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Free-living, Amphizoic and Opportunistic Amebas

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Amebas belonging to the genera *Naegleria, Acanthamoeba* and *Balamuthia* are free-living, amphizoic and opportunistic protozoa that are ubiquitous in nature. These amebas are found in soil, water and air samples from all over the world. Human infection due to these amebas involving brain, skin, lung and eyes has increased significantly during the last 10 years. The epidemiology, immunology, protozoology, pathology, and clinical features of the infections produced by these protozoa differ strikingly.

Infection by the pathogenic *Naegleria fowleri* is acquired by exposure to polluted water in ponds, swimming pools and man-made lakes. Raised temperatures during the hot summer months or warm water from power plants facilitate the growth of *N. fowleri*. *N. fowleri* is a thermophilic ameba that grows well in tropical and subtropical climates. The CNS infection, called Primary Amebic Meningoencephalitis (PAM), produced by *N. fowleri* is characterized by an acute fulminant meningoencephalitis leading to death 3-7 days after exposure. Victims are healthy, young individuals with a history of recent water-related sport activities. The portal of entry is the olfactory neuroepithelium. The pathologic changes are an acute hemorrhagic necrotizing meningoencephalitis with modest purulent exudate, mainly at the base of the brain, brainstem and cerebellum. Trophozoites can be seen within the CNS lesions located mainly around blood vessels. Thus far 179 cases have been reported; 81 in the USA alone.

***Balamuthia mandrillaris* and several species of *Acanthamoeba* are pathogenic "opportunistic" free-living amebas which cause Granulomatous Amebic Encephalitis (GAE) in humans and animals. GAE is an infection, usually seen in debilitated, malnourished individuals, in patients undergoing immunosuppressive therapy for organ transplants and in Acquired Immunodeficiency Syndrome (AIDS). The granulomatous component is negligible, particularly in immunocompromised individuals. Pathologically these amebas produce a patchy, chronic or subacute granulomatous encephalitis with the presence of trophozoites and cysts. The portal of entry is probably through the respiratory tract or an ulceration of the skin reaching the CNS by hematogenous spread. As of October 1, 1996, 166 cases (103 due to *Acanthamoeba* and 63 due to *Balamuthia)* of GAE have been reported from around the world. Of these 103 cases due to *Acanthamoeba* (72 have been reported in the USA alone, > 50 in AIDS). It is well known that several species of *Acanthamoeba* can also produce, chronic sight threatening ulceration of the cornea called *Acanthamoeba* keratitis (AK), mostly in contact lens wearers or in individuals with minor corneal abrasions. Hundreds of cases of AK have been documented world wide.**

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Introduction

Currently, we are witnessing a dramatic emergence and re-emergence of infectious diseases in both developing and developed countries. Some infectious diseases that are prevalent in the tropics are, in fact, being found much more frequently in the temperate regions. The designation of "tropical diseases", "tropical medicine" and "tropical neuropathology" is acquiring new meanings and a wider dimension. The perceived low prevalence of this disease in the tropics, however, is probably due to inexperience in the pre- and/or post-mortem diagnosis of the disease.

This paper will deal specifically with the free-living amebas and the diseases produced by them. Therefore, it is pertinent to start with some historical notes to understand better the evolution in the knowledge of these micro-organisms and the diseases they produce. The study of parasitic, free-living and amphizoic amebas started in the late 19th and the beginning of the 20th century. Fedor Aleksandrovich Lösch in St. Petersburg (Russia) in 1875 described the clinical symptoms and the relationship between the

Entamoeba histolytica and chronic dysentery in a 24 year old farmer (57). Eugene Penard in 1890 described a saprophytic limax ameba and called it *Hartmannella limax* (77). Simon Flexner in 1892 reported a case of a 62 year old white man from Virginia with a large jaw abscess communicating with the oral cavity probably due to a parasitic ameba (31).

F. Schardinger in 1899 isolated, described and named the *Amoeba gruberi* as an amebo-flagellate from the stools of a patient with dysentery. This was probably the first time that clinical symptoms were associated with pathogenicity and etiology (82). In spite of that, Fritz R. Schaudinn in 1903 stated that the parasitic *Entamoeba histolytica* caused amebic dysentery and was the only pathogenic ameba for man in contrast to the harmless *E. coli* (83). Erich Vahlkampf in 1905 published an elegant study on the *Amoeba limax* (93). Kurt Näegler in 1909 described in a well illustrated paper *Naegleria gruberi* as a non-pathogenic ameba (71). This free-living ameba is morphologically similar to the pathogenic *N. fowleri* (47). Max Hartmann in 1910 described with great detail the *Entamoeba histolytica* (40). Edouard Chatton and P. Lalung-Bonnaire in Paris described in 1912 a Vahlkampfia sp. ameba isolated from the stool of a patient with chronic diarrhea noting the absence of a flagellate state (15). These pioneer workers awoke the interest in protozoa, making basic discoveries recording their observations and building the foundations of protozoology and the biological characteristics of free-living, amphizoic and parasitic amebas.

Sir Aldo Castellani in Oxford (England) isolated in 1930 a free-living ameba growing in a yeast culture of *Cryptococcus pararoseus*. It was named by Douglas, *Hartmannella castellanii*, and placed it in the genus *Hartmannella*, but later was reclassified as *Acanthamoeba castellanii* (13,25). Jahnes, Fullmer and Li in 1957 reported the "spontaneous" contamination of a culture of monkey kidney cells by free-living amebas (45).

Clyde G. Culbertson and his collaborators in 1958 described an encephalitis in mice produced by an *Acanthamoeba* that contaminated a cell culture during the development of the polio vaccine (19,20). The polio vaccine was being produced at the Eli Lilly Laboratory in Indianapolis and each batch was being tested for safety on monkey kidney cell cultures. Plaques appeared in cell cultures inoculated with the vaccine as well as the uninoculated control suggesting an unknown virus. Cortisone treated monkeys and mice were inoculated with unfiltered, uncentrifuged and undiluted culture fluids. The animals developed prostrating illness and died (19).

Histological examination of the brain of these animals showed "unusual cells" responsible for this infection. Microscopic examination of the preserved culture fluids revealed free-living amebas, identified originally as *Acanthamoeba* spp. Lilly A-1 strain. But because of the confusion that existed regarding the taxonomic uncertainties and nomenclatural difficulties of *Acanthamoeba* and *Hartmannella* at that time, Culbertson designated these amoebae as belonging to *Hartmannella-Acanthamoeba* group or simply "H-A amoebae." It is now well established that *Hartmannella* and *Acanthamoeba* belong to distinct genera and that no true hartmannellid ameba is known to cause human CNS infection. All references to *Hartmannella* in human tissues should therefore be corrected to read as *Acanthamoeba* or *Balamuthia*. Culbetson's Lilly A-1 strain is now designated as *A. culbertsoni* in honor of Dr. Clyde G. Culbertson. *Acanthamoeba* had been found earlier to occur as an air-borne contaminant of cell cultures in other laboratories (44). Had it not been for animal inoculation this would have passed as simply another instance of cell culture contamination.

