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REVIEW

Diseases Due To Free-Living Amebas

Enfermedades por Amebas de Vida Libre

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ABSTRACT

Introduction: Acanthamoeba spp., Balamuthia mandrillaris and Naegleria fowleri are pathogenic free-living amoebae (FLA) and are commonly found in the environment, particularly in soil. This pathogenic FLA causes granulomatous amoebic encephalitis (GAE) or primary amoebic meningoencephalitis (PAM) that affects the central nervous system and can also cause keratitis and skin infections.
Objective: to determine the quantitative concentration of Acanthamoeba spp., B. mandrillaris and N. fowleri in soil samples collected from places where human contact is high by means of an assay and to reach the conclusion of contagion in these.
Method: a systematic review was performed in the following databases: PubMed; EMBASE; Cochrane; BVS, Cinahl and Web of Science. After the initial steps, the most relevant data were extracted from each article and compiled in full text in topics according to the chosen outcome.
Results: the qPCR assay detected a total of 45,71 % (n=16) of Acanthamoeba spp., 20 % (n=7) of B. mandrillaris and 17,4 % (n=6) of N. fowleri in five different soil sources. The quantitative concentration of Acanthamoeba spp., B. mandrillaris, 5 - 6×10^2, 47×10^4 to 39×10^3 , and 9×10^3 - 8×10^2 plasmid copies/gr, respectively. While the highest quantitative concentration of Acanthamoeba spp. and B. mandrillaris was determined in garden soil samples, N. fowleri was detected in potting soil samples. Three different genotypes T2 (18,75 %), T4 (56,25 %) and T5 (25 %) were identified from Acanthamoeba positive soil samples. The T4 genotype of Acanthamoeba was the most frequently detected genotype in soil samples and is also the most common genotype causing infection in humans and animals. To our knowledge, the present study is the first to identify the T5 genotype in soil samples from Turkey.
Conclusion: people and especially children should be aware of the hidden danger in the garden and potting soil samples they come into contact with most frequently. Public health awareness of human infections that may arise due to contact with soil should be increased. Public health specialists should raise awareness of this hidden danger in soil.

Keywords: Acanthamoeba spp; Balamuthia Mandrillaris; Pathogenesis of Amoebic Meningoencephalitis.

RESUMEN

Introducción: Acanthamoeba spp., Balamuthia mandrillaris y Naegleria fowleri son amebas patógenas de vida libre (FLA) y se encuentran comúnmente en el medio ambiente, particularmente en el suelo. Esta FLA patógena causa encefalitis amebiana granulomatosa (GAE) o meningoencefalitis amebiana primaria (PAM) que afecta al sistema nervioso central y también puede causar queratitis e infecciones de la piel.
Objetivos: determinar la concentración cuantitativa de Acanthamoeba spp., B. mandrillaris y N. fowleri en muestras de suelo recolectadas de lugares donde el contacto humano es alto mediante un ensayo y se quiere llegar a la conclusión de contagios en estos.

Método: se realizó una revisión sistemática en las siguientes bases de datos: PubMed; EMBASE; Cochrane; BVS, Cinahl y Web of Science. Después de los pasos iniciales, se extrajeron los datos más relevantes de cada artículo y se compilaron en texto completo en temas de acuerdo con el resultado elegido.
Resultados: el ensayo qPCR detectó un total de 45,71 % (n = 16) de Acanthamoeba spp., 20 % (n=7) de B. mandrillaris y 17,4 % (n=6) de N. fowleri en cinco fuentes de suelo diferentes. La concentración cuantitativa de Acanthamoeba spp., B. mandrillaris, 5 - 6×10^2, 47×10^4 a 39×10^3, y 9×10^3 - 8×10^2 copias de plásmido/gr, respectivamente. Mientras que la mayor concentración cuantitativa de Acanthamoeba spp. y B. mandrillaris se determinó en muestras de suelo de jardín, N. fowleri se detectó en muestras de suelo de macetas. Se identificaron tres genotipos diferentes T2 (18,75 %), T4 (56,25 %) y T5 (25 %) a partir de muestras de suelo positivas para Acanthamoeba. El genotipo T4 de Acanthamoeba fue el genotipo detectado con mayor frecuencia en muestras de suelo y también es el genotipo más común que causa infección en humanos y animales. Hasta donde sabemos, el presente estudio es el primero en identificar el genotipo T5 en muestras de suelo de Turquía.
Conclusión: las personas y especialmente los niños deben ser conscientes del peligro oculto en el jardín y las muestras de tierra para macetas que entran en contacto con más frecuencia. Se debe aumentar la conciencia de salud pública sobre las infecciones humanas que pueden surgir debido al contacto con el suelo. Los especialistas en salud pública deben concienciar sobre este peligro oculto en el suelo.

Palabras clave: Acanthamoeba spp; Balamuthia Mandrillaris; Patogénesis de la Meningoencefalitis Amebiana.

INTRODUCTION

Pathogenic free-living amoebae affecting the central nervous system are known to cause granulomatous amoebic encephalitis (GAE) or primary amoebic meningoencephalitis (PAM). Although hosts with impaired immunity are generally at increased risk of severe disease, amoebae such as Naegleria fowleri and Balamuthia mandrillaris can instigate disease in immunocompetent individuals, whereas Acanthamoeba species primarily infect immunocompromised individuals. Acanthamoeba can also cause a sight-threatening eye infection, primarily in contact lens wearers. However, infections due to pathogenic amoebae are considered rare. This is of particular concern, especially as global warming further exacerbates the problem.[1] Infections caused by Naegleria fowleri, Acanthamoeba spp., and Balamuthia mandrillaris result in various clinical manifestations in humans.

