

UC Davis

Dermatology Online Journal

Title

Cutaneous Balamuthia mandrillaris infection as a precursor to Balamuthia amoebic encephalitis (BAE) in a healthy 84-year-old Californian

Permalink

<https://escholarship.org/uc/item/8c8720qm>

Journal

Dermatology Online Journal, 23(7)

Authors

Lehmer, Larisa M
Ulibarri, Gabriel E
Ragsdale, Bruce D
[et al.](#)

Publication Date

2017

DOI

10.5070/D3237035732

Copyright Information

Copyright 2017 by the author(s). This work is made available under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives License, available at <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Peer reviewed

Cutaneous *Balamuthia mandrillaris* infection as a precursor to *Balamuthia amoebic encephalitis* (BAE) in a healthy 84-year-old Californian

Larisa M Lehmer, Gabriel E Ulibarri, Bruce D Ragsdale, James Kunkle

Affiliations: University of California Irvine Health, Department of Dermatology, Irvine, California, Central Coast Pathology Laboratory, San Luis Obispo, California, Western Diagnostic Services Laboratory, San Luis Obispo, California, Central Coast Dermatology, Arroyo Grande, California

Corresponding Author: Larisa M Lehmer, Department of Dermatology, UC Irvine Health, 118 Med Surg 1, Irvine, CA 92697-2400, Email: llehmer@uci.edu

Abstract

Soil and freshwater-dwelling amoebae may opportunistically infect the skin and evoke a granulomatous dermatitis that camouflages their underlying morphology. Amoebic infestations are incredibly rare in the U.S., predominantly occurring in the young, elderly, and immunocompromised. Sadly, because diagnosis is difficult and unsuspected, most cases are diagnosed at autopsy. The following case is of a healthy 84-year-old man with a non-healing nodulo-ulcerative cutaneous lesion on his left forearm that appeared following a gardening injury. Lesional punch biopsies repeatedly showed non-specific granulomatous inflammation with no pathogens evident histologically or by culture. Histopathologic diagnosis was made five months after initial presentation via identification of amoebic trophozoite forms in tissue from a large excisional specimen. Anti-amoebic therapy was initiated immediately. The patient experienced mental status changes three days following lesion excision, with evidence of a cystic mass in the left medial parieto-occipital lobe by CT. Both intraoperative brain biopsies and cutaneous tissue samples tested positive for *Balamuthia mandrillaris* by indirect immunofluorescent antibody assay performed at the Centers for Disease Control. The patient achieved a full recovery on a triple antibiotic regimen. Clinical suspicion and thorough histopathologic analysis may determine the difference between survival and death for a patient presenting with a treatment-refractory localized granulomatous lesion.

Keywords: Balamuthia mandrillaris; cutaneous balamuthiasis; granulomatous amoebic encephalitis; GAE; balamuthia amoebic encephalitis; BAE; immunocompromise; granulomatous dermatitis; immune senescence.

Introduction

Balamuthia mandrillaris (*B. mandrillaris*), along with *Naegleria*, *Sappinia* and several species of *Acanthamoeba*, are free-living amoebae found in fresh water and soil worldwide that may opportunistically infect the skin and/or central nervous system (CNS) of humans. The first sign of infection ranges from a stubborn indurated plaque or ulceration on the skin of the extremities and/or central face to florid encephalopathy [1]. Owing to its persistence in the skin, *B. mandrillaris* is commonly biopsied with only a non-specific granulomatous reaction apparent on most slides [2]. Once in the CNS amoebae cause a granulomatous amoebic encephalitis (GAE) which, until the publication of this report, was invariably fatal.

Although amoebic infection is more common in the immunocompromised population, (i.e., patients undergoing immunosuppressive therapy for solid organ transplantation, those with autoimmune deficiency syndrome (AIDS), immunocompetent individuals, the subject of the present report included, can also be affected [3]. We present an 84-year-old man from California who contracted cutaneous balamuthiasis of the left upper extremity. He was an active gardener and relatively healthy at the time of infection. However, at the time of infection he was

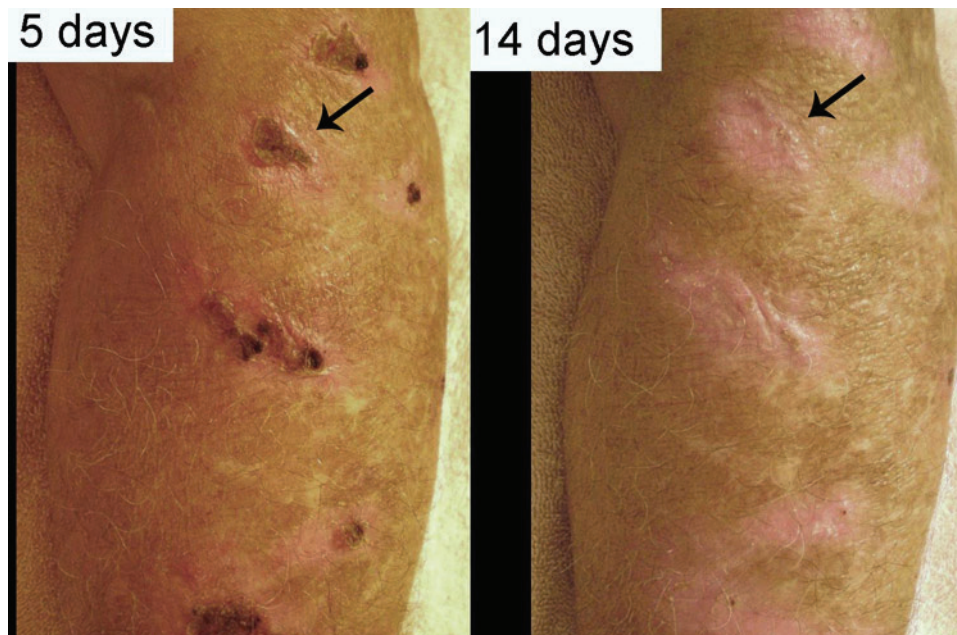


Figure 1. Early evolution of the patient's skin abrasions, as recorded on his wife's cell phone. The second abrasion from the top (arrow) eventuated in the ulcer of **Figure 2**.

undergoing ongoing maintenance therapy with rituximab for a hematologic dyscrasia five years in remission.

It is important to correlate social history, i.e., gardening or freshwater swimming, with pathogen exposure risk. Amoebae are known to enter the body via traumatic implantation of soil into the skin or by direct invasion of the superficial blood vessels in the nasal cavity after exposure to freshwater lakes, ponds, or rivers. This report describes a remarkable case of a small skin laceration that developed into a non-healing and enlarging cutaneous ulcer that evaded diagnosis for many months and grew to a size of 4-cm before *B. mandrillaris* was recognized in the histopathologic analysis of tissue obtained through excisional biopsy. The diagnosis was uncovered just as the patient started to display symptoms early amoebic encephalitis. Extraordinarily, this patient was treated and survived disseminated balamuthiasis, including *Balamuthia amoebic encephalitis* (BAE) and is back to his baseline functional status.