The discovery that this presumably innocuous, harmless and saprophytic free-living ameba, even when simply instilled intranasally could invade the olfactory mucosa, migrate to the brain and produce a rapidly fatal meningoencephalitis was incredible and perplexing. This finding suggested the possibility of the occurrence of infection in human beings and animals and the neuropathogenicity of free-living amebas. This important discovery provided the key to the recognition of human cases of amebic encephalitis in man in 1965 when Malcolm Fowler and Rodney F. Carter in Adelaide (Australia) reported the first 4 human cases of meningoencephalitis produced by free-living amebas (32). The virulent free-living ameba which produced the death in one Australian child in 1961 and in three additional children from the same district in 1965 proved to be *Naegleria fowleri* different from the *Acanthamoeba* that was originally described by Culbertson and his co-workers. The term Primary Amebic Meningoencephalitis (PAM) was coined by Cecyl G. Butt when he reported the "first" case from the USA in 1966 due to *N. fowleri* (10). Carter presented his memorable paper on January 20, 1972 at the Ordinary Meeting of the Royal Society of Tropical Medicine and Hygiene in London. Dr. P.C.C. Garnham, at that time, made the following remark: "Breaking a barrier is always a thrill; whether it is the "sound barrier" or the "barrier of host parasite specificity,... Dr. Carter's work on the amoebo-flagellates provides an example of overcoming an even more formidable obstacle. He has shown, in one of the most interesting papers presented to the Society, how the gulf between free-living and parasitic protozoa can be traversed to give rise to a fulminating infection in the human host..." (11). Since that memorable report, free-living amebas have been recognized with increasing frequency as important pathogens in normal individuals, but particularly in immunosuppressed hosts (1,47,48).

Position of *Naegleria*, *Acanthamoeba* and *Balamuthia* in the taxonomic scheme

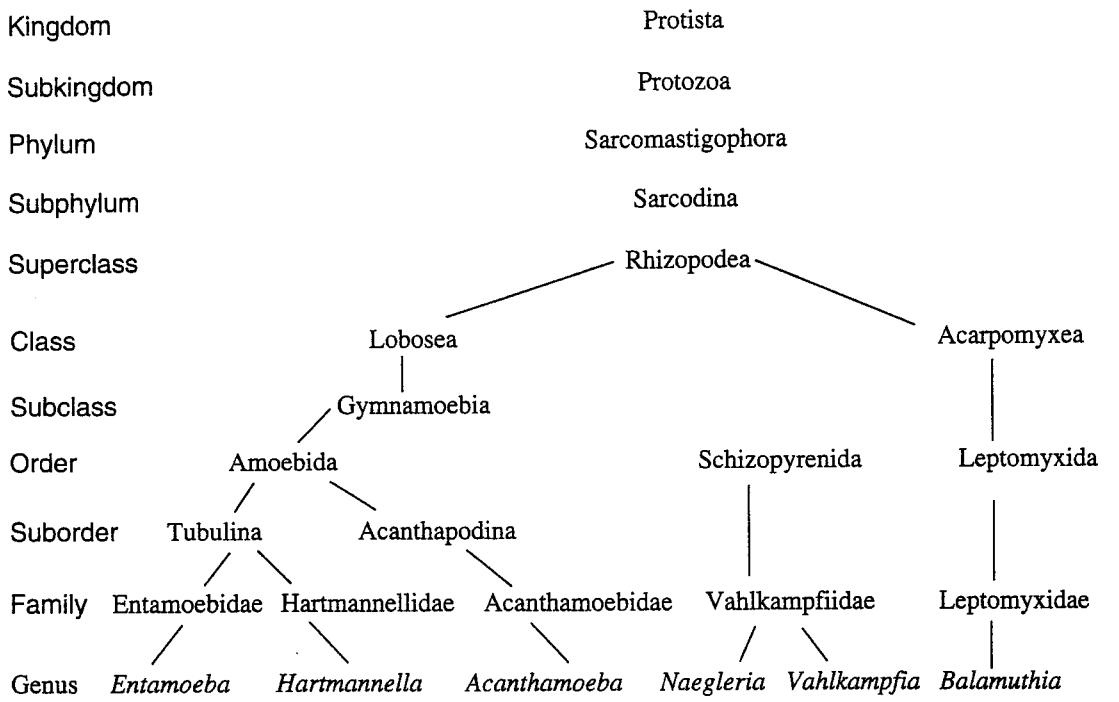

Table 1.

Evidently PAM was not a new disease, but had already occurred, although it had escaped recognition until the timely report from Australia. In 1968, Lubor Cerva and collaborators reported 16 fatal cases from Czechoslovakia that occurred from 1962 to 1965 (14). Also during 1968 eight cases of fulminant meningoencephalitis was reported from the Richmond (Virginia) area. Seven of these cases occurred in a cluster during the summers of 1951 and 1952 (12). A retrospective study from the Pathology Department files of the Medical College of Virginia dating back to 1920, done by John G. dos Santos, discovered that all these cases were due to *N. fowleri* and the first case occurred on July 15, 1937 (26).

Other cases of amebiasis were previously reported before the recognition of *Naegleria fowleri* pathogenicity. E.H. Derrick from Brisbane reported in 1948 a case of a 22 year old Japanese soldier who was captured on January 20, 1943 near Buna, New Guinea and died seven weeks later with disseminated "free-living amebas" proven to be due to *N. fowleri* after immunofluorescent antibody technique (IFAT) performed by Dr. W.P. Stamm (23). Also, James W. Kernohan et al in 1960 reported a case of a 6 year old girl from Tucson (Arizona) who died with a brain granuloma due to free-living ameba originally thought to be due to *Iodamoeba bütschlii*, but later proved to be due to *Acanthamoeba* sp (52).

D. Patras and J.J. Andujar in 1966 reported a case of a 59 year old drug addict from Texas who died in September 1964 after 18 days of neurological symptoms with meningoencephalitis due to a free-living ameba originally believed to be due to *Acanthamoeba* spp., but later recognized as *N. fowleri* by immunoperoxidase technique (75). Finally, W.St.C. Symmers in a retrospective study reported in 1969, two cases from Great Britain, of these, one had occurred in Essex, east of London in a young man who had died in 1909. The diagnosis was made by examination of a slide from a museum. The other was from a 10 year old girl from Belfast who had died in 1937 after a short clinical history following a swimming accident in a pool 10 days previously (89).

The first clearly identified human cases of GAE due to *Acanthamoeba* spp. were reported in 1972 by B.V. Jager and W.P. Stamm (44) and in 1973 by V.B. Robert and L.B. Rorke (79). The term Granulomatous Amebic Encephalitis (GAE) was proposed by A.J. Martinez to describe the histopathological features produced by *Acanthamoeba* spp. and to differentiate it from PAM (63).

Free-living or amphizoic amebas whether *N. fowleri* or the opportunistic species of Acanthamoeba and B. mandrillaris are preferentially neurotropic. On the other hand the parasitic *E. histolytica* rarely