Humans. These amoebae are found in water and soil throughout the world. Acanthamoeba spp. and B. mandrillaris cause granulomatous amebic encephalitis (GAE), which usually presents as a mass, whereas N. fowleri causes primary amebic meningoencephalitis.

(PAM). Acanthamoeba spp. can also cause keratitis, and both Acanthamoeba spp. and B. mandrillaris can cause skin and respiratory mucosal lesions. These amoebae can be challenging to diagnose clinically as these infections are rare and, if not suspected, may be misdiagnosed with other more common diseases. Microscopy remains the key first step in diagnosis. However, the amoeba may be mistaken for macrophages or other infectious agents if an infectious disease pathology or clinical microbiology expert is not consulted. Although molecular methods can help establish the diagnosis, these are only available at referral centers. Treatment requires a combination of antibiotics and antifungals, and even with timely diagnosis and treatment, mortality from neurological disease is exceptionally high. In this paper, we describe the epidemiology, presentation, diagnosis, and treatment of free-living amoeba infections.[2]

Free-living pathogenic amoebae such as Naegleria fowleri, Acanthamoeba spp., and Balamuthia mandrillaris are known to cause fatal “amoebic meningoencephalitis” by acquiring different routes of entry into the brain, the impression given is as if the brain damage is substantially due to enzymes and toxins produced by this amoeba. After a detailed review of the literature, an analysis of archived specimens, and our experimental trials, we establish that with N. fowleri, Acanthamoeba, and Balamuthia spp. Infections result in extensive brain damage that is, in fact, substantially caused by the host immune response and not by the amoeba. Due to the comparatively larger sizes of these pathogens and the prior exposure of the amoebal antigen to the human body, the host immune system launches an amplified response that not only breaches the blood-brain barrier (BBB) but also becomes the primary cause of brain damage in amoebic meningoencephalitis. It is our understanding that for N. fowleri, the host immune response is dominated by acute inflammatory cytokines, which is the case in the cases of Acanthamoeba and Balamuthia spp. The type IV hypersensitivity reaction fundamentally contributes to the disruption and leakage of the blood-brain barrier (BBB) and causes neuronal damage.[4]

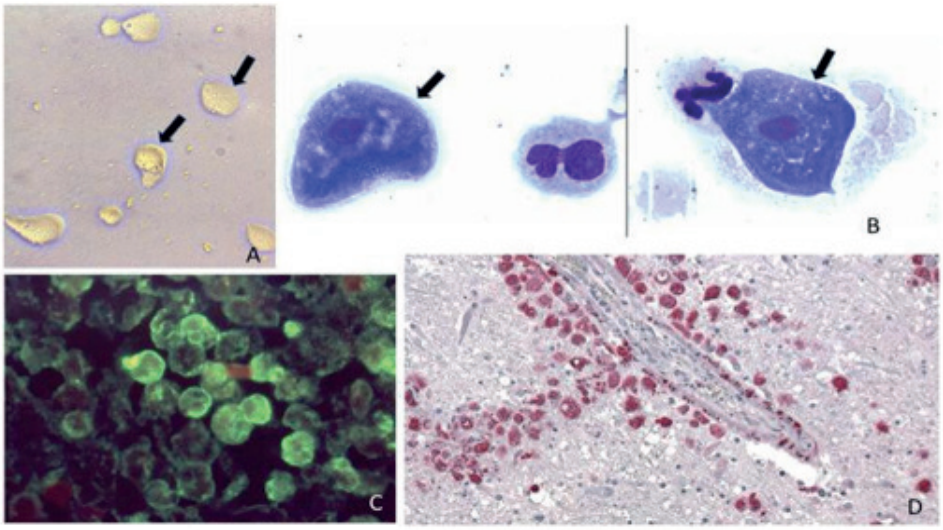

Figure 1. Different diagnostic methods used for free-living amoeba: (A) Trophozoites of free-living amoeba observed in culture using nutrient-free agar and bacterial lawn (arrows mark trophozoites). (B) Giemsa staining of cerebrospinal fluid from a patient with Acanthamoeba spp. Granulomatous amoebic encephalitis (arrows mark two trophozoites from the same slide but from different locations). (C) Immunofluorescence assay in brain tissue from a patient with N. fowleri (amoeba stained green). (D) An immunohistochemical assay in brain tissue from a patient with granulomatous amoebic encephalitis B. mandrillaris, with red staining, corresponds to the amoeba. Note that the amoeba surrounds a blood vessel. Panels A and C are from the Public Health Image Library, CDC.[3]

