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Disseminated *Acanthamoeba* infection in a heart transplant recipient treated successfully with a miltefosine-containing regimen: case report and review of the literature

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Abstract

Disseminated acanthamoebiasis is a rare, often fatal, infection most commonly affecting immunocompromised patients. We report a case involving sinuses, skin, and bone in a 60-year-old woman five months after a heart transplant. She improved with a combination of flucytosine, fluconazole, miltefosine, and decreased immunosuppression. To our knowledge this is the first case of successfully treated disseminated acanthamoebiasis in a heart transplant recipient and only the second successful use of miltefosine for this infection among solid organ transplant recipients. *Acanthamoeba* infection should be considered in transplant recipients with evidence of skin, central nervous system, and sinus infections that are unresponsive to antibiotics. Miltefosine may represent an effective component of a multi-drug therapeutic regimen for the treatment of this amoebic infection.

Case Presentation

A 60-year-old Pacific Islander presented to her primary care physician with rhinorrhea and sinus pressure five months after orthotopic heart transplantation.

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Disclaimer: The findings and conclusions herein are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Her past medical history was notable for non-ischemic cardiomyopathy requiring heart transplantation, gout, and chronic kidney disease. She had received thymoglobulin induction at the time of transplant and had been doing well on a maintenance immunosuppressive regimen of mycophenolate (1,000mg BID), tacrolimus (1.5mg BID), and prednisone (2.5mg daily). She was also on trimethoprim-sulfamethoxazole (800mg-160mg) three times weekly for *Pneumocystis* pneumonia (PCP) prophylaxis and had not experienced any episodes of rejection or opportunistic infections.

She was initially treated by her primary care physician for these sinus symptoms with a 10-day course of cephalexin and nasal fluticasone for presumed bacterial sinusitis. When her symptoms failed to improve, she received an additional 10 days of amoxicillin-clavulanate with guaifenesin and pseudoephedrine. During this time, she had several episodes of epistaxis and was referred for evaluation by an otolaryngologist. In the six-week period between antibiotic treatment and being evaluated by her otolaryngologist, she performed a tap water lavage of her sinuses on several occasions, as she had done in the past for nasal congestion, with minimal improvement of her symptoms. She had no fevers, chills or night sweats. She had not traveled outside of Northern California in several years and did not endorse any recent history of freshwater exposure or dust inhalation. Trained as an esthetician, she had not worked since before her transplant.

The patient was seen by an outpatient otolaryngologist two months after the onset of symptoms (seven months after transplant), and was noted on exam to have a friable and raised right nasal septal mass, with magnetic resonance imaging (MRI) of the sinuses showing right frontal, maxillary, and ethmoid opacification (Figure 1a). A nasal endoscopy was performed and biopsy was taken. Histopathological review was notable for an acutely inflamed atypical squamoproliferative lesion, raising concern for squamous cell carcinoma. Given the concern for malignancy, she was admitted for surgical resection of the mass. On admission, she was hemodynamically stable and afebrile. Labs showed a white blood cell count of 6200 cells/μL, hemoglobin of 8.5 g/dL, and creatinine of 2.81 mg/dL (baseline 2–3 mg/dL). Surgical resection was aborted when intraoperative frozen section revealed the presence of amoeba, and the patient was started on metronidazole and amphotericin B on the first post-operative day. Tissue sections were sent to the Centers for Disease Control and Prevention (CDC) for specific pathogen identification. Histopathologic examination and subsequent immunohistochemical testing revealed necrotizing granulomatous inflammation with intralesional *Acanthamoeba healyi*.

After consulting with local infectious disease specialists and the CDC, the patient was switched to fluconazole, flucytosine, and miltefosine on hospital day four. Miltefosine is not currently approved to treat acanthamoebiasis, but is available through the CDC's expanded access investigational new drug (IND) protocol. In this case, the drug was obtained through the IND protocol following patient consent and institutional review board approval. A second surgical debridement of the right nasal septum, including right total ethmoidectomy and right frontal sinusotomy, was performed on hospital day seven. Histopathological evaluation and immunohistochemical testing of specimens obtained during this procedure again demonstrated the presence of *Acanthamoeba healyi*. The CDC also performed serologic testing for *Acanthamoeba*, which was negative.