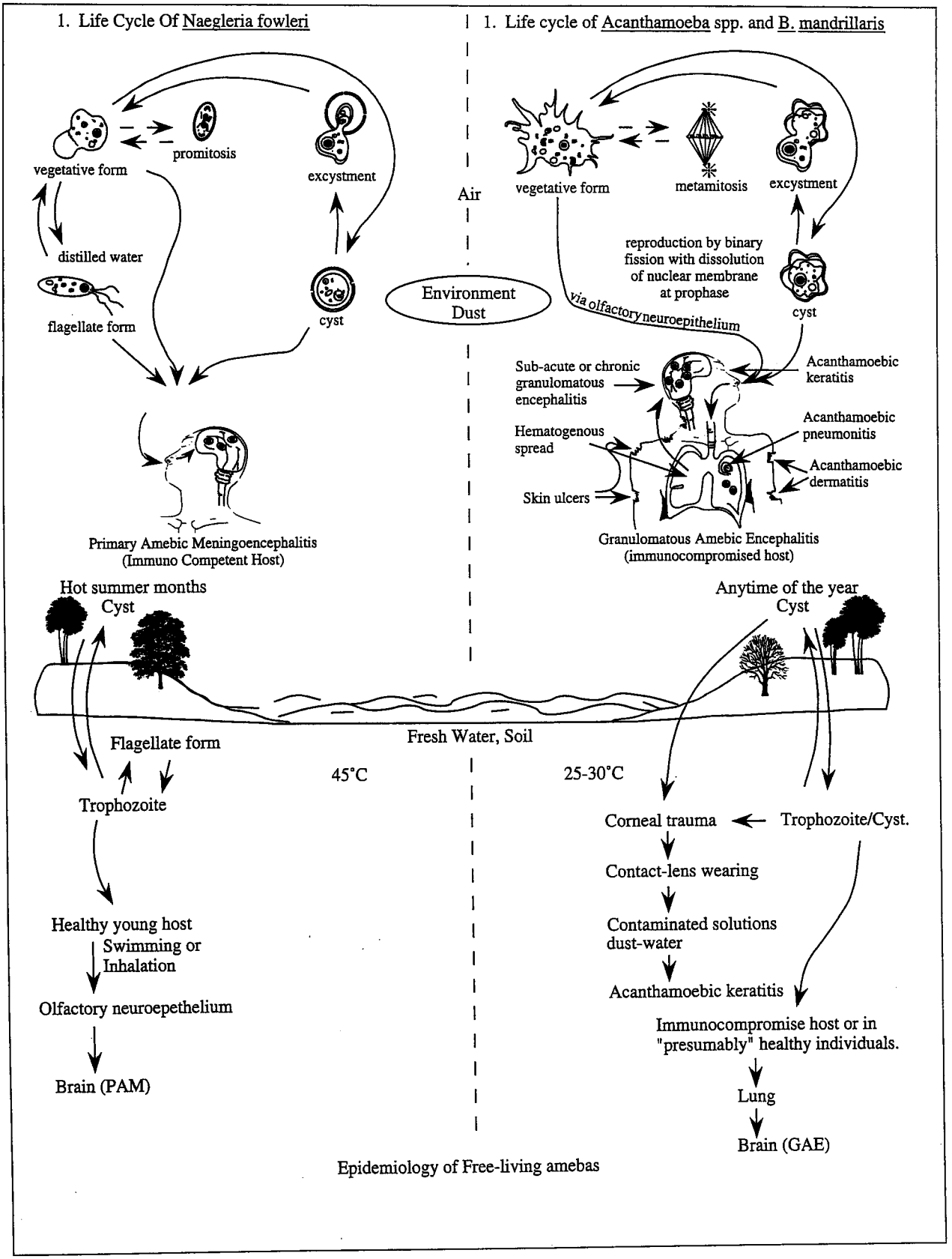
1. Life Cycle Of Naegleria fowleri
vegetative form
promitosis
excystment
distilled water
flagellate form
cyst
Primary Amebic Meningoencephalitis
(Immuno Competent Host)
1. Life cycle of Acanthamoeba spp. and B. mandrillaris
Air
Environment
Dust
vegetative form
metamitosis
excystment
reproduction by binary
fission with dissolution
of nuclear membrane
at prophase
via olfactory neuroepithelium
cyst
Sub-acute or chronic
granulomatous
encephalitis
Hematogenous
spread
Skin ulcers
Acanthamoebic
keratitis
Acanthamoebic
pneumonitis
Acanthamoebic
dermatitis
Granulomatous Amebic Encephalitis
(immunocompromised host)
Hot summer months
Cyst
Anytime of the year
Cyst
Fresh Water, Soil
Flagellate form
Trophozoite
45°C
25-30°C
Corneal trauma
Trophozoite/Cyst.
Contact-lens wearing
Contaminated solutions
dust-water
Acanthamoebic keratitis
Immunocompromise host or in
"presumably" healthy individuals.
Lung
Brain (GAE)
Healthy young host
Swimming or
Inhalation
Olfactory neuroepethelium
Brain (PAM)
Epidemiology of Free-living amebas

Table 2.

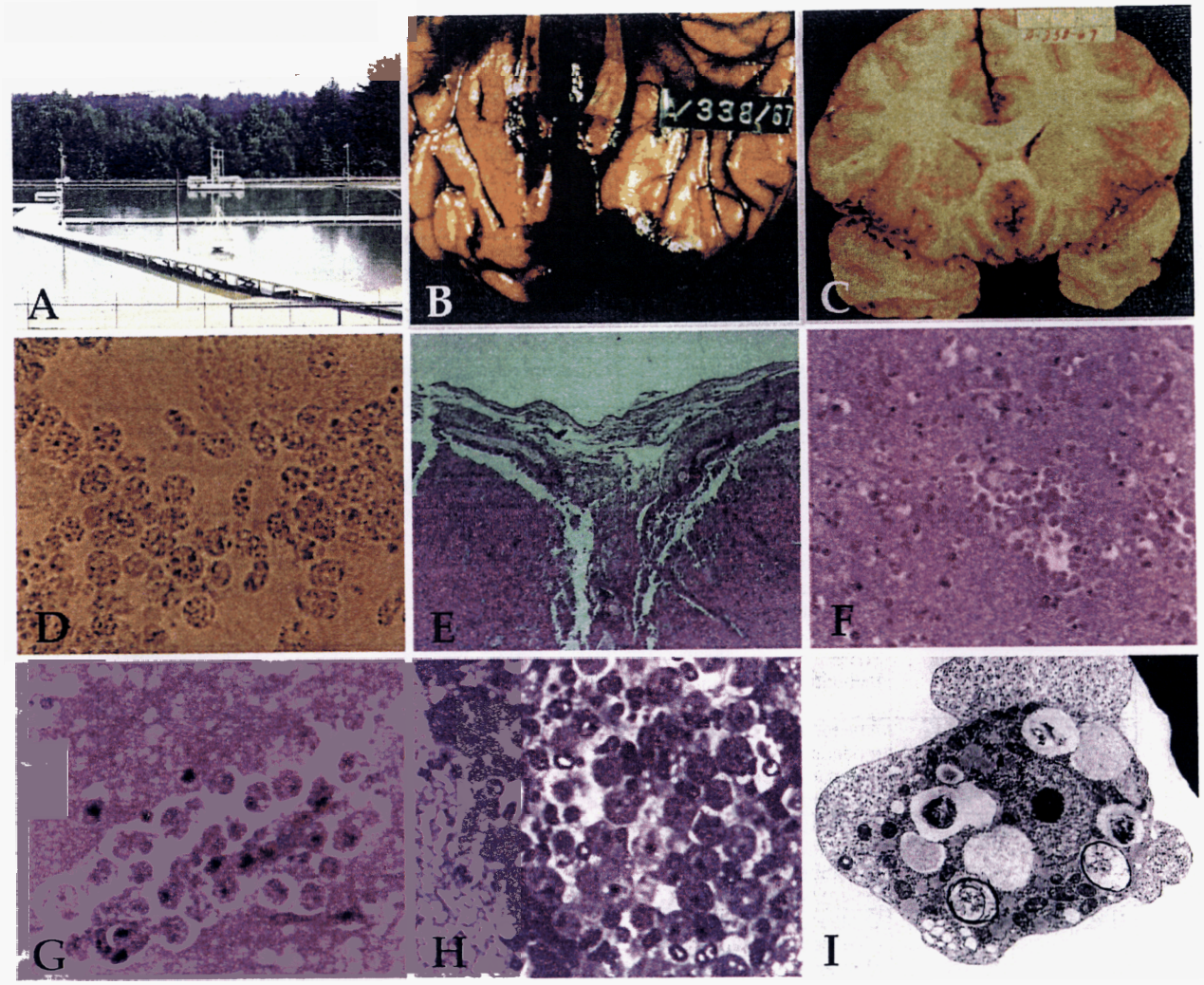

Figure 1. A. View of the man made "Lake Chester" south of Richmond, Virginia. Nine patients swam there before the development of clinical signs and symptoms of PAM. **B.** Gross appearance of the orbitofrontal cortex and the olfactory bulbs and tracts showing foci of recent hemorrhage and negligible amounts of purulent exudate around congested blood vessels. *Naegleria fowleri* was cultured and isolated. (From Medical College of Virginia A338-67) **C.** Coronal section at the level of the genu of the corpus callosum in a case of PAM due to *Naegleria fowleri*. There is pronounced hemorrhagic-necrotizing encephalopathy at the inferior aspect of the frontal lobes and cingulate gyri. Same case as Fig. 1B (with permission). **D.** Unstained "touch preparation" in a case of PAM. Numerous amebic trophozoites are detected (wet mount preparation X200). **E.** There is extensive leptomeningeal acute inflammatory exudate with disruption of the cerebral cortex in a typical case of PAM (H&E X150). **F.** There are clusters of amebic trophozoites around a blood vessel in a case of PAM. There is minimal inflammatory reaction within CNS parenchyma (H&E X250). **G.** There are numerous amebic trophozoites on the perivascular space from a typical case of PAM (Masson trichrome X400). **H.** Clusters of *N. fowleri* showing the conspicuous karisome surrounded by a clear nuclear halo (1 µm thick section, Toluidine blue X400). **I.** Ultrastructural features of a trophozoite of *Naegleria fowleri* showing cytoplasmic vacuoles, myelin bodies and the spherical dense karisome in the center of a finely granular nuclear chromatin (EM X6000).

