balamuthia mandrillaris* GAE.59 The donor frequently played out-doors and had confirmed soil and wading pool exposures.[59] Of the 4 organ transplant recipients, asymptomatic pediatric heart and liver transplant recipients were treated prophylactically for *Balamuthia* exposures and remained well; a 31-year-old female kidney transplant recipient died of PCR-confirmed *Balamuthia* GAE despite weeks of intensive care and therapy with multiple agents known to be effective for balamuthiasis; and a 27-year-old male recovered from PCR-confirmed *Balamuthia* GAE with permanent neurologic sequelae.[59]

In September 2010, the CDC reported a second PCR-confirmed cluster of organ transplant-transmit-ted *Balamuthia* GAE in Arizona.[60] The common organ donor, a 27-year-old Hispanic male landscaper, with a 6-month history of a non-healing skin lesion on his back attributed to an insect bite, died from a presumed stroke.[60] The donor's heart, liver, pancreas, and kidneys were transplanted into 4 recipients, 2 of whom, a kidney-pancreas recipient and a liver recipient, died of PCR-confirmed *Balamuthia* GAE.[60] Asymptomatic heart and kidney transplant recipients were placed on prophylactic combined antimicrobial therapy and have remained well.[60]

Discussion:

Why have free-living amoebic infections emerged as public health threats today?

Once considered non-pathogenic, free-living amoebae have emerged over recent decades as significant pathogenic threats to human health for several reasons including the following. (1) Free-living amoebae are widely distributed in soil and freshwater throughout the temperate and tropical world, have environmen-tally stable cyst forms for over-wintering, and have taken advantage of longer warm seasons to parasitize humans in outdoor pursuits. (2) Some free-living, become opportunistic pathogens in immunocompro-mised human hosts; but others may evade host responses in immunocompetent individuals. (3) Free-living amoebae are resistant to antimicrobial monotherapy and require combined therapy with a variety of antimicrobials and experimental agents. (4) Free-living amoebic infections are often difficult to diagnose unless suspected; the laboratory is alerted to the possibility of amebic forms in diagnostic specimens; and confirmatory immunological and molecu-lar tests are available, usually at distant reference labs. (5) Lastly, some ethnic groups, such as Hispanics, may be genetically predisposed to GAE because they can-not muster protective antibody responses to phylogenetically related Acanthamoeba spp. and Balamuthia mandrillaris.[47,48]

Conclusions and Recommendations

Fatal free-living amoebic encephalitis may be more common than initially reported and underdiagnosed, especially among organ donors with fatal encephalitis of uncertain etiologies. Although organs (liver, kid-neys) were harvested from a donor dying from PAM in 1995 and transplanted into 3 recipients without transplant-transmitted *N. fowleri* infections, donors dying from other causes of meningoencephalitis associated with granulomatous skin lesions and ring-enhancing intracebral lesions on neuroimaging studies should be screened by specialized diagnostic studies for other free-living amebic infections of the brain capable of hematogenous dissemination, including *Acanthamoeba*-associated GAE and *Balamuthia mandrillaris* GAE.[61]

Clinicians should suspect free-living amoebic infections of the CNS in refractory cases of meningoencephalitis initially managed as aseptic or bacterial infections, especially in patients predisposed to such infections by regions visited, behavioural practices, environmental or occupational exposures, ethnicity, or immunosuppression by chemotherapy or organ transplant anti-rejection therapy. Future investigations will be required to determine the significance of freshwater wakeboarding, popular among adolescents, as a significant recreational risk factor for PAM and to determine any dose-response effects of global warming on rising freshwater and topsoil temperatures and the multiplication and infectivity of aquatic and terrestrial free-living amebae.

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Table 3

A Descriptive Epidemiological Analysis of CDC-Confirmed Cases of Primary Amebic Meningoencephalitis -United States, 1937-2007 (N = 121)

Study periods compared		
# *Years*	NA	NA
# Cases in study (%) Annual period prevalence	84 (28%)	
{%%_	NA	NA
Case fatality rates (%) # Case clusters > 4/period	0.879	0.800% 0.001**
# Deaths /period	0/30 years	< 0.001**
	84/30 years	

NA: Not applicable.

* The Poisson distribution was used to model the number of case clusters per year and the number of deaths per year in order to compare rates during time periods, 1937-1976 vs. 1977-2007. Hypothesis tests were performed using the "ratio.test" function from the R package of the same name (R Development Core Team, 2010). This function was used to perform a two-sided exact rate ratio test assuming Poisson counts. A 5% significance level was used for all hypothesis tests.

** Statistically significant; a = 0.05, p < 0.05.

Table 4

The Demographic and Exposure Characteristics of CDC-Confirmed Cases of *Balamuthia mandrillaris* Granulomatous Amebic Encephalitis -United States, 1999-2010)

Gender (%)	Male (%)	Female (%)
N (%)	20 (71%)	8 (29%)
Mean age ± standard deviation (years)	21.82 ± 25.85	19.38 ± 27.06
Age range (years)	1.5-89	2-72
*Age significance test**	t = 0.223; P =0.825	
Ethnicity (%)	Hispanic (%)	Caucasian (%)
	14 (50%)	14 (50%)
Ethnicity in California (%)	9 (75%)	3 (25%)
	Exposure characteristics	
States of exposures (%)	Southern-tier**	Other US states
	24 (85%)	2 (15%)
California exposures (%)	California	Other US state
	12 (43%)	16 (57%)
Soil exposures (%)	Soil exposed	Not exposed
	9 (32%)	19 (68%)
Stagnant water exposures	Stagnant water	Not exposed
	2 (4%)	27 (96%)
Recent organ transplant		
	4 (8%)	2 (92%)
	Exposure characteristics	
Deaths (%)	Died	Survived
	22 (79%)	6 (21%)

Case fatality rate (%) 79% *Two-tailed t-test.