Case Synopsis

An 84-year-old male presented with a relatively asymptomatic but enlarging nodulo-ulcerative lesion on the left proximal forearm of two to three months' duration. It followed an initial "slip and fall" injury in his garden resulting in multiple abrasions at several sites on the left forearm, documented in a

cell phone image taken 5 days later (**Figure 1**). No medical attention was sought initially and the scrapes were cleaned with soap, water, and peroxide, and then dressed with gauze over polysporin ointment. Although the majority of the lacerations healed, one near the elbow worsened into a heaped-up, ulcerated mass.

Aside from the forearm lesion, he had no physical complaint. He denied any exotic travel, animal, or food exposure. His oncologic history was significant for melanoma in situ (0.2 mm thick) excised from his back 18 years prior without recurrence or metastasis and prostate cancer treated with

radiation 31 years prior, with 2 of 12 needle biopsies positive for Gleason Grade 8/10 at 25-year follow-up. Three years prior to presentation, he was diagnosed with monoclonal gammopathy of undetermined significance (MGUS) during a workup for peripheral neuropathy and started on rituximab at 750mg every two weeks. Therefore, recent increases in his prostate-specific antigen (PSA) raised concern for an albeit clinically unlikely possibility of metastatic prostate cancer. However, he only sought dermatologic attention after the persistent lesion had reached 4 cm in diameter (**Figure 2A**). A shave biopsy was obtained and submitted for dermatopathologic evaluation under clinical suspicion of primary skin cancer, lymphoma, or pyoderma.

Histopathology

Birefringent mineral particles recognized in the histopathologic background of a scar corroborated a history of soil-contamination at the inception of the lesion. However, unlike a silica granuloma, the lymphocyte-rich granulomatous inflammation with focal suppuration and patchy necrosis apparent in the shave biopsy, along with the clinical progression of lesion, indicated an infectious cause. Seven days after initial shave biopsy, a deeper punch biopsy was acquired near the same site followed by an excisional biopsy of a 2 cm rubbery nodule on the right upper inner breast two days later. Tissue obtained from the

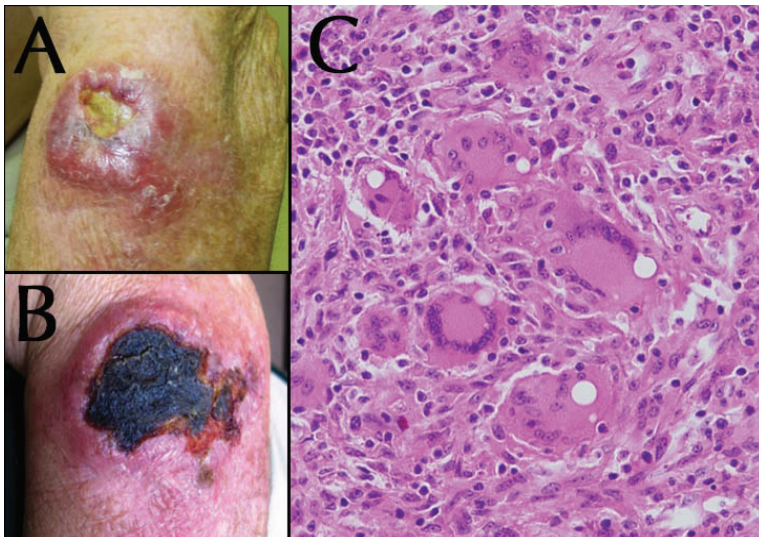


Figure 2A-C. Clinical progression of ulcer to a necrotic eschar prior to complete excision. A) Initial presentation to dermatology (2.5 months after injury). B) Progression to eschar with increase in surrounding erythema 7 weeks after initial presentation. C) Background, granulomatous inflammation in the ulcer base as shown in the excision specimen (H&E; 250x).

minocycline 100mg BID) and topical clobetasol. However, the lesion continued to increase in thickness and diameter.

Progressive enlargement of the ulcerated forearm mass motivated the patient to elect for complete excision of the lesion two months after his initial presentation, i.e., four months after the garden fall. Mycobacterial and fungal tissue cultures performed on samples of the large skin excision specimen were also negative. Histopathologic analysis of the 5.0 x 2.5 x 1.0-cm piece of cutaneous tissue revealed necrosis of the upper dermis characterized by a homogenous fibrin-inflated, partly desiccated eschar (**Figure 2B**). At the border between the necrotic change and underlying inflammation were thrombosed blood vessels deemed a result of surrounding inflammation versus a primary vasculitis with Touton-type

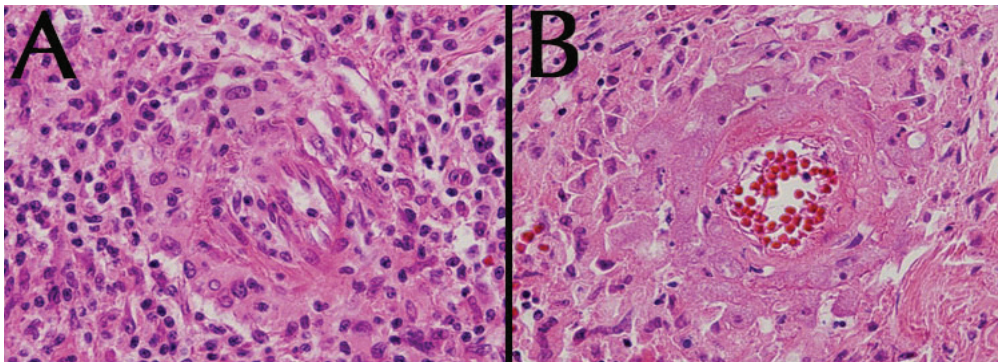


Figure 3A-B. Excisional Biopsy, inflammatory pattern (HE, 400x). Histiocytes (A) rim vessels in the skin excision specimen, a pattern mimicked by the bubbly cytoplasm of the amoebae (B).

giant cells present in clusters. (**Figure 3A**). This pattern of inflammation obscured the identification of amoebae which were circumferentially arranged around the small blood vessels (**Figure 3B**), i.e., their source of metabolites. Inflammation typified by giant cells and fibrosis extended into the subcutaneous fat, particularly along interlobular septa. It was only after 3 hours of scanning the several H&E slides of the indurated forearm ulcer excision that scattered rare amoebic trophozoites, only 3 to 5 per H&E slide, were identified.

chest lesion was submitted under a clinical diagnosis of angioliipoma. All three biopsies (two from the arm, and one from the chest) obtained in the span of nine days featured a lymphocyte rich diffuse granulomatous inflammation with patchy necrosis (**Figure 2C**). Results from immunohistochemical (IHC) staining for lymphocyte markers CD2, CD3, CD5, CD7, CD4, CD8, CD56, CD20, CD30, CD68, Ki-67 indicated a polymorphous infiltrate. Fite and PAS stains performed on all three specimens were negative for mycobacterial and fungal agents. Bacterial, fungal, and mycobacterial cultures of the second forearm biopsy (punch) and chest nodule biopsy were negative. Chest X-ray was unremarkable. In the absence of a definitive diagnosis, empiric therapy for atypical bacterial organisms was initiated with tetracyclines (doxycycline 50mg BID, then

versus a primary vasculitis with Touton-type giant cells present in clusters. (**Figure 3A**). This pattern of inflammation obscured the identification of amoebae which were circumferentially arranged around the small blood vessels (**Figure 3B**), i.e., their source of metabolites. Inflammation typified by giant cells and fibrosis extended into the subcutaneous fat, particularly along interlobular septa. It was only after 3 hours of scanning the several H&E slides of the indurated forearm ulcer excision that scattered rare amoebic trophozoites, only 3 to 5 per H&E slide, were identified.