Between hospital days one and four (before initiation of miltefosine therapy), the patient developed several subcutaneous nodules on her left flank and bilateral upper and lower extremities (Figure 2a). Skin biopsy of one of these lesions demonstrated *Acanthamoeba* by histopathological examination and immunohistochemistry (Figure 3a) performed at the California Department of Public Health, further confirmed by polymerase chain reaction (PCR) at CDC. Topical ketoconazole was added. A lesion over the right 3rd metacarpal started to drain in the next 48 hours, and imaging showed a lytic lesion of the right 3rd metacarpal (Figure 4a and 4b). The patient underwent incision and drainage of the digit by orthopedic surgery on hospital day nine, and the surgeon noted that the shaft was hollow and contained small exophytic growths, which were sent for pathologic evaluation. Amoebae were seen with Periodic acid–Schiff–diastase (PAS-D) and Grocott's methamine silver (GMS) stains, and *Acanthamoeba* was identified by PCR at the CDC.

The patient was then transferred to the tertiary care center where she had received her transplant for further management of antimicrobials and immunosuppression. In consultation with the CDC, the regimen of fluconazole, flucytosine, miltefosine, and topical ketoconazole was continued. Her immunosuppressive regimen was also altered: tacrolimus dosing was reduced from 1.5mg BID to 1mg BID with a target trough of 8–10 μg/L, and mycophenolate was reduced from 1,000mg BID to 750mg BID. Repeat brain MRI showed persistent severe pansinusitis but no evidence of bony destruction or central nervous system (CNS) extension of disease. The patient underwent a third superficial debridement of the right sinuses 12 days after her initial surgery and pathologic evaluation again demonstrated amoeba (Figure 3b). More extensive debridement was avoided to mitigate potential translocation of amoeba from the sinuses into the CNS.

Despite persistent demonstration of amoeba in the sinuses, the patient showed clinical improvement during her hospitalization: skin lesions visibly regressed in size and her sinus complaints diminished. Her C-reactive protein (CRP) levels, 76.6 mg/L at the time of transfer, also decreased substantially, dropping to 19.3 mg/L 10 days later. The patient was discharged after 25 days in the hospital, with the plan to maintain her decreased immunosuppressive regimen and the triple-drug therapy of fluconazole, flucytosine, and miltefosine for at least six months. At follow-up visits, she reported continued clinical improvement while on this triple therapy, although the patient did endorse significant nausea from the miltefosine. Liver function tests four months after discharge showed mildly elevated alkaline phosphatase (143–147 U/L) and an elevated gamma-glutamyl transferase (211 U/L), but no other laboratory abnormalities were observed. At six-months after discharge, her CRP was 1.5 mg/L, brain MRI showed resolution of sinus opacities, and her skin lesions had resolved completely. (Figure 2b). Her immunosuppressive regimen was increased to pre-infection levels at this time.

Although no cases of donor-derived *Acanthamoeba* infection have ever been reported, the California Transplant Donor Network was contacted because transplant-related transmission of another amoeba, *Balamuthia,* has been reported (1–3). The donor network concluded that no other infection or illness consistent with free-living amoeba infection had been reported in other organ recipients, and archived donor serum was negative for *Acanthamoeba* serology.

Discussion

Acanthamoeba are free-living amoebae found in soil, air, and numerous aquatic environments, including drinking water, seawater, and swimming pools (3). Human infection is most common in immunocompromised hosts and is transmitted by inhalation of *Acanthamoeba* cysts or inoculation by direct contact with skin or mucosal surfaces. This protozoan has been reported to cause cutaneous lesions, nasopharyngeal infections, pneumonia, pyelonephritis, and granulomatous amoebic encephalitis (GAE), which is fatal in 90% of cases (3–6). *Acanthamoeba* osteomyelitis is extremely rare, and has only been reported in one other solid organ transplant (SOT) patient (7).