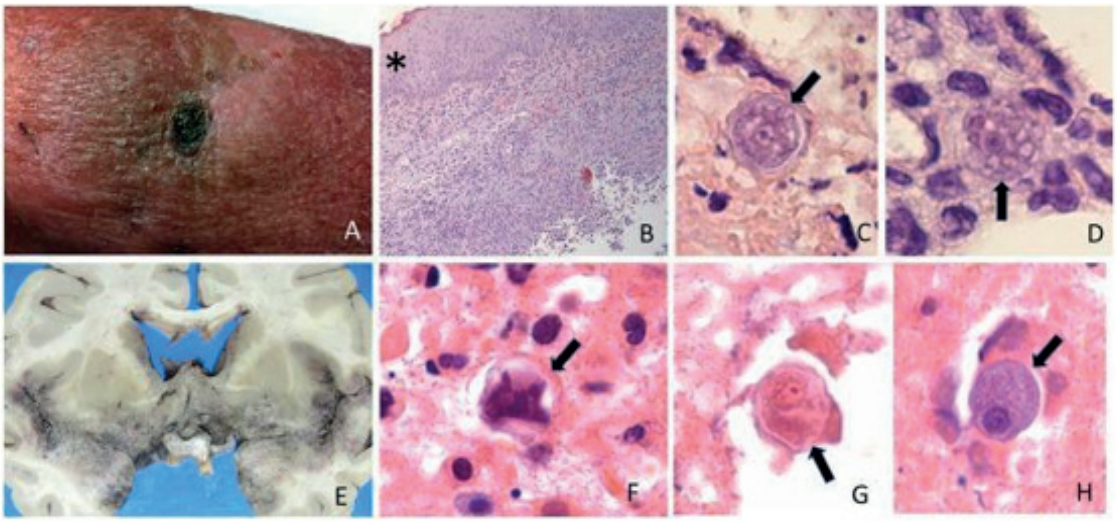

Figure 2. Skin and brain Acanthamoeba spp. Infections (A) Ulcerated necrotic lesion on the leg. (B) Hematoxylin and eosin stains show a thickened epidermis (*) and intense inflammatory infiltration throughout the dermis. Usually, the dermis should stain mostly homogeneous pink; in this photomicrograph, the dermis appears to have multiple blue dots corresponding to the inflammatory infiltrate. (C) Hematoxylin and eosin stain showing a double-walled cyst with a wrinkled outer wall (arrow marks the cyst). (D) Hematoxylin and eosin stain showing trophozoite (arrow marks trophozoite) with multiple vacuoles (stained light pink within the trophozoite) and two erythrocytes (stained dark red). Note the nuclear features of the cyst and trophozoite: the karyosome is prominent and central within the nucleus, and the nuclear chromatin is dispersed and not clumped. (E) Macroscopic photograph of the brain with granulomatous amoebic encephalitis. In this case, the base of the brain was the most affected, as evidenced by the brown/gray coloration distinguishable from the rest of the brain parenchyma. (F to H) Microscopic images of cysts and trophozoites corresponding to the same brain. (F) A collapsed cyst (arrow marks the cyst). (G) Cyst showing characteristic nuclear features (arrow marks cyst). (H) Trophozoite (arrow marks trophozoite). (G) Cyst showing characteristic nuclear features (arrow marks cyst). (H) Trophozoite (arrow marks trophozoite) (G) Cyst showing characteristic nuclear features (arrow marks cyst). (H) Trophozoite (arrow marks trophozoite).[3]

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The report of the death of a person from amoebic meningoencephalitis, the proverbial "brain-eating amoeba," Naegleria fowleri, acquired from a state park lake in Iowa in July 2022 has again set off seasonal alarms about this pathogen. While exceptionally rare, its near-universal mortality rate has terrified the public and made for good media copy. This review will address the free-living amoebae identified as causing CNS invasion in man, namely Naegleria fowleri, Acanthamoeba species, and Balamuthia mandrillaris.[5]

Among the many genera of free-living amoebae that exist in nature, members of only four genera have an association with human disease: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri and Sappinia diploidea. Acanthamoeba spp. and B. mandrillaris are opportunistic pathogens that cause infections of the central nervous system, lungs, sinuses, and skin, mainly in immunocompromised humans. Balamuthia is also associated with disease in immunocompetent children and Acanthamoeba spp. Cause an eye-threatening infection, Acanthamoeba keratitis, mainly in contact lens wearers. Of more than 30 species of Naegleria, only one species, N. fowleri, causes acute fulminant meningoencephalitis in immunocompetent children and young adults.[6]

This review focuses on free-living amoebae, widely distributed in soil and water, that cause opportunistic and non-opportunistic infections in humans: Acanthamoeba spp, Balamuthia mandrillaris, Naegleria fowleri and Sappinia diploidea. Diseases include primary amoebic meningoencephalitis (N. fowleri), granulomatous amoebic encephalitis, skin and nasopharyngeal infections (Acanthamoeba spp., Balamuthia mandrillaris, S. diploidea) and amoebic keratitis (Acanthamoeba spp). Acanthamoeba, Balamuthia, and Naegleria have been isolated repeatedly; S. diploidea has been reported, only once, from a brain infection. Antimicrobial therapy for these infections is usually empirical, and patient recovery is often problematic. N. fowleri is very sensitive to the antifungal agent amphotericin B, but delayed diagnosis and the fulminant nature of the disease result in few survivors. Encephalitis and other infections caused by Acanthamoeba and Balamuthia have been treated, with varying success, with antimicrobial combinations including anti-sterol directed azoles (clotrimazole, miconazole, ketoconazole, fluconazole, itraconazole), pentamidine isethionate, 5-fluorocytosine, and sulfadiazine. The use of drug combinations addresses resistance patterns that may exist or develop during treatment by ensuring that at least one of the drugs is effective against amoebae. Favorable drug interactions (additive or synergistic) are another potential benefit.