The organisms' finely granular cytoplasm and nucleus with prominent nucleolus (**Figure 4A**) had previously been mistaken for granular histiocytes with necrotic cell remnants. Once identified, amoebae could also be appreciated in the cytoplasm of multinucleated histiocytic giant cells (**Figure 4B**). Occasional trophozoite and cyst forms were binucleate (**Figure 4C**), indicating *Balamuthia* over *Acanthamoeba* [4]. Slender pseudopodia were visible on close inspection (**Figure 4D**). Some of the cyst forms mimicked fungal spherules. In Toluidine blue, trophozoites stained more darkly than the reactive cellular background

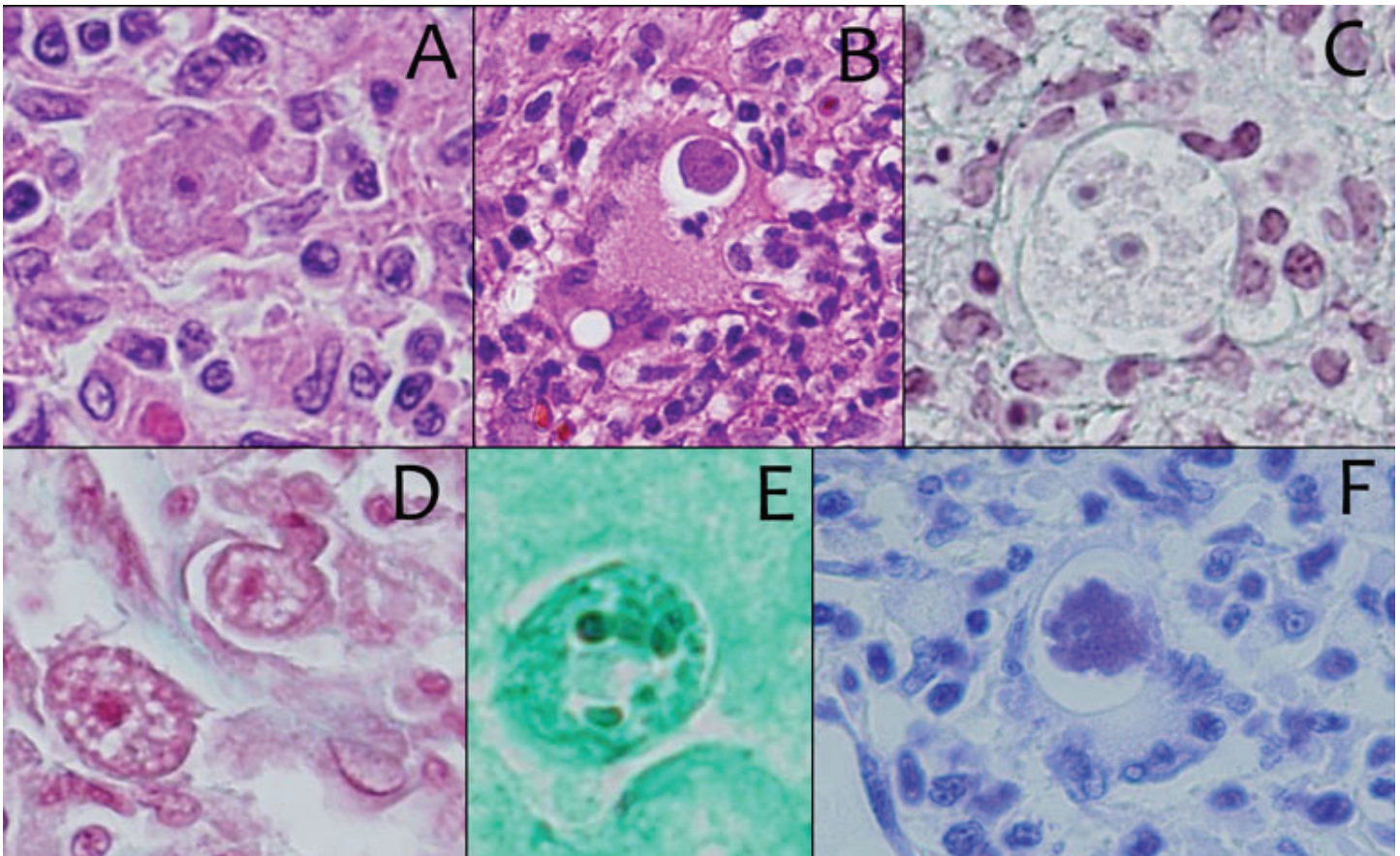


Figure 4A-F. Various appearances of *Balamuthia* from the arm ulcer. A, B) H&E (400x). C, D) Masson trichrome (800x). E) GMS (800x). F) Toluidine blue (400x).

(**Figure 4F**). PAS, GMS, and Gomori's green trichrome stain offered no improved distinction compared to conventional H&E staining. A notable testament to the infrequency of this subtle pathogen, is that none of the 13 color photomicrographs illustrating granulomatous inflammation included in the three original dermatopathology reports captured a single amoeba. Re-examination of the slides from the three prior biopsies demonstrated very few amoebic trophozoite or cyst forms.

Three days after complete excision of the ulcer, the patient was hospitalized for four days of mild confusion, disorientation, and headache. His son confirmed gradual changes in personality occurring over prior months. Physical exam revealed apraxia, visual agnosias, and mild aphasia. Cerebrospinal fluid (CSF) analysis demonstrated only moderate lymphocytosis with no eosinophilia, although a mild eosinophilia was detected in peripheral blood. A CT scan of the head on a General Electric Four Slice Lightspeed CT Scanner displayed moderate size changes in the mid to posterior basilar region

concerning for vasogenic edema in addition to global signs of underlying microvascular disease. A follow-up CT head with contrast displayed bilateral areas of abnormal enhancement with a cystic mass with nodule apparent in the left medial parieto-occipital lobe. In addition, moderate gyral enhancement was noted throughout the right temporal posterior parietal lobe, consistent with vasogenic edema. An MRI (General Electric 1.5 Tesla Vantage) of the brain with and without contrast indicated bilateral rim-enhancing occipital lobe lesions in the cortical/subcortical region with gyral enhancement present for the right-sided lesion with the left medial occipital lesion representing a juxtaposed enhancing lesion (**Figure 5**). The radiologic differential diagnosis included inflammation/infection, neoplasm and ischemia/infarct.