involves the brain. Amebic abscesses of the brain due to *E. histolytica* occur as a secondary involvement from a primary source in the colonic mucosa leading to pulmonary and liver abscesses (56).

Classification

Free-living amebas of the genera *Naegleria, Acanthamoeba* and *Balamuthia* are mitochondria bearing eukaryotic protists and have been included in the superclass Rhizopodea and classified further as shown in Table 1. Since they are capable of living as parasites or as free-living organisms, they are often called amphizoic amebas. These amebas can produce basically two types of disease in the central nervous system (CNS) and other organs: a fulminant, rapidly progressing CNS infection called PAM produced by *Naegleria fowleri* and a chronic slowly progressive disease called GAE, produced by several species of *Acanthamoeba* and by *Balamuthia mandrillaris* (60).

Reports of infections involving brain, skin, lung and eyes have increased markedly during the last decade. This increase coincides with the identification, culture and isolation of the causative organism. The only species of *Naegleria* known to cause human

Comparison of clinical, protozoological and pathological features of Granulomatous Amebic Encephalitis (GAE), Primary Amebic Meningoencephalitis (PAM), and *Acanthamoeba* Keratitis (AK)

FEATURES	GAE	PAM	AK
Etiologic Agents	*Acanthamoeba* spp. *Balamuthia mandrillaris* Trophozoites: 15-35 µm Cysts: 12-30 µm	*Naegleria fowleri;* Trophozoites: 10-20 µm Cysts: 8-15 µm	*Acanthamoeba castellanii, culbertsoni, polyphaga, hatchetti* and *rhysodes*
Predisposing Factors	Immunodeficiency; AIDS; Debilitating chronic diseases.	Previous good health; Exposure to contaminated freshwater or dust.	Good health, corneal trauma, contaminated contact lens wearing
Epidemiology	Worldwide distribution; Any time of the year.	Worldwide distribution; Hot summer months.	Worldwide distribution Anytime of the year
Usual Portals of Entry	Lungs; skin; olfactory neuroepithelium.	Olfactory neuroephithelium.	Corneal abrasion
Incubation Period	Probably weeks to months.	1 to 15 days.	Probably days
Clinical Course; Prognosis	Subacute or chronic (several weeks to months); Fatal 100%.	Acute; Fulminant, death (4 to 6 days after onset of symptoms); Fatal 98%.	Subacute or chronic; Good if properly treated
Clinical Symptoms and Signs	Personality changes; confusion; sommolence; irritability; seizures; hemiparesis, aphasia; cranial nerve palsies; diplopia; nausea; headache; dizziness.	Headache; nausea; vomiting; stiff-neck; fever; coma.	Ocular discomfort; severe ocular pain; typical corneal ring "infiltrate" photophobia; blurred vision
Differential Diagnosis	Acute pyogenic (bacterial) meningitis	Brain tumors, abscesses, TB or fungal meningitis, cerebral hematomas	Herpes keratitis; Bacterial or fungal keratitis
Cerebrospinal Fluid	Amebas rarely observed; moderate mononuclear pleocytosis; elevated protein; low glucose.	Trophozoites usually present; polymorphonuclear pleocytosis; elevated protein; low glucose.	Unremarkable
Gross Pathology	Multiple, necrotic foci in brain; focal chronic leptomeningitis; space - occupying lesions.	Hemorrhagic necrosis: orbito frontal cortex, olfactory bulbs, and base of brain; acute leptomeningitis.	Ulceration of corneal epithelium. Conjunctival congestion
Histopathology	Cysts and trophozoites, often perivascular; mononuclear cells with or without multinucleated giant cells; vasculitis.	Trophozoites only (does not encyst in human host), often perivascular; polymorphonuclear leukocytes and hemorrhagic necrosis.	Acute and chronic inflammation of corneal tissue. Presence of amebic trophozoites and cysts.
Extracranial Involvement	Lungs, cornea, skin, Agonal dissemination throughout the body.	None known.	None known
Treatment (*)	Ketoconazole cream; Itraconazole; Miconazole; Sulfametazine; Pentamidine IV and topical; Chlorhexidine gluconate	Amphotericin B	Polyhexamethylene biguamide, Propamidine isethionate

Table 3. (*)See: The Medical Letter, Issue 961;November 10, 1995.

*Modified from Martinez, AJ and Janitschke K. Amöbenenzephalitis durch Naegleria und Acanthamoeba. Vergleich und Gegenüberstellung der Organismen und der Erkrankungen. Immunität und Infektion. 1979;7, 57-64 and Martinez AJ and DeJonckheere JF (1981) Les Infections par les amibes libres. Bull Inst Pasteur 79:171-205.

infection is *Naegleria fowleri* (also called *Naegleria invadens* and *Naegleria aerobia* which is morphologically identical to the non-pathogenic *Naegleria gruberi*. Among the other species of *Naegleria, N. andersoni, N. australiensies, N. jadini* and *N. lovanensis* (48) only *N. australiensis* has been found to be pathogenic to mice. The opportunistic species of *Acanthamoeba* includes *Acanthamoeba castellanii, A. polyphaga, A. mauritaniensis, A. palestinensis, A. hatchetti, A. culbertsoni, A. astronyxis, A. royreba, A. divionensis, A. rhysodes, A. healyi, A. jacobsi, A. terricola, A. lenticulata* and *A. griffini*. An important aspect of the biology of free-living amebas is that different strains of the same species differ in their degree of pathogenicity or virulence (49,63,64,66). So far *Hartmannella* has not been found to be pathogenic for human beings or animals.