Potential in vitro drug testing of clinical isolates points to strain and species differences in sensitivity, so it cannot be assumed that any drug is effective against all amoebae.[7] The anticancer agent miltefosine and the antifungal drug voriconazole were tested in vitro against Balamuthia mandrillaris, Acanthamoeba spp., and Naegleria fowleri. All three amoebae are etiologic agents of chronic (Balamuthia, Acanthamoeba) or fulminant (Naegleria) encephalitis in humans and animals and, in the case of Acanthamoeba, amoebic keratitis. Balamuthia exposed to miltefosine concentrations <40 microns survived, whereas concentrations of > or = 40 microns were generally amoebacidal, with varying sensitivity among strains. At metastatic drug concentrations, recovery of drug effects may take up to 2 weeks.[8]

Acanthamoeba is an opportunistic protozoan that exists widely in nature and is distributed primarily in soil and water. Acanthamoeba generally exists in two forms: trophozoites and cysts. The trophozoite stage is one of growth and reproduction. In contrast, the cyst stage is characterized by cellular dormancy, which commonly results in human infection, and the lack of effective monotherapy after initial infection leads to chronic disease.[9]

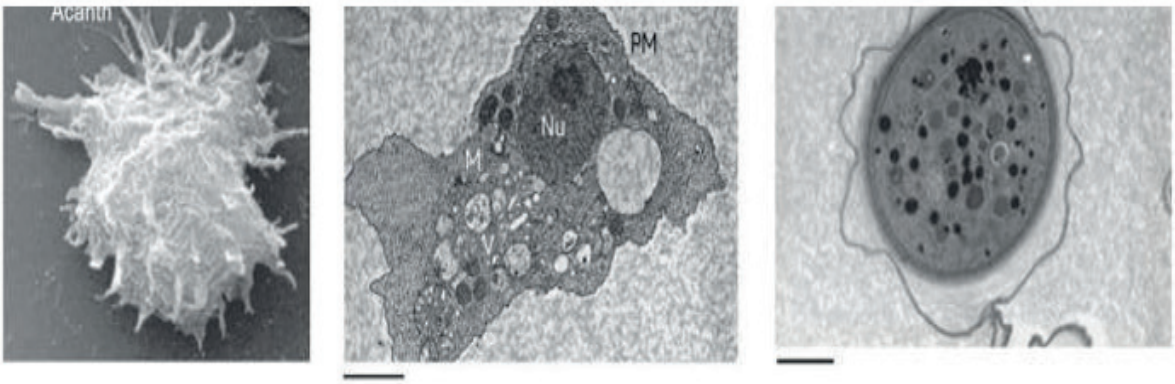

Figure 3. The structure of Acanthamoeba. (A) Scanning electron micrograph of an Acanthamoeba trophozoite showing many spiny pseudopodia around the entire cell surface. Acantho, acanthopod. (B) Transmission electron micrograph of Acanthamoeba trophozoite stage. Nu, nucleus; V, vacuoles; M, mitochondria; PM, plasma membrane. (C) Transmission electron micrograph of an Acanthamoeba cyst. OL, outer layer; IL, inner layer.[9]

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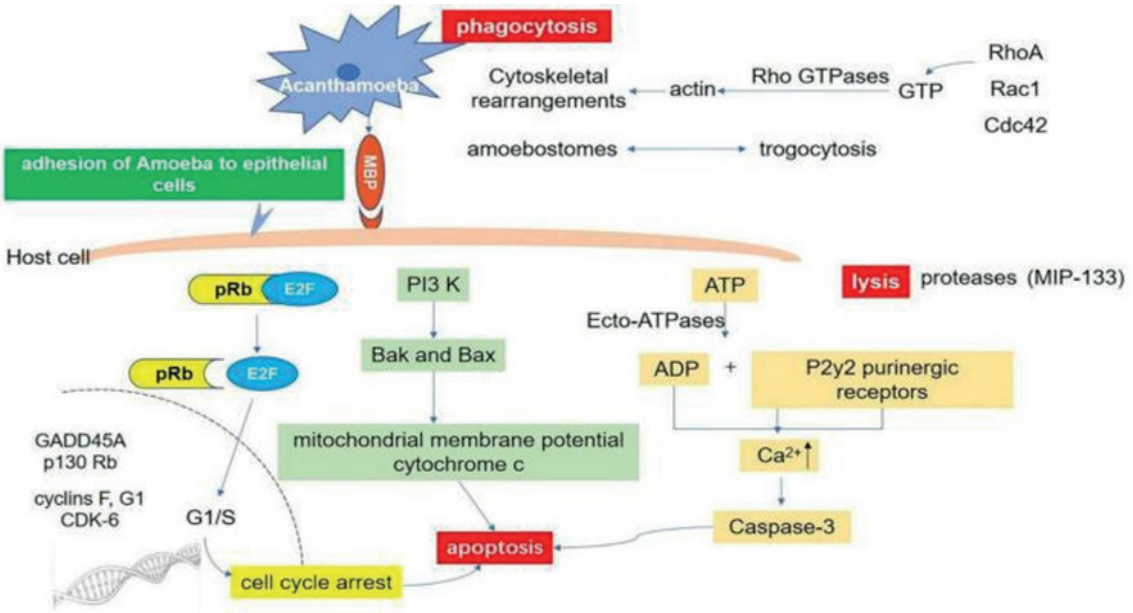

Figure 4. Molecular signaling pathways of host-parasite interaction.