Even with the knowledge of the recently discovered metastatic amoebic skin infection (left arm to subcutaneous chest), a brain biopsy was necessary to rule-out the possibility of metastatic prostate carcinoma. An image-guided right parietal craniotomy

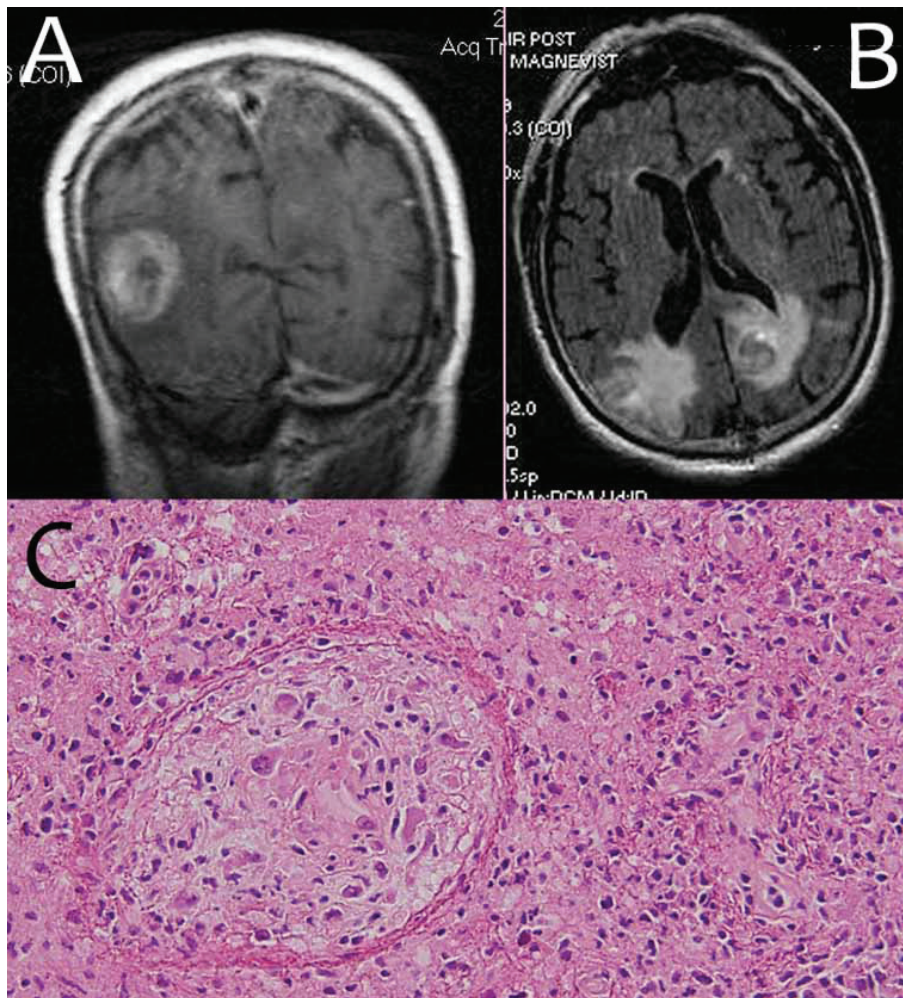


Figure 5A-C. Neurologic involvement. A, B) Brain abscesses visualized on MRI. C) Granulomatous inflammation in the brain biopsy (250x).

indirect immunofluorescent (IFA) anti-*B. mandrillaris* antibody (**Figure 6**) and found to be non-reactive to the anti-*Acanthamoeba* antibody. Serial serum specimens obtained following craniotomy were negative for *B. mandrillaris* by IFA serologic testing. Antibody titers remained stable at 1:32 for a number of months peaking at 1:62 on two occasions with eventual decline to 1:8 eight months after definitive diagnosis (**Table 1**).

Levetiracetam for seizure prophylaxis was initiated postoperatively. A postoperative CT scan of the head confirmed decreasing edema in right parietal region and subtle improvement in the left occipital area. A non-contrast CT head at two-month follow-up showed moderate interval improvement in the bilateral low attenuation cerebral lesions. In response to the nature of his underlying diagnosis, his antibiotic regimen was updated to azithromycin, flucytosine, and sulfadiazine.

and gross total resection of an intracerebral abscess was performed on hospital day 7 (10 days after large left arm excision) with histopathology consistent with that seen in the prior cutaneous specimens, a brisk granulomatous reaction camouflaging rare amoebic organisms.

Unstained paraffin sections of the brain specimen and the resected elbow lesion were sent to the CDC in Atlanta, GA. Pathology reports indicated many amoebae clustered around capillaries in the cutaneous specimen with very few amoebae appreciated in individual brain sections. Both brain and skin tissue were positive for *B. mandrillaris* as assessed by

Follow-up

Eight months after the inciting fall and four months

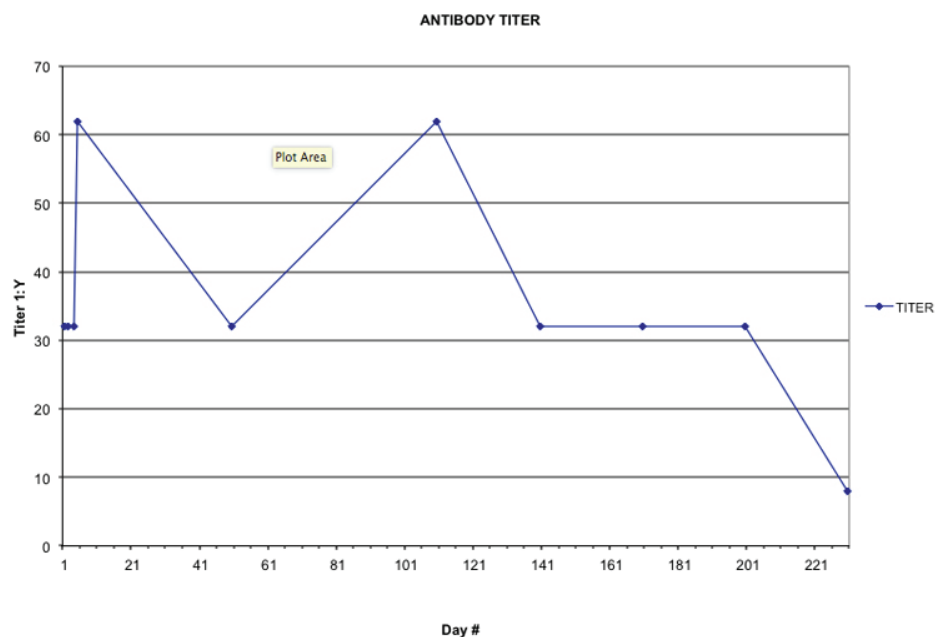


Table 1. Serum antibody titers to *Balamuthia mandrillaris* determined by indirect immunofluorescence microscopy.