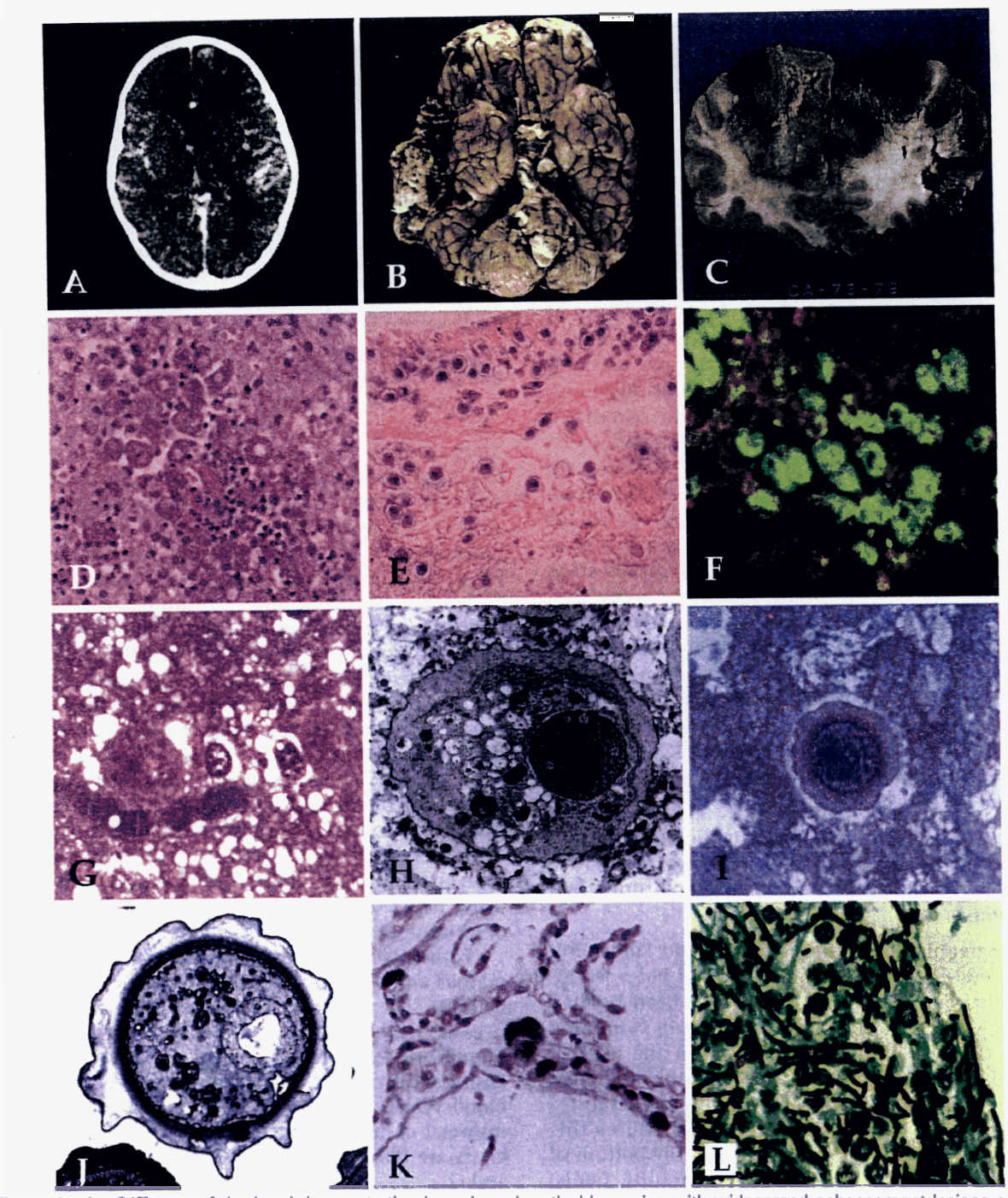

Figure 2. A. CAT scan of the head demonstrating irregular subcortical lucencies with widespread enhancement lesions on both cerebral hemispheres from a 2 1/2 year old boy with GAE. **B.** Base of the cerebral hemispheres, cerebellum and brainstem, from the same case. There are multifocal hemorrhagic lesions on both cerebral hemispheres and a large necrotic area on the right frontoparietal lobe (courtesy of E. Yunis, M.D., Children's Hospital of Pittsburgh). **C.** Coronal section of frontal lobes, demonstrating extensive areas of hemorrhagic encephalomalacia involving cerebral cortex and subcortical white matter. **D.** Granulomatous Amebic Encephalitis due to *B. mandrillaris*. There are numerous amebic trophozoites associated with modest lymphocytic response (H&E X450). **E.** Granulomatous Amebic Encephalitis due to *Acanthamoeba castellanii*. There are numerous amebic trophozoites and cysts within perivascular space (H&E X250). **F.** Strong positivity demonstrating amebic trophozoites, using antiserum against *A. Rhyzodes* (IFAT X400). **G.** Trophozoite of *Acanthamoeba culbertsoni* within infected CNS tissue (1 µm thick section, Toluidine blue X600). **H.** Ultrastructural appearance of a trophozoite of *Balamuthia mandrillaris* within CNS tissue of an experimentally infected mouse (X5000). **I.** Cyst of *B. mandrillaris* showing a thick wall (1 µm thick section, Toluidine blue X800). **J.** Ultrastructural feature of a cyst of *B. mandrillaris* showing an undulating outer wall, a dense inner wall and a clear mesocyst (EM X5000). **K.** Section of the lung from a 38 year old white man, immunosuppressed after a kidney transplant. He died of GAE due to *A. castellanii*. There are cytomegalovirus infected cells within lung parenchyma (CMV, IPA X250). **L.** Section of the lung, same case as K showing hyphae of *Candida albicans* and amebic trophozoites and cysts (Grocott Methenamine Silver X250).

Free-living Amebas as Human Pathogens

Naegleria fowleri. Morphology and Life Cycle. N. fowleri has three stages in its life cycle: the trophozoite, the flagellate and the cyst (Table 2). The vegetative or trophozoite form of *N. fowleri* measures approximately 15 to 25 µm (Fig. 1I). In culture they are active and constantly change in size and shape. When rounded, like within the CNS tissue in PAM, they measure about 8 to 12 µm in diameter. The cytoplasm is finely granular with multiple mitochondria, lysosomes and vacuoles and rounded pseudopods or lobopodia that are related to motility of the vegetative form. The nucleus is usually centrally placed with clumps of compact, finely granular chromatin containing a dense, spherical nucleolus. In histological preparations the nuclear chromatin appears as a clear halo with a centrally located nucleolus or karyosome or endosome. The flagellate form is usually pear shaped and biflagellated. Occasionally, however, multiple flagella (up to 10) have been seen. The flagellate organism is a non-feeding stage and reverts back to the trophic stage (Fig. 1I). The cysts are spherical, 8 to 12 µm in diameter, composed of dense wall with one or two flat pores. PAM cysts are not found within infected CNS tissues. *N. fowleri* reproduce by binary fission. The mechanism of nuclear division is promitotic, wherein, the nucleolus and the nuclear membrane persists throughout the cell division. The nucleolus elongates and forms dumbbell-shaped structure before dividing into nucleoli (49).

The trophozoite of *N. fowleri* is thermophilic and can grow and multiply at temperatures of 40 - 45° C. They encyst in response to adverse environmental conditions.

Geographic distribution: Ecology and Epidemiology. *Naegleria fowleri* is ubiquitous and has a worldwide distribution (Fig. 3). They have been isolated from the soil, dust in air, fresh water from swimming pools, (Fig. 1A) hot springs, and from the nose of normal individuals (22,99). They multiply and grow well in tropical climates and in hot temperatures between 40 to 45°C. In colder temperatures this ameba probably encyst and remains in the bottom of sediments of lakes, rivers, and swimming pools. They feed on bacteria and other sources of organic matter. In fact they are the main predators controlling bacterial populations in soils (96).

Primary Amebic Meningo-encephalitis (PAM). As of October 1, 1996 more than 175 cases of PAM have been reported worldwide. Eighty-one cases have been reported from the USA alone (Fig. 3).

Clinical manifestations. *N. fowleri* can produce fulminant, usually fatal acute meningoencephalitis in children and young adults, usually in excellent health who have been swimming and diving during the hot summer months or in heated swimming pools. The clinical course of PAM is rapid and the incubation period is short, only 3-5 days. The disease is characterized by severe frontal headache, fever, nausea and vomiting and stiff neck (Table 3). This is followed by coma, occasionally seizures and then death due to cardiorespiratory failure as a consequence of severe cerebral edema (10,19,32).

Differential diagnosis. The differential diagnosis should include acute purulent bacterial meningoencephalitis (Table 1). PAM cannot be differentiated clinically from acute bacterial infection. The positive diagnosis of PAM should be based on the correlation between the clinical signs and symptoms, laboratory and radiographic findings in the context of the patient's history and epidemiologic risk (60).