All 22 reported cases of acanthamoebiasis in SOT patients (including the present case) are described in Table 1. Given that exposure is common but diagnosis is difficult to establish, these reported cases may underrepresent the true disease burden in SOT recipients. To our knowledge this is only the second reported case of *Acanthamoeba* infection in a heart transplant recipient and the first report of successful treatment (8). Other cases have been reported in kidney (n=8), lung (n=8), liver (n=2), and multi-organ (n=2) recipients. The mean age of patients for these cases was 50 (range 31–64), and mean time to infection following transplant was 18 months (range three months-six years). Immunosuppressive regimens have most commonly included tacrolimus, cyclosporine, mycophenolate and/or prednisone. In three cases (including this case), the patient received thymoglobulin as part of his or her post-transplant immunosuppression regimen. Our patient's use of tap water for nasal lavage was initially regarded as a possible source of amoeba, particularly given her history of repeated tap water irrigations in the past. However, upper respiratory symptoms pre-dated her use of nasal irrigation in the acute setting, and this mechanism of amoebic infection has only been reported in two cases of *Naegleria fowleri* infection in the United States (9). Immunocompromised patients should nonetheless avoid the use of non-sterile water for nasal irrigation given the potential for amoebic infection.

Diagnosis of *Acanthamoeba* infection can be challenging. The majority of cases listed in Table 1 relied on histopathologic or immunofluorescence staining of involved tissue or fluid, and most cases (including ours) confirmed the diagnosis by sending samples to the CDC for immunofluorescence testing to detect *Acanthamoeba* trophozoites and cysts. Additionally, diagnosis can be made by culture or PCR from tissue or fluid specimens. Serologic testing is available, however the sensitivity and specificity of this assay is not well described.

Optimal treatment for acanthamoebiasis is not well established. Lack of susceptibility data for *Acanthamoeba* makes choice of antibiotic particularly challenging. Thus, a 'kitchen sink' approach relying on multiple pharmacologic mechanisms of action is often employed (10). There are six previous case reports of successfully treated *Acanthamoeba* infection in SOT patients, as noted in Table 1. Effective regimens have included sterol-targeting azoles (fluconazole, itraconazole, ketoconazole), pentamidine isethionate, trimethoprim-sulfamethoxazole, sulfadiazine, 5-fluorocytosine, azithromycin, amphotericin B, and miltefosine (10–16). Miltefosine, an aklylphosphocholine drug initially approved by the United States Food and Drug Administration (FDA) for the treatment of visceral leishmaniasis, also has *in vitro* activity against free living amoeba, including *N. fowleri,*

Balamuthia mandrillaris, and *Acanthamoeba* spp. (12). It has been available in the United States (from CDC) under an expanded access IND protocol for use in the treatment of free-living ameba infections since 2013. There are five case reports of its use in treatment of *Acanthamoeba*, only two of which resulted in successful treatment (17–22). Topical chlorhexidine or ketoconazole has also been used for cutaneous lesions. Overall prognosis for this infection remains poor: only six of 21 previous SOT cases were reported as cured, and no therapeutic regimen has shown consistent efficacy. Of those that survived, only one had CNS involvement, and only two had disease involving more than one organ. This would suggest that the absence of encephalitis and/or disseminated disease may be important prognostic factors.

Our patient developed disseminated infection, which included the sinuses, skin, and bone but fortunately without evidence of CNS disease. Dissemination appeared to progress despite initial therapy with amphotericin B and metronidazole for four days and subsequent conversion to fluconazole, flucytosine, and miltefosine. Continued use of this triple-drug therapy and concurrent reduction in immunosuppression were followed by steady improvement in the second and third weeks of hospitalization. We speculate that improvement of the patient's skin lesions and absence of new bone invasion resulted from this combination of anti-amoebic therapy and prompt decrease in the patient's immunosuppressive regimen. Following discharge, the patient missed numerous appointments but did report adhering to her drug regimen, making it difficult to assess the progress of her improvement. Given a lack of interval imaging and evaluation, adequate duration of triple-drug therapy to resolve the infection was unclear, and a prolonged course was maintained. After six months of therapy, sinus symptoms and MRI lesions had completely resolved, and cutaneous lesions were not visible. Given this improvement and the patient's ongoing nausea, miltefosine was discontinued eight months after discharge. Shortly after discontinuation of miltefosine, the patient's absolute neutrophil count (ANC) began to drop; this was attributed to bone marrow suppression from flucytosine. Her ANC reached a nadir of 1660 cells/μL and she was switched to a regimen of fluconazole and trimethoprim-sulfamethoxazole, which she has been on for four months. Her ANC returned to baseline (> 4000 cells/μL) and she has remained clinically stable at 16 months after discharge.