**8 Southern-tier US states: AZ, CA, FL, GA, KY, MS, SC, TX.

Figure 1

The life cycle of *Naegleria fowleri,* causative agent of primary amebic meningoencephalitis (PAM). Source: US Centers for Disease Control and Prevention(CDC). Available at www.dpd.cdc.gov/DPDx/HTML/ImageLibrary. No copyright permission required.

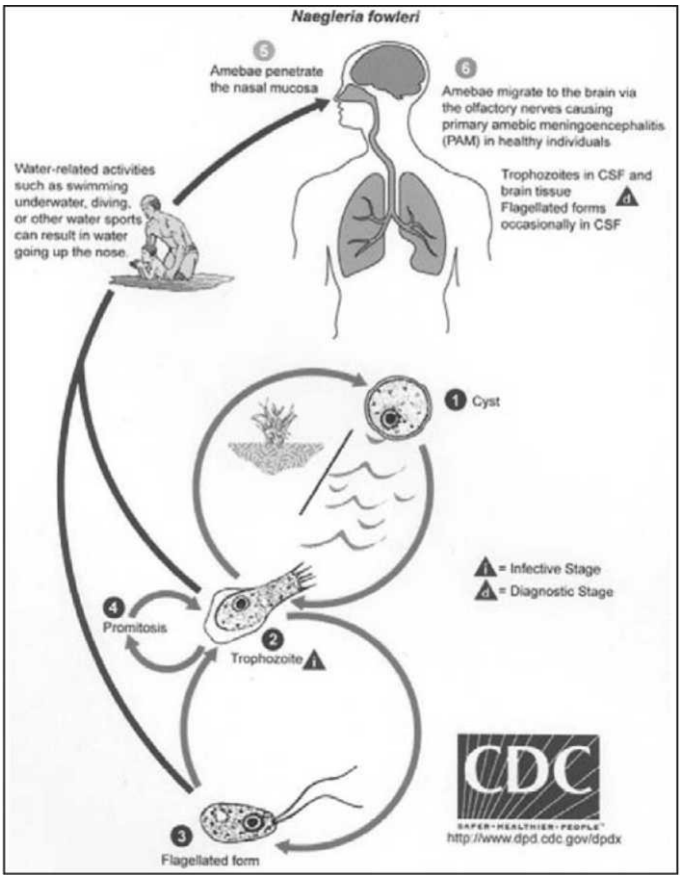

Table 3

A Descriptive Epidemiological Analysis of CDC-Confirmed Cases of Primary Amebic Meningoencephalitis -United States, 1937-2007 (N = 121)

Study periods compared		
# *Years*	NA	NA
# Cases in study (%) Annual period prevalence	34 (28%)	87 (72%)
{%%_	NA	NA
Case fatality rates (%) # Case clusters > 4/period	0.879	0.121
	0.001**	
# Deaths /period	0/30 years	30/30 years
	< 0.001**	

NA: Not applicable.

* The Poisson distribution was used to model the number of case clusters per year and the number of deaths per year in order to compare rates during time periods, 1937-1976 vs. 1977-2007. Hypothesis tests were performed using the "ratio.test" function from the R package of the same name (R Development Core Team, 2010). This function was used to perform a two-sided exact rate ratio test assuming Poisson counts. A 5% significance level was used for all hypothesis tests.

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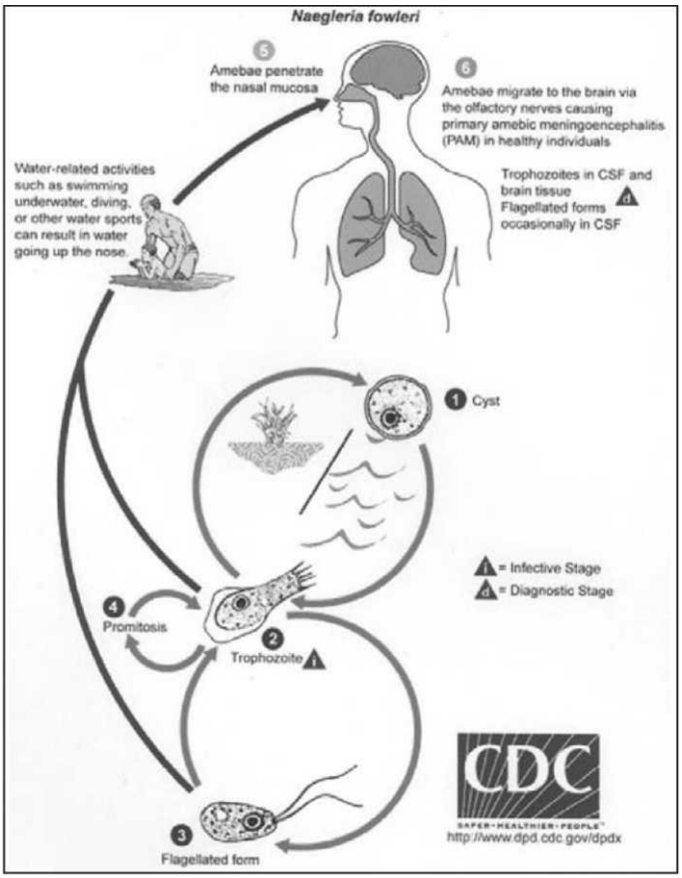

Exposure characteristics

Deaths (%) Died Survived

Figure 4

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