Laboratory methods. The definitive diagnosis of PAM is based on the visual detection of the amebic trophozoite in the cerebrospinal fluid or brain tissue. Opening pressure is generally high with pleocytosis and no bacteria. It is important to be aware that the amebic trophozoites may be easily mistaken for macrophages or epithelial cells. The diagnosis of PAM can be made in the emergency room by examining fresh cerebrospinal fluid under the ordinary light microscope. Motile amebic trophozoite can be observed moving in the CSF when one or two drops of unstained CSF is placed on a glass slide with coverslip and examined under low power of a light microscope with lowered diaphragm, phase-contrast or dark-field illumination (Fig. 1D). Smears may be stained with Giemsa or Wright demonstrating the typical amebic trophozoite. Gram stain is not helpful in the diagnosis. Computed tomography (CAT) shows cerebral edema. Retrospective diagnoses have been made by examination of histological sections using hematoxylin and eosin or immunoperoxidase or immunofluorescent antibody techniques (65).

Pathological features. PAM shows a swollen, edematous and congested cerebral hemisphere and cerebellum. The olfactory bulbs and orbitofrontal cortices are necrotic and hemorrhagic (Figs. 1B and 1C). The fibrinopurulent exudate is usually scant. Histologically amebic trophozoites are found around blood vessels within the Virchow-Robin spaces with minimal or no polymorphonuclear cells (10,11,94). Cysts are not usually seen in CNS tissue (Figs. 1E,F and G).

Pathogenesis. *Naegleria fowleri* gain access to the body through the olfactory neuroepithelium. Amebic trophozoites or cysts may be present in contaminated water or may be inhaled from the dust and air. The sustentacular cells of the olfactory neuroepithelium are capable of active phagocytosis. The amebic trophozoites travel up the mesaxonal spaces of the unmyelinated fila olfactoria of the olfactory nerves and cross the cribriform plate and reach the subarachnoid space where they continue to multiply and proliferate within the subarachnoid space. There they penetrate into the CNS tissue producing hemor-

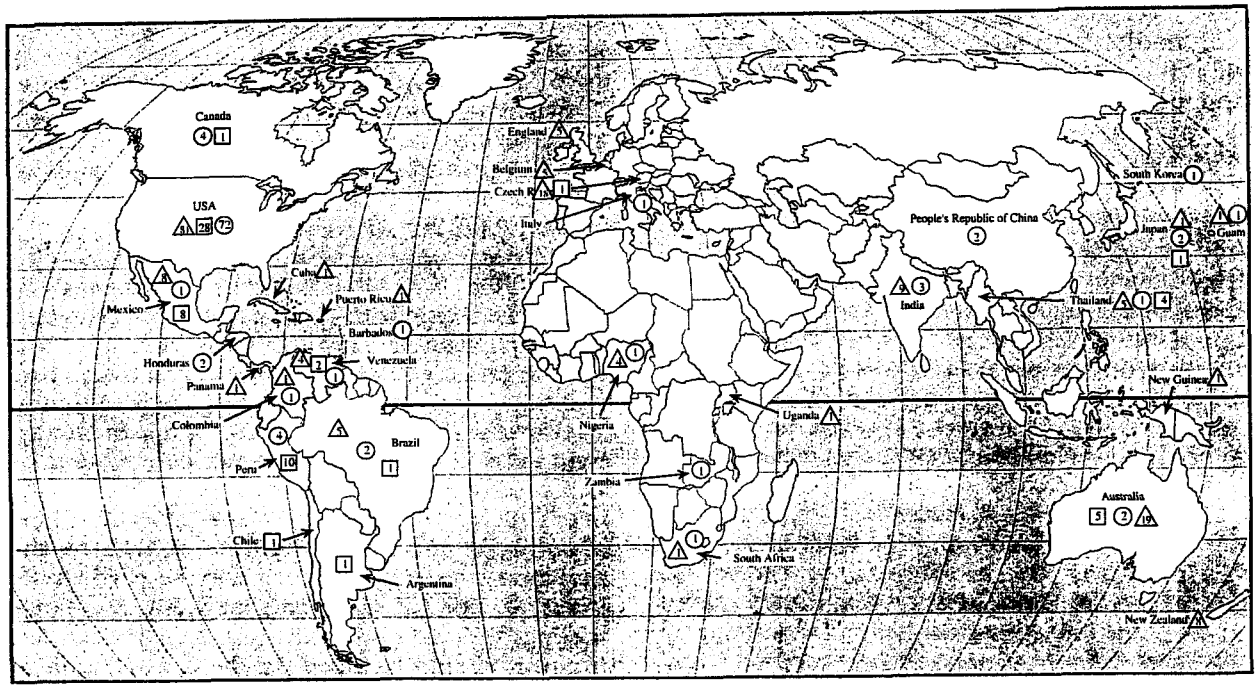

Figure 3.

rhagic necrosis and edema, the characteristic histopathologic features of PAM. The amebas induce necrosis of CNS tissues through direct ingestion with the help of production of lysosomal hydrolases and phospholipase that degrade myelin (48).

Treatment and prognosis. PAM due to *N. fowleri* is an acute, fulminant and fatal meningoencephalitis that probably does not elicit a protective cellular or humoral immune response. Early diagnosis is essential for successful medical treatment and good outcome. When the diagnosis is early the patient can be treated with high-doses of intravenous and intrathecal amphotericin B in combination with miconazole (Table 3). No more than four patients have been documented to survive PAM. They were treated early in the clinical course of the disease. No significant neurological sequelae has been noted in the few patients that survived (86).

Prevention and control. *N. fowleri* is susceptible to 1 mg/L or less of chlorine. Hence, proper disinfection of the water in swimming pools, whirlpools and Jacuzzis with chlorine and no diving or splashing water into the nostrils appears to be reasonable measures to avoid contracting PAM. The National Health and Medical Research Council of Australia has recommended the use of chlorine in swimming pools at 1 mg/L if the water temperature is less than 26°C and at least 2 mg/L if the temperature exceeds 26°C and 3 mg/L for temperatures over 28°C. The poor prognosis of PAM with its devastating consequences urges strict vigilance from public health authorities. Early diagnosis and prompt therapy are logical steps in the management of cases of PAM (86).

Acanthamoeba spp and Balamuthia mandrillaris as opportunistic pathogens. GAE may be caused by several species of *Acanthamoeba* and *B. mandrillaris*, mainly in immunosuppressed or immunodeficient individuals, such as in chronic alcoholism, pregnancy, HIV/AIDS, systemic lupus erythematosus or in bone marrow suppression due to chemotherapy (1). However, there have been described cases of GAE without evidence of immunodeficiency (97,98).

Morphology and Life Cycle. The vegetative or trophozoite form of *Acanthamoeba* and *B. mandrillaris* are morphologically similar in fixed tissue sections and measure from 15 to 35 μm in diameter (Fig. 2H). The vegetative form or trophozoite of *Acanthamoeba* possess an abundant cytoplasm with multiple elongated mitochondria, lysosomes, ribosomes and vacuoles. Spine-like processes or filamentous pseudopodia (acanthopodia) that constantly extend and retract from the surface of the body are characteristic. Amebas have a centrally placed nucleus with a

prominent round nucleolus. The cysts measure between 15 to 20 µm in diameter and are double walled. The outer wall, the ectocyst is rippled or wrinkled whereas the inner wall, the endocyst, is stellate, polygonal, round or oval (Figs. 2I and J). Pores or ostioles, covered with an operculum, are present at the junction of the ecto and endocysts. The optimum temperature for growth is about 25°C, though many isolates are known to grow at higher temperatures (Table 2). The trophozoites divide by binary fission. Nuclear division is by conventional mitosis wherein the nucleolus and the nuclear membrane disappears. Cysts are formed as a result of adverse environmental conditions. Marked variation exists between strains regarding shape and size of the cysts. *B. mandrillaris* will not row on bacteria-coated non-nutrient agar plate but grows on mammalian cells and tissue culture monolayers in contrast to *Acanthamoeba* spp that grow on mainly Gram negative bacteria-coated non-nutrient agar plates as well as mammalian cell culture.