Summary

Acanthamoebiasis is a rare, potentially fatal infection in SOT patients. Here we describe the second reported occurrence of disseminated acanthamoebiasis in a heart transplant recipient. This is also the first instance of successful treatment with a miltefosine-containing regimen for such a patient with disseminated disease, albeit without amebic encephalitis. Our patient demonstrated an extremely rare lytic lesion of bone, along with more typical sinus and skin involvement. Miltefosine appears to show some promise in the treatment of free-living amoebic infection, although it should be noted that regimens including this investigational drug have only led to cure in three out of six *Acanthamoeba* SOT case reports where it was used. Furthermore, miltefosine had limited effect in individuals with granulomatous amebic encephalitis, where infection was uniformly fatal. This ubiquitous protozoan should be considered as a cause of disseminated infection in immunosuppressed individuals who do

not respond initially to antibiotics. Although reported outcomes are poor, and the optimal anti-amoebic regimen is not well defined, prompt diagnosis of *Acanthamoeba* infection, reduction of immunosuppression, and initiation of multi-drug therapy may provide the best chance for successful treatment.

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Abbreviations

CDC	Centers for Disease Control and Prevention
IND	investigational new drug
CNS	central nervous system
CRP	C-reactive protein
SOT	solid organ transplant
MRI	magnetic resonance imaging
ANC	absolute neutrophil count

References

1. Centers for Disease C, Prevention. Balamuthia mandrillaris transmitted through organ transplantation --- Mississippi, 2009. MMWR. Morbidity and mortality weekly report. Sep 17; 2010 59(36):1165–1170. [PubMed: 20847719]
2. Centers for Disease C, Prevention. Notes from the field: transplant-transmitted Balamuthia mandrillaris --- Arizona, 2010. MMWR. Morbidity and mortality weekly report. Sep 17.2010 59(36):1182. [PubMed: 20847722]
3. Satlin MJ, Graham JK, Visvesvara GS, et al. Fulminant and fatal encephalitis caused by *Acanthamoeba* in a kidney transplant recipient: case report and literature review. Transpl Infect Dis. 2013; 15(6):619–26. [PubMed: 24010955]
4. Trabelsi H, Dendana F, Sellami A, Sellami H, Cheikhrouhou F, Neji S, et al. Pathogenic free-living amoebae: epidemiology and clinical review. Pathol Biol. 2012; 60:399–405. [PubMed: 22520593]
5. Murakawa G, McCalmont T, Altman J, Telang G, Hoffman M, Kantor G, et al. Disseminated acanthamoebiasis in patients with AIDS. Arch Dermatol. 1995; 131:1291–6. [PubMed: 7503573]
6. Khan N. *Acanthamoeba* and the blood-brain barrier: the breakthrough. J Med Microbiol. 2008; 57:1051–7. [PubMed: 18719172]
7. Steinberg J, Galindo R, Kraus E, Ghanem K. Disseminated acanthamoebiasis in a renal transplant recipient with osteomyelitis and cutaneous lesions: case report and literature review. Clin Infect Dis. 2002; 35:e43–9. [PubMed: 12173148]
8. Barete S, Combes A, de Jonckheere J, Datry A, Varnous S, Martinez V, et al. Fatal disseminated *Acanthamoeba lenticulata* infection in a heart transplant patient. Emerg Infect Dis. 2007; 13(5):736–8. [PubMed: 17553253]
9. Yoder J, Straif-Bourgeois S, Roy S, et al. Primary Amebic Meningoencephalitis Deaths Associated With Sinus Irrigation Using Contaminated Tap Water. Clin Infect Dis. 2012; doi: 10.1093/cid/cis626