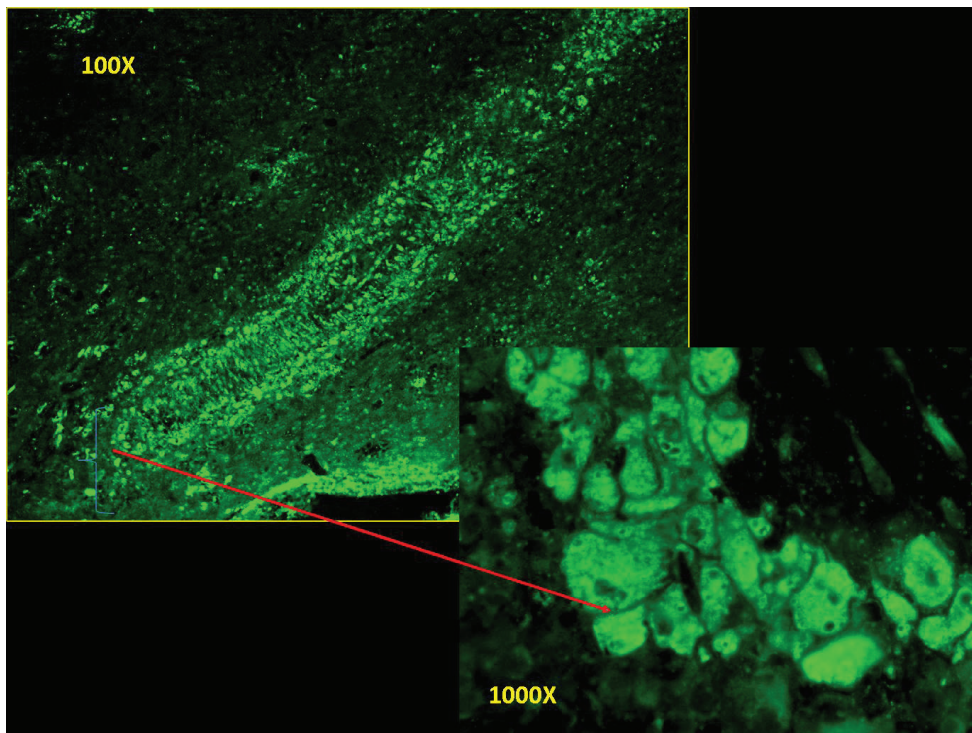


Figure 6. Indirect immunofluorescent antibody labeling of the brain biopsy with anti-*Balamuthia mandrillaris* antibody of amoebae clustered along the sides of blood vessels has its counterpart in the skin excision specimen (**Figure 3B**).

post-brain surgery, the patient remained stable at his baseline functional status continuing maintenance therapy with flucytosine and levetiracetam without adverse effect. At one-year follow-up his primary care provider reports he is alive and well.

Case Discussion

It was in Germany, far removed from the epicenters of disease located in Lima, Peru and the U.S. states California and Texas, [3] that Nasse first described cutaneous amoebiasis in 1892. Except for skin involvement by the intestinal parasitic amoeba *Entamoeba histolytica* [5], cutaneous infection by free-living amoebae, i.e. *Balamuthia*, *Acanthamoeba*, or *Naegleria*, is extremely rare [6]. However, these organisms abound in the environment and have been detected in soil, water, and even air samples from all over the world. *Acanthamoeba* spp. and *Balamuthia mandrillaris* (*B. mandrillaris*) are opportunistic pathogens. Although they are able to invade any organ [7-9], they are most commonly found in the skin and central nervous system (CNS), [10]. Infection by *B. mandrillaris* is referred to as balamuthiasis.

Balamuthiasis was first described in 1990 by Visvesvara et al. [11] as the cause of a fatal CNS infection in a

pregnant mandrill baboon at the California San Diego Zoo. Since its recognition, over 200 cases of balamuthiasis have been reported [12]. South American physicians are most familiar the condition and therefore adept at instituting prompt treatment [13]. Local, frequently insidious trauma, often precedes the appearance of cutaneous infection with subclinical micro-abrasions serving as likely ports of entry [14]. The most common presentation of *B. mandrillaris* cutaneous amoebiasis is a rubbery plaque or ulceration on the central face, particularly the nose [15]. If left untreated, lesions may progress to diffuse infiltration and deformity. Simultaneous extremity and/or oral lesions, particularly of the hard palate, may arise.

Regional satellite lesions, such as the chest lesion in the present case, may appear as well. Hence, the patient in this report experienced a 'classic' course: compromise of the skin barrier complicated by soil contamination leading to a localized, ulcerated skin infection followed by hematogenous dissemination to nearby skin and the CNS [16].

Tissue invasion is achieved by *B. mandrillaris* via the secretion of digestive enzymes [17]. After penetrating through cutaneous blood vessels, the organisms travel to the CNS. In the CNS *B. mandrillaris* induces human brain microvascular endothelial cells to release the pro-inflammatory cytokine interleukin 6, which results in granulomatous amoebic encephalitis (GAE), referred to more specifically as *Balamuthia* amoebic encephalitis (BAE), [18]. GAE is a chronic condition with an incubation period from roughly two days to upward of 2-years [17]. Once the acute phase develops, death occurs in a matter of days [19]. Initial neurological symptoms are encephalitic and include altered mental status, headache, fever, and photophobia [20]. GAE is often mistaken for neurotuberculosis or neurocysticercosis clinically; brain tumor and fungal or viral encephalitides are other potential diagnoses in the differential diagnosis

[14]. However, *B. mandrillaris* is not readily isolated from CSF. To date, only one such attempt at isolation has been successful and was obtained on autopsy [16]. CSF analysis reveals lymphocytic pleocytosis with mild to severe elevation ($\geq 1,000\mu\text{g}/\text{dl}$) of protein and normal to low glucose concentrations [21]. Seizure, lethargy, and anisocoria, indicate increased intracranial pressure, i.e., disease progression. In the United States, the prognosis of BAE is poor, with a mortality rate approaching 95% (7 survivors of 150 reported cases), [17]. Only a few patients worldwide are reported to have survived BAE [12, 22-24].

Even in the instance of premortem diagnosis, the disease is most often fatal [22]. Of the 60 cases of *Balamuthia amoebic encephalitis* (BAE) diagnosed in the United States, the majority were identified at autopsy [23, 26, 27]. Histopathologic diagnosis is rare because amoebae are camouflaged by the granulomatous inflammatory response of the surrounding tissue [31]. BAE is likely under diagnosed by U.S. clinicians and pathologists owing to its nonspecific symptoms and unfamiliar appearance of amoebas in tissue sections, respectively [28]. Granulomatous lesions are a hallmark of BAE, but they may be absent in the immunosuppressed [23, 29]. Predisposing factors include very young [30] or old age, Hispanic ethnicity [31], chronic health problems, malnutrition, contact with soil or water, and prior treatment with corticosteroids [11, 23, 31].