The trophozoites of *B. mandrillaris* are larger than those of *Acanthamoeba* spp. The cysts of *B. mandrillaris* appear to be similar to those of *Acanthamoeba* at the light microscope level. However, the cyst wall ultrastructurally is tripartite and has an outer thin irregular layer, the ectocyst, a thick, electron-dense inner layer, the endocyst, and a middle amorphous fibrillar layer, the mesocyst (98). During mitosis, the nucleolus and the nuclear membrane remain intact initially but as mitosis continues both nucleolus and nuclear membranes disappear (98) (Figs. 2I and J).

Geographic distribution: ecology and epidemiology. *Acanthamoeba* spp. are widespread in nature (Fig. 3). So far *B. mandrillaris* has not been isolated from nature, but it is suspected they have the same habitat of *Acanthamoeba* spp. and *N. fowleri*. *Acanthamoeba* spp. has been found living in association with *Legionella pneumophilia* and they have often been found to contaminate bacterial, fungal and mammalian cell cultures in laboratories (30). *Acanthamoeba* spp. are ubiquitous and have been isolated from cooling towers, air conditioner filters, ocean waters, ponds, sewage, lagoons, rivers, dust, eye wash stations, pipe lines and even from nasal passages and mouth from normal individuals (22,99).

Granulomatous Amebic Encephalitis (GAE). The clinical manifestations are characterized by headache, personality changes, slight fever, seizures, hemiparesis, cranial nerve palsies, depressed level of consciousness and coma (Table 3). The clinical signs and symptoms are mainly those of a focal or localized encephalopathy with severe meningeal irritation and encephalitis (Table 1). The clinical presentation is insidious and may mimic a bacterial leptomeningitis, tuberculous meningitis or a viral encephalitis (18,63-66,80,81). GAE is a chronic, clinically protracted illness.

Immune response - immunodeficiency states. Normal human serum may contain inhibitors that may prevent growth of *Acanthamoeba* . Should host defenses become impaired, then these amebas multiply and disseminate throughout the body including the CNS resulting in a chronic illness. Diagnosis of the infection is difficult, the prognosis is grim and treatment may be futile and problematic, mainly in HIV/AIDS patients or others with severe or absent humoral or cell mediated immune response (11,3,8,17,24,33,35,47,53,67,70,74,92,100).

Differential diagnosis - Laboratory methods. The CSF findings may be of value in the diagnosis of GAE. Pleocytosis with abundant lymphocytes and polymorphonuclear leukocytes is the rule (14). The opening pressure is slightly elevated and the glucose is usually low with moderately high protein. Diseases that should be considered in the differential diagnosis of GAE include any space occupying mass such as a brain hemorrhage or tumor, or low density lesions such as brain infarct. GAE may also mimic hemorrhagic infarcts. Diagnosis of GAE in the immunocompromised patient may be particularly difficult. The CT scans and magnetic resonance imaging (MRI) are nonspecific (Fig. 2A). The positive diagnosis should be based on the constellation of clinical symptoms (especially in AIDS) correlated with the laboratory and radiographic findings. Computed tomography (CAT scan) and magnetic resonance imaging (MRI) are of limited value in the diagnosis. Enhancing and non-enhancing lesions have been observed in some cases (58,84) (Fig. 2A). The definitive diagnosis is also based on the visual detection of amebic trophozoites and cysts within brain lesions. H&E is usually sufficient to detect the amebic structures. Gomori methenamine silver stains the cyst wall black, while PAS-H stains the cyst wall brightly red. Immunoperoxidase and immunofluorescent antibody methods are helpful in species differentiation.

Pathological features. In GAE the cerebral hemispheres show moderate edema. The leptomeninges may contain a moderate amount of purulent exudate, with minimal cloudiness over the most affected cortical areas. In the less affected regions, the leptomeninges are transparent (36-39) (Fig. 2B).

There are foci of encephalomalacia and softening associated with discrete or confluent areas of hemorrhagic necrosis in occipital, parietal, temporal, or—less often—frontal lobes (Fig. 2C). The lesions are usually multifocal, located in the posterior fossa structures, midbrain, thalamus, brainstem, corpus callosum, and cerebellum. The cervical portion of the spinal cord is occasionally affected. Both *Acanthamoeba* spp. and *B. mandrillaris* produce a chronic granulomatous encephalitis with both trophozoites and cysts within the necrotic cerebral tissue. Though histopathological features may vary

from case to case regarding location, extent, composition of cellular elements, and density of trophozoites and cysts (Figs. 2D,E and G) the lesions however, are most numerous in the basal ganglia, midbrain, brainstem, and cerebral hemispheres (63-66).

Usually there is a focal, rather modest, chronic or subacute leptomeningitis, predominantly in close proximity to parenchymal lesions. Gliosis around necrotic areas is usually mild. Phagocytic (microglial) nodules and foci of recent hemorrhages may be seen, usually within the necrotic areas. In some cases there is severe angiitis (panarteritis), with perivascular cuffing by lymphocytes, some plasma cells, few macrophages, and rare eosinophils. Fibrinoid necrosis and thrombosis may be present in some instances. Amebic trophozoites and cysts may be found piercing the vascular wall (Fig. 2E). At postmortem, involvement of viscera other than the CNS may be found, suggesting an agonal hematogenous dissemination of trophozoites and cysts. The organs involved may be subcutaneous tissue and skin, liver, lungs (Figs. 2K and L), kidneys, adrenals, pancreas, prostate, lymph nodes, and myometrium (91,92). In cases of severe immunodeficiency the inflammatory reaction is negligible and occasionally there is a rather acute, inflammation with abscess formation. Some cases originally attributed to *Acanthamoeba* spp. have been found due to *B. mandrillaris* using immunofluorescent techniques (98) (Fig. 2F).

Pathogenesis. The pathogenesis of GAE is complex and remains poorly understood. In GAE the immunity is predominantly T-cell mediated, therefore the diminution of CD4+ and T helper lymphocytes enables the proliferation of free-living amebas. There are some clues that help to elucidate the pathogenetic mechanism of the CNS involvement such as the pneumonitis containing amebic trophozoites and cysts (Fig. 2L). Ulceration of the skin containing both amebic trophozoites and cysts suggest also the portal of entry into the bloodstream. In experimental animals the olfactory neuroepithelium has also been found to be a possible portal of entry (20,46). The incubation period of GAE is unknown but is probably longer than 10 days. The ability of acanthamoebas to produce necrosis of brain tissue is probably due to an enzymatic action induced by lysosomal hydrolases and a phospholipase that can degrade phospholipids of the myelin sheaths (21). In the CNS tissue, both trophozoites and cysts are often seen around blood vessels and within necrotic brain tissue. In patient's with AIDS and other immunocompromised situations the chronic inflammatory reaction is minimal and without multinucleated giant cells. Pathogenic free-living amebas may secrete several enzymes such as aminopeptidases, hydrolases, esterases and acid and alkaline phosphatases and dehydrogenases which directly or indirectly contributes to CNS tissue damage (21,69).

Treatment, supportive care and prognosis. So far there is no effective treatment for GAE. The majority of cases have been diagnosed at postmortem. The main feature associated with poor prognosis is the state of immunosuppression (70). Adjunctive therapy usually is not sufficiently effective because of the underlying disease process such as AIDS (Table 1). Some of the complications of the immunosuppression per se require specific management of anemia, renal failure, respiratory distress syndrome, hypoglycemic state, hyponatremia, all of these and others must be managed accordingly. Though sulfadiazine, pentamidine, propamidine and ketoconazole appear to be effective in vitro, it is questionable whether these drugs are useful because of the host's impaired immune system. The prophylaxis of GAE is difficult because of several factors: a) no single drug is effective against both troph and cysts of *Acanthamoeba;* b) other opportunistic microorganisms may invade the host and; c) increased risk of multi-drug toxicity and interactions among different therapeutic modalities and its effects on quality of life and prolonged survival (88).