10. Schuster F, Visvesvara G. Opportunistic amoebae: challenges in prophylaxis and treatment. Drug Resist Updat. 2004; 7:41–51. [PubMed: 15072770]
11. Walochnik J, Duchene M, Seifert K, Obwaller A, Hottkowitz T, Wiedermann G, et al. Cytotoxic activities of alkylphosphocholines against clinical isolates of *Acanthamoeba* spp. Antimicrob Agents Chemother. 2002; 46:695–701. [PubMed: 11850250]
12. Schuster FL, Guglielmo BJ, Visvesvara GS. In-vitro activity of miltefosine and voriconazole on clinical isolates of free-living amebas: *Balamuthia mandrillaris Acanthamoeba* spp. and *Naegleria fowleri*. J Eukaryot Micribiol. 2006; 53:121–6.
13. Slater C, Sickel J, Visvesvara G, Pabico R, Gaspari A. Successful treatment of disseminated *Acanthamoeba* infection in an immunocompromised patient. N Engl J Med. 1994; 331:85–7. [PubMed: 8208270]
14. Oliva S, Jantz M, Tiernan R, Cook DL, Judson MA. Successful treatment of widely disseminated acanthamoebiasis. South Med J. 1999; 92(1):55–7. [PubMed: 9932828]
15. Vernon SE, Acar BC, Pham SM, Fertel D. *Acanthamoeba* infection in lung transplantation: report of a case and review of the literature. Transpl Infect Dis. 2005; 7:154–7. [PubMed: 16390406]
16. Walia R, Montoya J, Visvesvara G, Booton G, Doyle R. A case of successful treatment of *Acanthamoeba* infection in a lung transplant recipient. Tranpl Infect Dis. 2007; 9:51–4.
17. Aichelburg A, Walochnik J, Assadian O, Prosch H, Steuer A, Perneczky G, et al. Successful treatment of disseminated *Acanthamoeba* sp. infection with miltefosine. Emerg Infect Dis. 2008; 14:1743–6. [PubMed: 18976559]
18. Young AL, Leboeuf NR, Tsiouris SJ, Husain S, Grossman ME. Fatal disseminated *Acanthamoeba* infection in a liver transplant recipient immunocompromised by combination therapies for graft-versus-host disease. Transpl Infect Dis. 2010; 12(6):529–37. [PubMed: 20604904]
19. Zamora A, Henderson H, Swiatlo E. *Acanthamoeba* encephalitis: A case report and review of therapy. Surg Neurol Int. 2014; 5:68. [PubMed: 24991471]
20. Salameh A, Bello N, Becker J, Zangeneh T. Fatal granulomatous amoebic encephalitis caused by *Acanthamoeba* in a patient with kidney transplant: A case report. Open Forum Infect Dis. 2015; 2(3):1–4.
21. Kandakuri S, Morgan M, Zakowski P, et al. Transplant patient with skin nodules. Am J Dermatopathol. 2015; 37:229–231. [PubMed: 25699978]
22. Centers for Disease Control and Prevention. Investigational Drug Available Directly from CDC for the Treatment of Infections with Free-Living Amebae. MMWR. 2013; 62:666. [PubMed: 23965830]
23. Martinez J. Acanthamoebiasis and Immunosuppression. J Neuropathol Exp Neurol. 1982; 41(5): 548–57. [PubMed: 7108568]
24. Van Hamme C, Dumont M, Delos M, Lachapelle JM. Cutaneous acanthamoebiasis in a lung transplant patient. Ann Dermatol Venereol. 2001; 128(11):1237–40. [PubMed: 11908170]
25. Duarte AG, Sattar F, Granwehr B, Aronson JF, Wang Z, Lick S. Disseminated acanthamoebiasis after lung transplantation. J Heart Lung Transplant. 2006; 25(2):237–40. [PubMed: 16446227]
26. Readinger A, Blumberg M, Colome-Grimmer MI, Kelly E. Disseminated *Acanthamoeba* infection with sporotrichoid nodules. Int J Dermatol. 2006; 45(8):942–3. [PubMed: 16911379]
27. McKellar MS, Mehta LR, Greenlee JE, et al. Fatal granulomatous *Acanthamoeba* encephalitis mimicking a stroke, diagnosed by correlation of results of sequential magnetic resonance imaging, biopsy, in vitro culture, immunofluorescence analysis, and molecular analysis. J Clin Microbiol. 2006; 44(11):4265–9. [PubMed: 16988022]
28. Mendez O, Kanal E, Abu-Elmagd KM, et al. Granulomatous amebic encephalitis in a multivisceral transplant recipient. Eur J Neurol. 2006; 13(3):292–5. [PubMed: 16618348]
29. Gene AH, Gardner PA, Couce Matovelle ME. Rapidly expanding brain mass. Transpl Infect Dis. 2007; 9:211–213. [PubMed: 17605736]
30. Mutreja D, Jalpota Y, Madan R, Tewari V. Disseminated *Acanthamoeba* infection in a renal transplant recipient: a case report. Indian J Pathol Microbiol. 2007; 50(2):346–8. [PubMed: 17883068]
31. Fung KT, Dhillon AP, McLaughlin JE. Cure of *Acanthamoeba* cerebral abscess in a liver transplant patient. Liver Transpl. 2008; 14(3):308–12. [PubMed: 18306348]