Adhesion via spiny pseudopods and adhesins (such as MBP) is the basis for Acanthamoeba to establish infection. Once adhesion is complete, the intracellular signal transduction process is activated and triggers cascading effects such as phagocytosis of target cells, protease secretion, and apoptosis, resulting in direct pathological damage. Phagocytosis: There are three thoroughly studied pathways involved in this process. The RhoA pathway leads to stress fiber formation, Rac1 activation, which triggers plaque foot formation, and Cdc42 activation, which promotes filamentous foot formation. Apoptosis: There are at least three pathways related to apoptosis following Acanthamoeba infection: cellular apoptosis driven by ecto-ATPases, interference with the expression of important genes that regulate the cell cycle, and the phosphatidylinositol 3-kinase (PI3K)-mediated apoptosis pathway. Lysis: Acanthamoeba secretes a variety of proteases involved in cell lysis. A serine protease (MIP133) has been identified as a key component in Acanthamoeba pathogenesis.[9] Acanthamoeba spp. are frequently the etiologic agents of severe sight-threatening keratitis called Acanthamoeba keratitis.

The contact lens storage solution of a patient with keratitis of unknown genesis was evaluated using our diagnostic tools to detect potentially pathogenic free-living amoebae (FLA). In the context of this routine screening, culture methods and a triple quantitative real-time polymerase chain reaction (qPCR) targeting Acanthamoeba spp, Naegleria fowleri, and Balamuthia mandrillaris were used. Although culture did not detect amoebae, qPCR specifically detected B. mandrillaris DNA. This ALF is known to be the etiologic agent of a fatal form of encephalitis in humans and other mammals, Balamuthia amoebic encephalitis (BAE). A fragment of the 18S rDNA gene was amplified from the sample and showed 99 % sequence identity with the B. mandrillaris sequences from GenBank. To our knowledge, this is the first report of B. mandrillaris associated with contact lenses. Although no viable amoebae were obtained through culturing efforts, verification of B. mandrillaris DNA in contact lens storage solution demonstrates the ease with which this pathogen could come into close contact with humans.[10]

We describe a series of three cases of encephalitis in a family group with fatal outcomes occurring in the Tumbes region between December 2019 and February 2020. Acanthamoeba sp. was identified in the cerebrospinal fluid sample of case 1. All three cases had a history of entering a swimming pool months before the disease. There is no previous record of ALF encephalitis in the Tumbes region, and its occurrence in a familial cluster has never been reported.[11] Granulomatous amoebic encephalitis caused by free-living amoebae is a rare condition that is difficult to diagnose and treat and is usually fatal. Anti-amebic treatment is often delayed because clinical signs and symptoms can obscure the likely causative agent and mislead appropriate diagnostic testing. Four genera of free-living amoebae are associated with human infection: Naegleria, Acanthamoeba sp., Balamuthia, and Sappinia. Two children were admitted with a diagnosis of acute encephalitis. The history of contact with swimming pools and rivers supports the suspicion of infection by free-living amoebae. In both cases, a brain biopsy was performed, and histology confirmed granulomatous amoebic encephalitis with the presence of amoebic trophozoites.[12] We present the first case of granulomatous amoebic encephalitis of Balamuthia mandrillaris definitively acquired in Africa. Our case emphasizes the nonspecific initial dermatologic features, the delay in confirming the diagnosis, the difficulties in accessing recommended medication, and the

uncertainty about the optimal treatment of a disease with a frequently fatal outcome.[13]

Case presentation

A 54-year-old man was admitted to the hospital after experiencing acute onset of numbness and weakness in the left extremity. Due to the initial consideration of the intracranial tumor, surgical excision of the right parietal lesion was performed. However, the patient presented with headache accompanied by diplopia and difficulty walking, and a new lesion was found in the left occipital-parietal lobe two weeks after the first operation. High-throughput next-generation sequencing (NGS) detected the presence of high copy reads of the B. mandrillaris genome sequence in the patient's blood, cerebrospinal fluid (CSF), and brain tissue. Pathological investigation of the brain tissue showed granulomatous changes and scattered amoebic trophozoites around the blood vessels with high magnification. The patient was reoperated for progressive confusion due to subfalcine herniation of the left cerebral hemisphere. The right parietal lobe lesions decreased in size after the first surgery, and the left occipital lobe lesions and surfacing herniation did not improve two months after the second surgery. The patient was transferred to the local hospital for continued treatment with sulfamethoxazole and azithromycin. After five months after the second surgery, the patient showed good recovery with a mild headache. Moreover, the left occipital lobe lesions and sunfalcine hernia did not improve two months after the second surgery. The patient was transferred to the local hospital for continued treatment with sulfamethoxazole and azithromycin. After five months after the second surgery, the patient showed good recovery with a mild headache. Moreover, the left occipital lobe lesions and sunfalcine hernia did not improve two months after the second surgery. The patient was transferred to the local hospital for continued treatment with sulfamethoxazole and azithromycin. After five months after the second surgery, the patient showed good recovery with mild headache.[14]