Prevention and control. Prevention of GAE is difficult because the overwhelming majority of infections with *Acanthamoeba* spp. or *B. mandrillaris* have occurred in hosts with weakened immune systems such as AIDS. Periodic inspection of hot water tanks, air filters, plumbing systems and in-line filters used for purifying portable water supplies and eye wash stations is recommended as these systems are known to harbor amebas. These amebas are opportunistic and are able to multiply in patients with impaired humoral or cell mediated immunity.

Acanthamoeba keratitis (AK). *Acanthamoeba* keratitis is a vision-threatening chronic inflammation of the cornea caused by various species of *Acanthamoeba* (6,51). The first reported case of AK occurred in 1973 in a Texas rancher with minimal trauma to the right eye (51). The number of cases of AK has increased gradually since then (90). It is estimated that more than 700 cases have occurred as of October 1, 1996.

Clinical manifestations. AK is characterized by severe ocular pain associated with photophobia, blurred vision and conjunctiva congestion. A typical 360° stromal ring infiltrate, recurrent corneal epithelial breakdown, and a corneal lesion refractory to the antiviral, antibacterial and antimycotic drugs is a hallmark of *Acanthamoeba* keratitis. Waxing and waning clinical course is usually a clue in the diagnosis of AK (72). *Herpes simplex* keratitis should be considered in the differential diagnosis mainly due to the dendriform-appearing lesion of the cornea.

During the early years, AK has been associated with corneal trauma. It is now known, based on an epidemiologic study that the principal risk factor for AK is the use of soft contact lenses together with the use of homemade saline solution (Table 1).

Laboratory methods. *Acanthamoeba* trophozoites

and cysts can be recovered from corneal scrapings or biopsies, isolated, cultured and identified. The biopsy or scraped material should be processed for culture, isolation and staining with H&E, Gomori methenamine silver, hemacolor, Periodic acid Schiff (PAS), Masson Trichrome, immunofluorescence technique (IFA) or calcofluor white. Immuno-fluorescence technique and the calcofluor white requires the use of a fluorescence microscope. The former do not. Recent studies suggest confocal microscopy may be used to arrive at a clinical diagnosis (16). Biopsy tissue can be fixed in Karnovsky's fixative and embedded in plastic and stained with Toluidine blue for light microscopic examination and additional ultrastructural studies.

Pathological features. AK is characterized by a moderate or minimal chronically inflamed corneal stroma. Both polymorphonuclear leukocytes, eosinophils and lymphocytes may be present along with amebic cysts and occasional trophozoites. Macrophages, plasma cells, rare multinucleated foreign body giant cells may be present.

Pathogenesis. *Acanthamoeba* spp. probably gains access to the corneal stroma through a physical gap in the corneal epithelium, as happens with minor trauma or abrasion of the covering epithelium. Contact lens may also cause mechanical or hypoxic trauma which may allow the invasion and destruction of corneal stroma by *Acanthamoeba*. The tear film with its reduced antimicrobial activity and the possibility of secondary bacterial contamination could also be factors in the establishment of AK. It is possible that the amebic trophozoite secrete collagenolytic and proteinase enzymes and these enzymes may play a role in the pathogenesis of AK (69).

Treatment and prognosis. Recently, however, AK has been treated successfully with poly-hexa-methylene biguanide (PHMB) (Table 3). Ophthalmic solution 0.02% of PHMB is the treatment of choice (55). Some cases of AK have been treated successfully with a 0.1% eye drops of propamidine isethionate (Brolene), in combination with topical polymyxin B, miconozole nitrate, gramicidin, neomycin or neosporin (7,9,95). Pharmacological interactions should be kept in mind when several drugs are used simultaneously. In addition, some patients may develop hypersensitivity to medications such as neomycin and propamidine. Cysts of *Acanthamoeba* spp. are very resistant to antibacterial, antifungal and antiviral agents. Ketoconazole (Nizoral) and clotrimazole appears to be effective in vitro (50). Magainins (peptide isolated from the African clawed frog skin) have an amoebastatic and amebicidal activity which is enhanced when used in combination with a solution of silver nitrate (85). Topical steroids should be used with caution. Penetrating keratoplasty and corneal grafting and debridement has been used in some cases (29). Cryotherapy has been used in some cases without success (43). Recurrent AK has been reported in some cases after corneal transplants, because of the presence of amebic cysts in the corneal stroma. AK must be eradicated before surgery. Otherwise the infection may recur in the new graft. The natural history of AK is corneal perforation, vascularized corneal scar with impaired vision and even loss of the eye (27,54,55).

Prevention and control. Contact lens wearers should be instructed carefully to use sterile solutions after disinfecting lenses as recommended by the manufacturer or health care professionals; cleaning and disinfecting the lenses each time they are removed and washing hands before each time the lenses are handled. Contact lenses should not be used during swimming or while performing water sports (42,78,79,90).

Acanthamoeba pneumonitis. In some patients with GAE the pulmonary parenchyma has been found to contain amebic trophozoites and cysts in focal areas, producing lesions consistent with *Acanthamoeba* pneumonitis (64) (Fig. 2L). This finding suggest the lower respiratory tract as a portal of entry of the amebic microorganism and subsequently into the CNS by hematogenous spread (64). This was also noted in the experimental animal models (61,62).

Acanthamoeba spp. dermatitis and ulcerations. Skin lesions characterized by nodules and ulcerations containing amebic trophozoites and cysts have been reported in a number of cases (41,69,81,87). This is another possible "portal of entry" of the ameba into the body and subsequent spread to other regions of the body by hematogenous route (74,91).

Free-living Amebas as Animal Pathogens

Infections in small and large mammals: Pathogenic free-living amebas particularly species of *Acanthamoeba* and *B. mandrillaris* as well as *N. fowleri* can produce CNS infection similar to human, when intranasally or intracerebrally inoculated in mice, guinea pigs and rabbits (46,61,62). Mouse is quite susceptible to the *N. fowleri* infection. This makes this animal an ideal murine model for PAM and GAE (19). The transgenic mouse has been used as an example of immunodeficient animal, mimicking the histopathological changes in AIDS patients (46).

Larger mammals like cattle, sheep, horses, dogs, kangaroos and especially non-human primates (Baboon-Mandrill, Lowland gorilla) have been reported naturally and spontaneously infected with *Acanthamoeba* spp. and *B. mandrillaris* (2,4,5,28,34, 68,73,76,98). The medical and veterinary as well as the public health implications of this finding is obvious, demonstrating the ubiquitous presence of these amebas in nature as parasites or as pathogens with high virulence capable of producing lethal diseases in wild and domestic animals. A South American Tapir

has been the first case of spontaneous Primary Amebic Meningoencephalitis (PAM) due to *N. fowleri* in an animal living in a zoological park (59).

Summary and Future Projections

The true frequency and real incidence of free-living amebic infections is not exactly known. The numbers given here should be considered an approximation or an estimate at best. These numbers are probably under-represented. The main reason is that the autopsy rate in many countries is either minimal or nonexistent. The high mortality rates in diseases produced by free-living and opportunistic amebas has increased the interest of public health authorities and medical personnel, in understanding the natural history of the disease and its pathogenesis in hopes of improving therapeutic modalities and preventive measures.

The use of animal models may help in this endeavor and deserves further scrutiny (19,20). It is likely that there will be reports of more cases of GAE, particularly in immunodeficient patients, due to increasing use of instrumentation such as indwelling catheters, broad spectrum antibiotics and chemotherapeutic agents to avoid organ rejection after transplantation and HIV/AIDS. Therefore microorganisms that were considered harmless and innocuous commensals will be able to multiply and become opportunists causing severe disease.

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