32. Afshar K, Boydking A, Ganesh S, Herrington C, McFadden PM. Rapidly fatal disseminated acanthamoebiasis in a single lung transplant recipient. Ann Transplant. 2013; 18:108–11. [PubMed: 23792510]

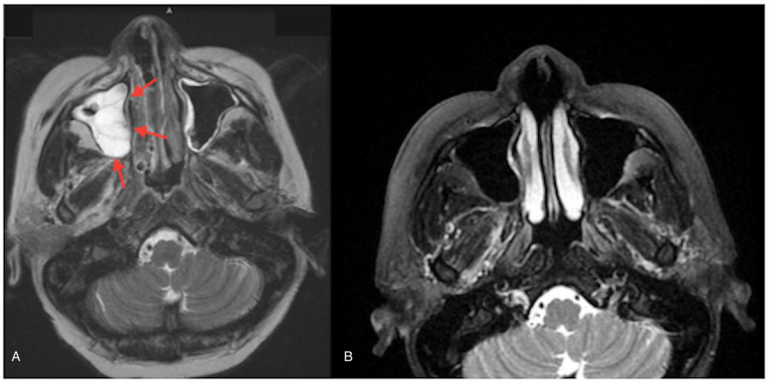

Fig. 1.

A) MRI of sinuses before debridement showed opacification consistent with observed mass (red arrows). B) MRI of sinuses 6 months after discharge demonstrated clearance of previous opacifications.

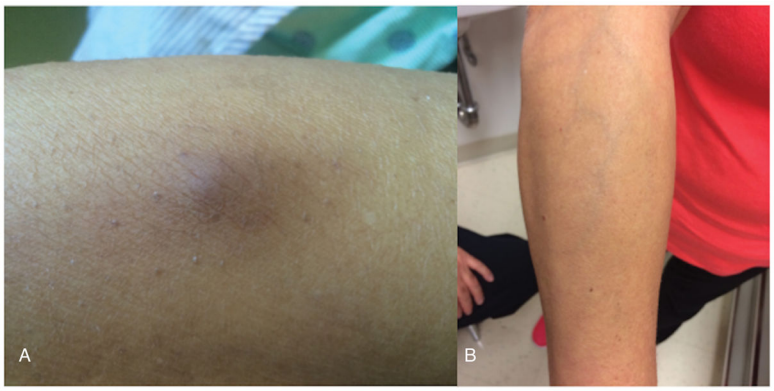

Fig. 2.

A) Cutaneous lesion of the right forearm photographed 8 days after initial eruption. B) Photograph of the right forearm at 8-month follow up visit demonstrated resolution.