Occupational contact with soil or during gardening is a significant risk factor for *Balamuthia amoebic encephalitis* [11]. Infection has been noted following contact with soil via playing with potting soil [33], digging a drain [21], gardening [34], pruning roses [35], riding in open vehicles over dusty desert terrain [23], and amputation of a leg in a farm accident with resultant wound contamination [36]. Water has also been implicated in several cases [23]. There are now a handful of cases of *B. mandrillaris* infection following organ transplantation [37, 27, 25], including disease clusters in the event of transmission from an infected donor to multiple recipients [38]. Despite nearly constant environmental exposure, vanishingly few instances of human *B. mandrillaris* infections have been reported, suggesting low virulence [39].

The patient presented was a relatively healthy

84-year-old. However, immune senescence associated with his age alone put him at risk for *B. mandrillaris* infection. Additionally, while the pathogenesis of *B. mandrillaris* remains under investigation, IgG and IgM antibodies against the amoeba are present in serum and his 5-year course of rituximab, an anti-B cell immunotherapy shown to suppress humoral immunity with long-term use [40], may have contributed. Rituximab is mentioned in a case of *Acanthamoeba* associated GAE (diagnosed postmortem) in a 66-year-old woman with hepatitis C-related cryoglobulinemic vasculitis [41]. GAE developed after rituximab infusions had begun and corticosteroid dose was tapered. The authors postulated that *Acanthamoeba* was already present in the brain at the time of the first rituximab infusion, which precipitated the unusually rapid course of the encephalitis.

Cutaneous amoebiasis in AIDS is the most common and often presenting sign of disseminated infection [42]. However, it often manifests as subcutaneous nodules that mimic mundane clinical entities [43, 44]. Encouragingly, cutaneous disease in the absence of CNS involvement is increasingly recognized, especially in the setting of chronic, nonhealing skin lesions in patients with AIDS [45-48].

Diagnosis

Aside from specific serologic testing available only through a national reference laboratory and culture performed by placing fresh tissue on a plate of live cells (because, unlike other free-living amoebae, *Balamuthia* does not grow on bacteria-coated non-nutrient agar plates), the diagnosis of an amoebic infection is made through direct visualization of trophozoites and/or cysts in tissue (**Figure 3B**), [49].

Histopathology

A macrophage-predominant granulomatous tissue reaction pattern was present in all cutaneous and nervous specimens obtained in the case presented. Granulomatous inflammation is notably absent in skin lesions taken from immunocompromised patients with severe balamuthiasis and CNS involvement [50, 51], with only occasional multinucleated giant cells present [52]. Therefore, an inability to produce granulomata around the amoebas is thought to contribute to dissemination of the organism [53].

Irregular to oval 12-60µm trophozoites are often arranged in perivascular array. Triple-encapsulated 15-30µm cysts, which appear dual-walled by light microscopy, may be more easily discerned but do not always accompany trophozoite forms in histological sections [54]. The nuclei of both cysts and trophozoites are prominent and contain distinct nucleoli. Acanthopodia, cellular projections that aid in motility, may be distinguishable. Other histologic features include leukocytoclastic or necrotizing vasculitis, panniculitis, and acute and/or chronic inflammation [3]. However, the diagnosis of cutaneous amoebiasis eludes initial histopathologic analysis as non-specific in nearly all cases, present report included, as trophozoite forms are more often mistaken for large histiocytes amid necrotic debris. Reliable diagnosis depends on a high degree of suspicion by the pathologist and the recognition of the consistent morphological features of the organisms, as revealed by standard hematoxylin-eosin (HE) staining. The use of special stains is problematic as amoebic cyst walls pick up Gomori methenamine silver and periodic acid-Schiff stains in a pattern reminiscent of fungal yeast cells. Although it is difficult to differentiate *B. mandrillaris* from *Acanthamoeba* spp. in tissue sections by light microscopy, characterization is possible via antibody-mediated immunofluorescence or transmission electron microscopic analysis of cyst morphology [55]. Finally, amoebae may mimic other microbiologic phenomena histopathologically including *Rhinosporidium seeberi*, *Cryptococcus neoformans*, *Prototheca wickerhamii* and *Blastomyces dermatitidis* [47].

Treatment

Amoebae are multidrug resistant and the optimal antimicrobial therapy has yet to be determined [21]. Encephalitis and other infections caused by *Acanthamoeba* and *Balamuthia* have been treated, more or less successfully, with antimicrobial combinations including sterol-targeting azoles (clotrimazole, miconazole, ketoconazole, fluconazole, itraconazole), pentamidine isethionate, 5-fluorocytosine, and sulfadiazine. Miltefocine, an alkylphosphocholine used to treat visceral leishmaniasis, has also been reported to reverse *Balamuthia* CNS involvement [56]. The use of drug combinations addresses resistance patterns that may exist or develop during treatment. Amoebicidal

drugs have been identified in laboratory trials, but the in vivo efficacy of these drugs remains unknown owing to toxicity at therapeutic dosages [30]. Another issue complicating treatment is that it has yet to be determined whether *Balamuthia* cysts are able to remain dormant in the brain and other tissues and enable a relapse of infection after completion of effective treatment [30]. Further investigation into the immune systems role in warding off opportunistic amoebae may open the door to immune modulation as a potential therapeutic alternative [53].

Conclusion

Cutaneous and/or bone lesions that fail to show improvement with broad-spectrum antibiotic therapy in the young, elderly, or immunosuppressed, should raise suspicion for cutaneous amoebiasis. Timely histopathologic evaluation for evidence of free-living amoebae may determine the difference between survival and death for a patient presenting with a treatment-refractory localized granulomatous lesion.

References

1. Bravo FG, Seas C. *Balamuthia mandrillaris* amoebic encephalitis: an emerging parasitic infection. *Curr Infect Dis Rep* 2012;14:391-6. [PMID: 22729402].
2. Bravo FG, Alvarez PJ, Gotuzzo E. *Balamuthia mandrillaris* infection of the skin and central nervous system: an emerging disease of concern to many specialties in medicine. *Curr Opin Infect Dis* 2011;24:000-000. [PMID: 21192259].
3. Chang OH, Liu F, Knopp E, Muehlenbachs A, Cope JR, Ali I, Thompson R, George E. Centofacial *Balamuthia* infection: case report of a rare cutaneous amoebic infection. *J Cutan Pathol* 2016;43:892-7. [PMID: 27251900].
4. Garcia LS. Chapter 5: Free-Living Amebae. In: *Diagnostic Medical Parasitology*, 4th Edition. (ASM Press: Herndon, VA) 2001, page 120.
5. Moran P, Rojas L, Cerritos R, Zermeño V, Valadez A, Montes de Oca G, Reyes MA, Gonzalez E, Partida O, Hernandez E, Miriam N, Portillo T, Gudiño M, Ramiro M, Ximenez C. Cutaneous Amebiasis: the importance of molecular diagnosis of an emerging parasitic disease. *Am J Trop Med Hyg* 2013 Jan 9;88(1):186-90. [PMID: 23208883].
6. Visvesvara GS, Schuster FL. Opportunistic free-living amoebae, part I. *Clin Microbio Newsletter* 2008;30(20):151-8.
7. Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amoebae. *Brain Pathol* 1997;7:583-98. [PMID: 9034567].
8. Shuster FL, Dunnebacke TH, Booton GC, Yagi S, Kohlmeier CK, Glaser C, Vugia D, Bakardjiev A, Azimi P, Maddux-Gonzalez M, Martinez AJ, Visvesvara GS. Environmental isolation of *Balamuthia mandrillaris* associated with a case of amoebic encephalitis. *J Clin Microbiol* 2003 July;41:3175-80. [PMID: 12843060].
9. Niyiyati M, Lorenzo-Morales J, Rezaeian M, Martin-Navarro CM, Haghi AM, Maciver SK, Valladares B. Isolation of *Balamuthia mandrillaris* from urban dust, free of known infectious involvement. *Parasitology Research* 2009;106(1):279-81. [PMID: 19685076].
10. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic