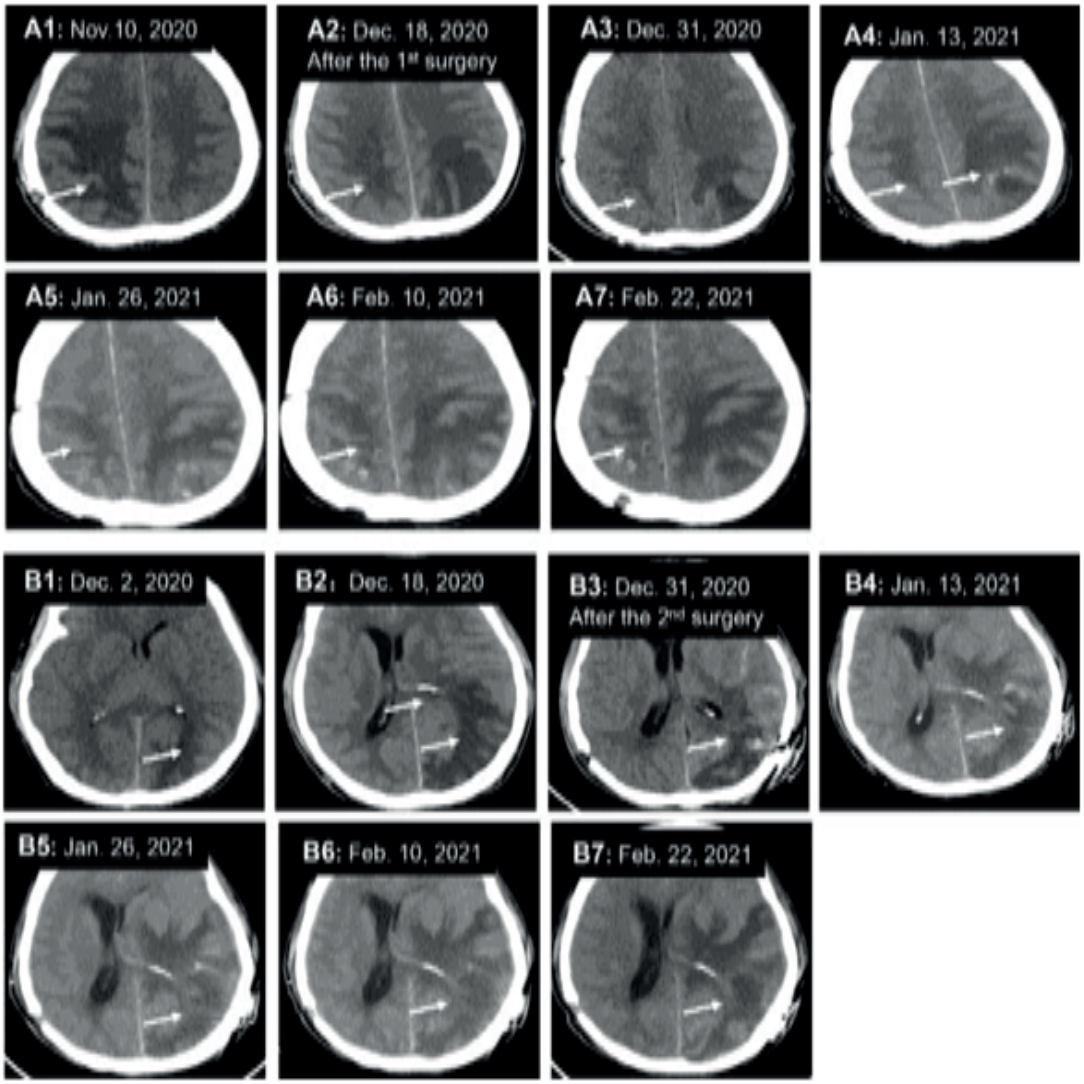

Figure 5. The dynamic changes of the CT head. A The original lesion in the right parietal lobe was getting smaller; B a new lesion that occurred in the left occipital lobe worsened.[14]

Background

Granulomatous amoebic encephalitis (GAE) is a rare infection of the central nervous system caused by Balamuthia mandrillaris or Acanthamoeba species. Diagnosis is challenging due to the nonspecific clinical presentation, cerebrospinal fluid analysis, and radiological features. To date, there is no effective treatment for GAE.[14]