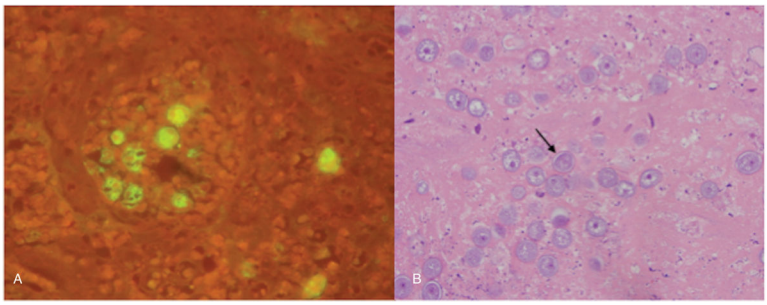

Fig. 3.

A) Immunohistochemical stain of skin biopsy showed *Acanthamoeba* cysts by immunofluorescence (performed at the CDC). B) Biopsy sample from repeat sinus debridement showed cysts consistent with *Acanthamoeba* spp. 12 days after the patient's initial surgery.

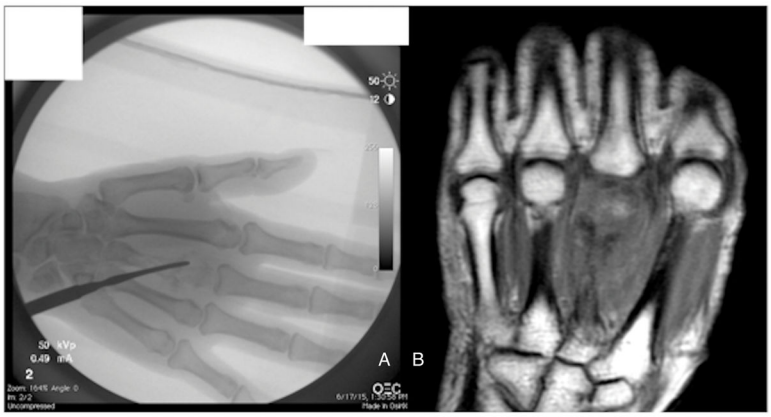

Fig. 4.

a) Radiograph of the right hand revealed a lytic lesion of the third metacarpal. b) MRI of the hand following debridement showed hollow cavity due to bone destruction.

Table 1

Reported cases and outcomes of *Acanthamoeba* infection in solid organ transplant (SOT) patients

Year	Patient Age/Gender	Location	Organ	Time to infection (months)	Immunosuppresive regimen	Type of Infection	Treatment Regimen	Diagnosis confirmation	Outcome	Reference
1982	38/M	Pennsylvania	Kidney	30	Azathioprine, methylprednisolone	Skin, lung, brain	Broad-spectrum antibiotics	Histopathologic staining – biopsy specimen	Died	23
1994	31/M	Texas	Kidney	10	Azathioprine, cyclosporine, prednisone	Skin	Pentamidine, topical chlorhexidine/ketoconazole	IF staining of biopsy tissue section	Cured	13
1999	39/F	South Carolina	Lung	72	Azathioprine, prednisone, tacrolimus	Skin	5-fluorocytosine, itraconazole, pentamidine, topical chlorhexidine/ketoconazole	Histopathologic staining of abscess fluid *	Cured	14
2001	38/M	France	Bilateral lung	36	Methylprednisolone, tacrolimus	Skin	Itraconazole pentamidine, topical chlorhexidine/ ketoconazole	IF staining of tissue at autopsy	Died	24
2002	61/F	Maryland	Kidney	12	Mycophenolate mofetil, prednisone, tacrolimus	Skin, bone	Amikacin, amphotericin B, azithromycin, imipenem	Culture of tissue at autopsy	Died (postmortem dx)	7
2005	49/F	Florida	Bilateral lung	7	Mycophenolate mofetil, prednisone, tacrolimus	Sinus	Amphotericin B, caspofungin, voriconazole	IF staining of biopsy tissue section	Cured	15
2006	60/M	Texas	Bilateral lung	9	Mycophenolate mofetil, prednisone, Tacrolimus	Skin, lung, brain	Amphotericin B, ciprofloxacin, imipenem, itraconazole, vancomycin	Histopathologic staining at autopsy *	Died (postmortem dx)	25
2006	60/M	Texas	Lung	Not reported	Azathioprine, prednisone, tacrolimus	Skin, lung, brain	Broad-spectrum antibiotics	IF staining and PCR analysis of brain biopsy specimen	Died	26
2006	51/M	Utah	Kidney	3	Mycophenolate mofetil, prednisolone, tacrolimus	Skin, brain	Amphotericin B, azithromycin, flucytosine, metronidazole, pentamidine, rifampin, sulfadiazine	IF staining of tissue at autopsy	Died (post-mortem dx)	27
2006	40/M	Pennsylvania	Multiple organs	9	Thymoglobulin, tacrolimus	Brain	Not reported	IF staining of tissue at autopsy	Died (postmortem dx)	28
2007	40/M	Spain	Multiple organs	9	Tacrolimus	Brain	Not reported	Histopathologic staining at autopsy	Died (postmortem dx)	29
2007	52/F	Florida	Lung	36	Mycophenolate mofetil, prednisone, tacrolimus	Skin	Amphotericin B, voriconazole	IF staining of biopsy tissue section	Cured	16
2007	39/M	France	Heart	22	Cyclosporine, mycophenolate mofetil, prednisone	Skin, lungs, kidneys	5-fluorocytosine, itraconazole, pentamidine	IF staining of biopsy tissue section, confirmed by culture and PCR	Died	8
2007	36/F	India	Kidney	48	Not reported	Brain, lungs, pancreas	Broad-spectrum antibiotics	IF staining of biopsy tissue section	Died (postmortem dx)	30
2008	41/M	United Kingdom	Liver	14	Azathioprine, cyclosporine, prednisone	Brain	co-trimoxazole, rifampicin, surgical resection	IF staining of biopsy tissue section	Cured	31