- free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunol Med Microbiol* 2007; 50:1-26. [PMID: 17428307].
11. Visvesvara GS, Martinez AJ, Schuster FL, et al. Leptomyxid amoeba, a new agent of amebic meningoencephalitis in humans and animals. *J Clin Microbiol* 1990;28:2750-6. [PMID: 2280005].
 12. Schuster FL, Visvara GS. *Balamuthia mandrillaris*. In: *Emerging Protozoan Pathogens*. Khan NA, ed. London, England: Taylor and Francis Group; 2008: 71-118.
 13. Seas RC, Bravo PF. Amebic granulomatous encephalitis due to *Balamuthia mandrillaris*: fatal disease increasingly recognized in Latin America [Spanish]. *Rev Chilena Infectol* 2006 Sep;23(3):197-9. [PMID: 16896491].
 14. Pritzker AS, Kim BK, Agrawal D, Southern PM Jr, Pandya AG. Fatal granulomatous amebic encephalitis caused by *Balamuthia mandrillaris* presenting as skin lesion. *J Am Acad Dermatol* 2004;50(2 Suppl):38-41. [PMID: 14726864].
 15. Bravo, FG, Cabrera, J., Gotuzzo, E., Visvesvara, GS. Cutaneous manifestations of infection by free-living amoebas. In: *Tropical Dermatology*. eds. SK Tyring, O. Lupi, and UR Hengge. Elsevier: Philadelphia, PA. 2006, pp. 49–55.
 16. Helton J, Loveless M, White CR Jr. Cutaneous acanthamoeba infection associated with leukocytoclastic vasculitis in an AIDS patient. *Am J Dermatopathol* 1993;15:146-9. [PMID: 8494115].
 17. Magana ML, Fernandez-Diez J, Magana M. Cutaneous Amebiasis in Pediatrics. *Arch Dermatol* 2008;144:1369-1372. [PMID: 18936402].
 18. Jayasekera S, Sissons J, Tucker J, Rogers C, Nolder D, Warhurst D, Alsam S, White JM, Higgins EM, Khan NA. Post-mortem culture of *Balamuthia mandrillaris* from the brain and cerebrospinal fluid of a case of granulomatous amoebic meningoencephalitis, using human brain microvascular epithelial cells. *J Med Microbiol* 2004;53:1007-12. [PMID: 15358823].
 19. Schuster FL, Yagi S, Gavali S, Michelson D, Raghavan R, Blomquist I, Glastonbury C, Bollen Aw, Scharnhorst D, Reed SL, Kuriyama S, Visvesvara GS, Glaser CA. Under the Radar: *Balamuthia* Amebic Encephalitis. *Clin Infect Dis* 2009;48:879-87. [PMID: 19236272].
 20. Barratt JLN, Harkness J, Mariotte D, Ellis JT, Stark D. Importance of nonenteric protozoan infections in immunocompromised people. *Clin Microbiol Rev* 2010 Oct;23(4):795-836. [PMID: 20930074].
 21. Tavares, M. Correia da Costa JM, Carpenter SS, Santos LA, Afonso C, Aguiar A, Pereira J, Cardoso AI, Schuster FL, Yagi S, Sriram R, Visvesvara GS. Diagnosis of first case of *Balamuthia* amoebic encephalitis in Portugal by immunofluorescence and PCR. *J Clin Microbiol* 2006; 44:2660-2663. [PMID: 16825409].
 22. Cary LC, Maul E, Potter C, Wong P, Nelson PT, Given C 2nd, Robertson W Jr. *Balamuthia mandrillaris* meningoencephalitis: survival of a pediatric patient. *Pediatrics* 2010;125(3):699-703. [PMID: 20123772].
 23. Martinez DY, Seas C, Bravo F, Legua P, Ramos C, Cabello AM, Gotuzzo E. Successful treatment of *Balamuthia mandrillaris* amoebic infection with extensive neurological and cutaneous involvement. *Clin Infect Dis* 2010;51(2):e7-11. [PMID: 20550438].
 24. Doyle JS, Campbell E, Fuller A, Spelman DW, Cameron R, Malham G, Gin D, Lewin SR. *Balamuthia mandrillaris* brain abscess successfully treated with complete surgical excision and prolonged combination antimicrobial therapy. *J Neurosurg* 2011;114(2):458-62. [PMID: 21073255].
 25. Centers for Disease Control and Prevention. *Balamuthia mandrillaris* transmitted through organ transplantation – Mississippi, 2009. *MMWR* 2010; 59:1165-70; C; CDC Notes from the field Transplant-transmitted *Balamuthia mandrillaris* – Arizona 2010. *MMWR* 2010;59:1182. [PMID: 20847722].
 26. Intalapaporn P, Suankratay C, Shuangshoti S, Phantumchinda K, Keelawat S, Wilde H. *Balamuthia mandrillaris* meningoencephalitis: the first case in Southeast Asia. *Am J trop Med Hyg* 2004; 70:666-9. [PMID: 15211011].
 27. Katz JD, Ropper AH, Adelman, L, Worthington M, Wade P. A case of *Balamuthia mandrillaris* meningoencephalitis. *Arch Neurol* 2000; 57:1210-2. [PMID: 10927804].
 28. Schuster FL, Visvesvara GS. Opportunistic Amoebae: Challenges in Prophylaxis and Treatment. *Drug Resist Update* 2004;7:41-51. [PMID: 15072770].
 29. Anzil AP, Rao C, Wrzolek MA, Visvesvara GS, Sher JH, Kozlowski PB. Amebic meningoencephalitis in a patient with AIDS caused by a newly recognized opportunistic pathogen. *Arch Pathol Lab Med* 1991;115:21-5. [PMID: 1987909].
 30. Ghosh PS, Ghosh D. Necrotizing granulomatous meningoencephalitis due to *Balamuthia* in an immunocompetent child. *Neurology* 2011;77(8):801-2. [PMID: 21860010].
 31. Schuster FL, Glaser C, Honarmand S, Maguire JH, Visvesvara GS. *Balamuthia* amoebic encephalitis risk, Hispanic Americans [letter]. *Emerg Infect Dis* 2004 Aug;10(8):1510-2. [PMID: 15503402].
 32. Zagardo MT, Castellani RI, Zoarski GH, Bauserman SC. Granulomatous amoebic encephalitis caused by leptomyxid amoebae in an HIV-infected patient. *Am J Neuroradiol* 1997;18:903-8. [PMID: 9159368].
 33. Bakardjiev A, Azimi PH, Ashouri N, Ascher DP, Janner D, Schuster FL, Visvesvara GS, Glaser C. Amebic encephalitis caused by *Balamuthia mandrillaris*: report of four cases. *Pediatr Infect Dis J* 2003; 22:447-52. [PMID: 12792389].
 34. Jung, S, Schelper RL, Visvesvara GS, Chang HT. *Balamuthia mandrillaris* meningoencephalitis in an immunocompetent patient: an unusual clinical course and a favorable outcome. *Arch Pathol Lab Med* 2004;128:466-468. [PMID: 15043486].
 35. Deetz, TR, Sawyer MH, Billman G, Schuster FL, Visvesvara GS. Successful treatment of *Balamuthia* amoebic encephalitis: presentation of 2 cases. *Clin Infect Dis* 2003; 37:1304-1312. [PMID: 14583863].
 36. Gordon SM, Steinberg JP, DuPuis MH, Kozarsky PE, Nivkerson JF, Visvesvara GS. Culture isolation of *Acanthamoeba* species with amoebic meningoencephalitis, including two patients with AIDS. *Clin Infect Dis* 1992; 15:1024-30. [PMID: 1457633].
 37. Peleg AY, Husain S, Kwak EJ, Silveira FP, Ndirangu M, Tran J, Shutt KA, Shapiro R, Thai N, Abu-Elmagd K, McCurry KR, Marcos A, Paterson DL. Opportunistic infections in 547 organ transplant recipients receiving alemtuzumab, a humanized monoclonal CD-52 antibody. *Clin Infect Dis* 2007;44:204-12. [PMID: 17173218].
 38. Gupte AA, Hocevar SN, Lea AS, Kulkarni RD, Schain DC, Casey MJ, Zendejas-Ruiz IR, chung WK, Mbaey C, Roy SL, Visvesvara GS, da Silva AJ, TALLAJ J, Eckhoff D, Baddley JW. Transmission of *Balamuthia mandrillaris* through solid organ transplantation: utility of organ recipient serology to guide clinical management. *Am J Transplant* 2014 June;14(6):1417-24. [PMID: 24840013].
 39. Siddiqui R, Khan NA. *Balamuthia mandrillaris*: morphology, biology, and virulence. *Trop Parasitol* Jan-Jun 2015;5(1):15-22. [PMID: 25709948].
 40. Eisenberg R. Update on rituximab. *Ann Rheum Dis* 2005;64(Suppl4):iv55-7. [PMID: 16239389].
 41. Meersseman W, Lagrou K, Sciort R, Jonckheere JD, Haberler C, Walochnik J, Peetermans WE, van Wijngaerden E. Rapidly fatal *Acanthamoeba* encephalitis and treatment of cryoglobulinemia. *Emerg Infect Diseases* 2007;13:469-471. [PMID: 17552103].
 42. Chandrasekar PH, Nandi PS, Fairfax MR, Crane LR. Cutaneous infections due to *Acanthamoeba* in patients with acquired immunodeficiency syndrome. *Arch Intern Med* 1997;157:569-72. [PMID: 9066462].
 43. Capetanakis J, Doxiades T. Perianal ulcers and secondary manifestations due to amoebic infection. *Am J Proctol*. 1966;17:58-63. [PMID: 5902492].
 44. Rosenberg AS, Morgan MB. Disseminated acanthamoebiasis