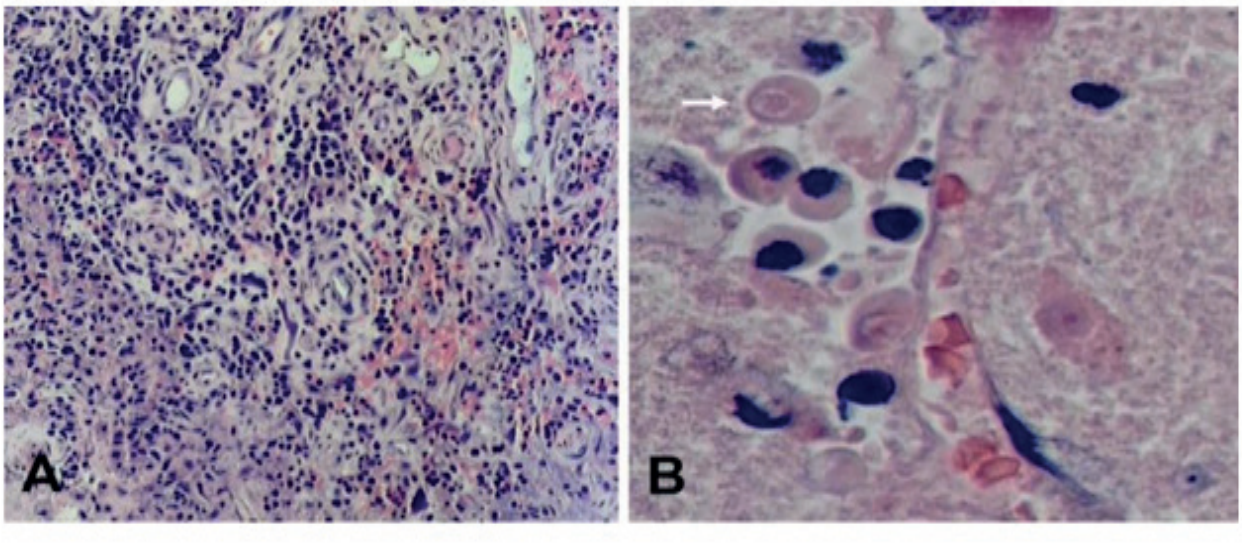

Figure 6. Histological examinations. A Granulomatous changes and inflammatory perivascular infiltrate (×100); B Amoea trophozoite (×400).[14]

Objective

When this disease is suspected, a correct clinical diagnosis is required since these infections are rare, and there is a risk of misdiagnosis with other, more common diseases. That is why the primary objective is to detect the disease early.

METHOD

Study design: the present study is a systematic review of the literature that seeks to answer when to think about free-living amoebas and thus prevent their presence.

Study population: this study is oriented to the general population, especially to hosts with altered immunity who are generally at higher risk of severe disease.

Inclusion criteria

We considered articles eligible for our systematic review if they met the following conditions:

1. Original articles
2. Published in English
3. Investigated the study of When to think about free-living amoebae and thus subsequently prevent their presence.
4. Reported exclusively on humans
5. Both retrospective and prospective clinical case studies.

Exclusion Criteria

1. Patients undergoing treatment for other pathologies.
2. Patients presenting other associated diseases
3. Articles in which another adverse effect is treated.

Selection and sample size

The selected samples are those human patients without age or sex limits who have participated in the studies required for this systematic review.

Scope of the study

University setting, at the Inter-American Open University.

Operational description of the variables

Patient age: quantitative variable

Form of contagion of the disease: variable, lakes, rivers, swimming pools.

The evolution of the disease in the patient is variable, including eye infections, respiratory, and neurological disease.

Treatment: variable, antibiotics, antifungals.

Proposed intervention and instrument(s) for data collection.

It is based on the search for and review of bibliographic materials to analyze the research question.

RESULTS

The data analysis was obtained from a search in PubMed with the terms Mesh on free-living amoebas AND their prevention; first of all, we started by choosing those articles that had a title that was relevant to the objectives of the study and according to the topic, then a more detailed reading of the articles was carried out and once selected, the inclusion and exclusion criteria were applied, thus obtaining those that were useful to carry out this systematic review, from the totality of the search 16 articles corresponding to the research work were selected.

Data analysis plan

The corresponding bibliographic citations were searched for in validated scientific search engines such as Pubmed, Cielo, Sociedad Argentina de infectología, and Mesh.

DISCUSSION

When this type of free-living amoebae should be suspected, in recent years, there has been an increase in active infections, starting in 1958, increasing to more than 2000 cases until 2020, and decreasing in 2021.