Year	Patient Age/Gender	Location	Organ	Time to infection (months)	Immunosuppresive regimen	Type of Infection	Treatment Regimen	Diagnosis confirmation	Outcome	Reference
2010	63/M	New York	Liver	12	Alemtuzumab, Cyclophosphamide, Daclizumab, Doxorubicin, Etoposide, mycophenolate mofetil, Prednisone, Rituximab, Tacrolimus, Vincristine	Skin, lung, brain	Amphotericin B, caspofungin, flucytosine, **miltefosine**, pentamidine, voriconazole, topical ketoconazole	IF staining of biopsy tissue section	Died	18
2013	62/M	California	Bilateral lung	6	Mycophenolate mofetil, prednisolone, tacrolimus	Skin, brain	Amikacin, flucytosine, pentamidine, intrathecal amphotericin B	Light microscopy and PCR of CSF fluid	Died	32
2013	58/M	New York	Kidney	24	Methylprednisolone, mycophenolate mofetil, prednisone, rituximab, tacrolimus, thymoglobulin	Brain	Broad-spectrum antibiotics, ganciclovir, pyrimethamine, sulfadiazine, voriconazole	IF staining of tissue at autopsy	Died (postmortem dx)	3
2014	63/M	Mississippi	Kidney	6	Mycophenolate mofetil, tacrolimus	Brain	Azithromycin, fluconazole, flucytosine, **miltefosine**, sulfadiazine	Histopathologic staining – brain biopsy specimen *	Died	19
2015	64/F	Arizona	Kidney	7	Mycophenolate mofetil, prednisone, tacrolimus	Brain	Azithromycin, fluconazole, flucytosine, **miltefosine**, pentamidine, sulfadiazine	IF staining of brain biopsy section	Died	20
2015	59/F	California	Lung	10	Not reported	Skin, sinus	Inpatient: 5-flucytosine, azithromycin, bactrim, intranasal pentamidine; Discharge: azithromycin, bactrim, **miltefosine,** voriconazole	IF staining of biopsy tissue section	Cured	22
2015	60/F	California	Heart	5	Mycophenolate mofetil, prednisone, tacrolimus, thymoglobulin	Skin, sinus, bone	Fluconazole, flucytosine, **miltefosine**	IF staining of biopsy tissue section	Cured	24

IF: immunofluorescence; PCR –polymerase chain reaction; CSF –cerebrospinal fluid

* Histochemical staining not specified