- presenting as lobular panniculitis with necrotizing vasculitis in a patient with AIDS. *J Cutan Pathol* 2001; 28:307-13. [PMID: 11401678].
45. Culbertson CG, Ensminger PW, Overton WM. Amebic cellulocutaneous invasion by *Naegleria aerobia* with generalized visceral lesions after subcutaneous inoculation: an experimental study in guinea pigs. *Am J Clin Pathol*. 1972;57:375-86. [PMID: 5018608].
 46. Tan B, Weldon-Linne CM, Rhone DP, Penning CL, Visvesvara GS. Acanthamoeba infection presenting as skin lesions in patients with the acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 1993;117:1043-6. [PMID: 8215828].
 47. May LP, Sidhu GS, Buchness MR. Diagnosis of Acanthamoeba infection by cutaneous manifestations in a man seropositive to HIV. *J Am Acad Dermatol* 1992;26:352-5. [PMID: 1569257].
 48. Levine S, Goldstein AE, Dahdouh M, Blank P, Hoffman C, Gropper CA. Cutaneous Acanthamoeba in a patient with AIDS: a case study with a review of new therapy; quiz 386. *Cutis* 2001;67:377-80. [PMID: 11381852].
 49. Sison JP, Kemper CA, Loveless M, McShane D, Visvesvara GS, Deresinski SC. Disseminated Acanthamoeba infection in patients with AIDS: case reports and review. *Clin Infect Dis* 1995;20:1207-16. [PMID: 7620001].
 50. Anderlini P, Przepiorka D, Luna M, Langford L, Andreef M, Claxton D, Deisseroth AB. Acanthamoeba meningoencephalitis after bone marrow transplantation. *Bone Marrow Transplant* 1994;14:459-61. [PMID: 7994273].
 51. Wiley CA, Safrin RE, Davis CE, Lampert PW, Braude AI, Martinez AJ, Visvesvara GS. Acanthamoeba meningoencephalitis in a patient with AIDS. *J Infect Dis* 1987;155:130-3. [PMID: 3794297].
 52. Tavares, M. Correia da Costa JM, Carpenter SS, Santos LA, Afonso C, Aguiar A, Pereira J, Cardoso AI, Schuster FL, Yagi S, Sriram R, Visvesvara GS. Diagnosis of first case of Balamuthia amoebic encephalitis in Portugal by immunofluorescence and PCR. *J Clin Microbiol* 2006; 44:2660-2663. [PMID: 16825409].
 53. Marciano-Cabral F, Puffenbarger R, Cabral GA. The increasing importance of Acanthamoeba infections. *J Eukaryot Microbiol* 2000;47:29-36. [PMID: 10651293].
 54. Visvesvara GS. Classification of Acanthamoeba. *Rev Infect Dis* 1991;13:5369-72. [PMID: 2047665].
 55. Visvesvara GS, Schuster FL. Opportunistic free-living amebae, part II. *Clin Microbio Newsletter* 2008;30(21):159-66.
 56. Martinez D, Seas C, Bravo F, Legua P, Ramos C, Cabello AM, Gotuzzo E. Successful treatment of Balamuthia mandrillaris amoebic infection with extensive neurological and cutaneous involvement. *Clin Infect Dis* 2010;51(2):e7-e11. [PMID: 20550438].