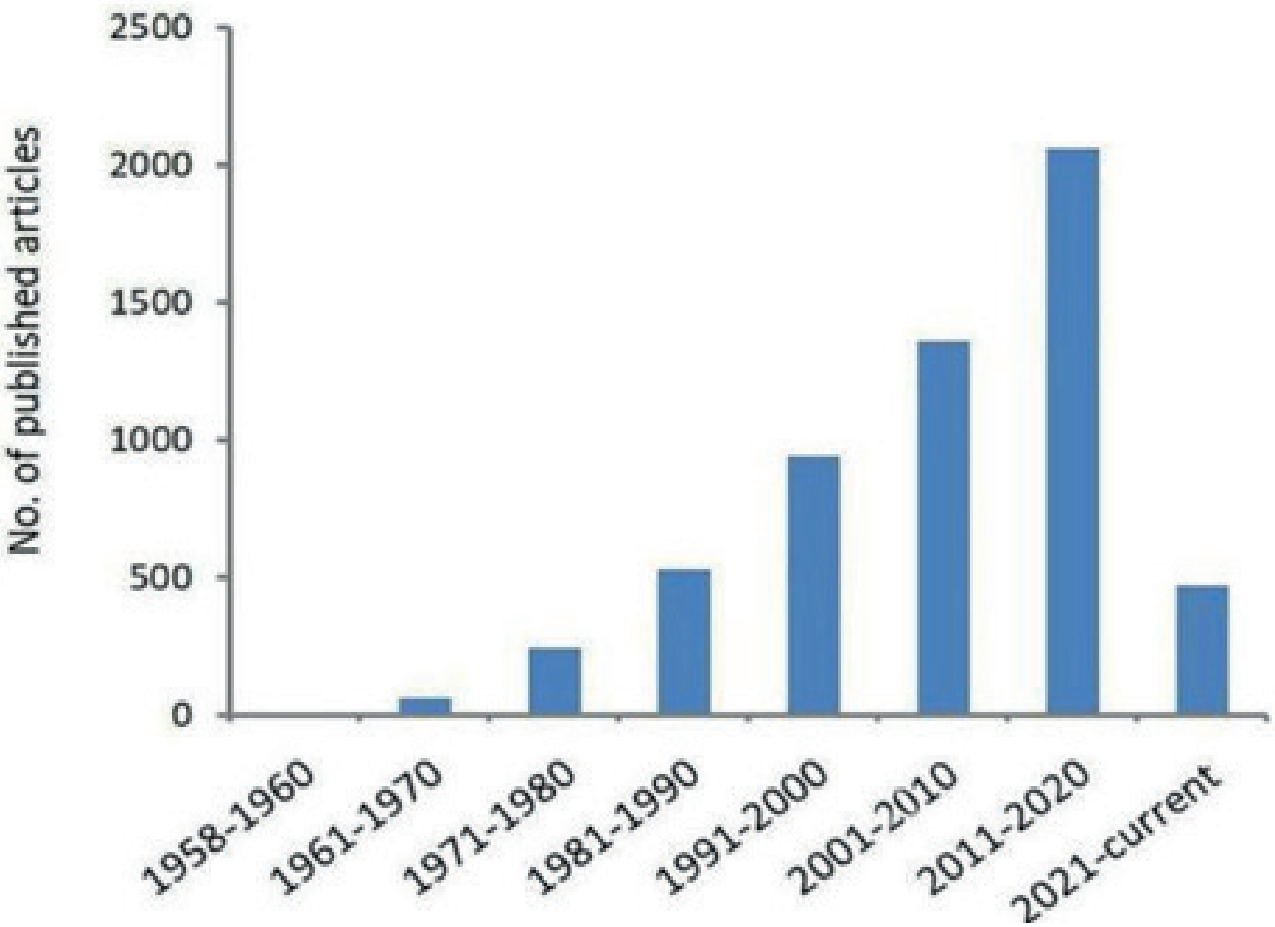

Figure 7. Number of publications related to Acanthamoeba. Growing scientific interest in the field of Acanthamoeba as determined by published articles cataloged in PubMed over time.

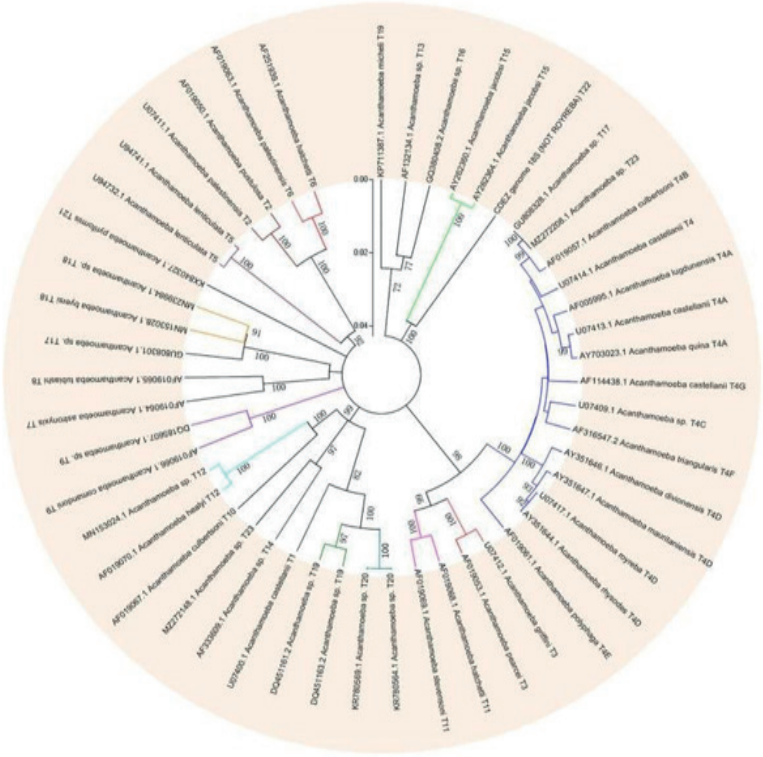

Figure 8. Phylogenetic relationship of 49 different genotypes or subtypes of Acanthamoeba T1-T23 based on the "complete" 18S rRNA gene sequence. The tree was constructed using the Neighbor-Joining algorithm in MEGA 4.

Figure 9. High-throughput next-generation sequencing (NGS) results. Balamuthia mandrillaris with 112 sequence copy reads in serum (A), 539 sequence copy reads in CSF (B), and 3723 sequence copy reads in brain tissue (C).

CONCLUSIONS

Having ruled out the most common causes of meningoencephalitis and keratitis, it is important to search for epidemiological factors that may lead to the suspicion of this type of amoebae. The number of epidemiological reports is increasing, so it is essential to maintain a high index of suspicion in cases compatible with meningoencephalitis or keratitis in which the infection of these free-living amoebae may be suspected.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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