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Risk for Transmission of *Naegleria fowleri* From Solid Organ Transplantation

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Primary amebic meningoencephalitis (PAM) caused by the free-living ameba (FLA) *Naegleria fowleri* is a rare but rapidly fatal disease of the central nervous system (CNS) affecting predominantly young, previously healthy persons. No effective chemotherapeutic prophylaxis or treatment has been identified. Recently, three transplant-associated clusters of encephalitis caused by another FLA, *Balamuthia mandrillaris*, have occurred, prompting questions regarding the suitability of extra-CNS solid organ transplantation from donors with PAM. During 1995–2012, 21 transplant recipients of solid organs donated by five patients with fatal cases of PAM were reported in the United States. None of the recipients developed PAM, and several recipients tested negative for *N. fowleri* by serology. However, historical PAM case reports and animal experiments with *N. fowleri*, combined with new postmortem findings from four patients with PAM, suggest that extra-CNS dissemination of *N. fowleri* can occur and might pose a risk for disease transmission via transplantation. The risks of transplantation with an organ possibly harboring *N. fowleri* should be carefully weighed for each individual recipient against the potentially greater risk of delaying transplantation while waiting for another suitable organ. In this article, we present a case series and review existing data to inform such risk assessments.

Keywords: Ameba, amoeba, disseminated, *Naegleria*, primary amebic meningoencephalitis, transplant

Abbreviations: CDC, United States Centers for Disease Control and Prevention; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; FLA, free-living ameba; H&E, hematoxylin and eosin stain; ICP, intracranial pressure; IHC, immune alkaline phosphatase staining, a form of immunohistochemical testing; IIF, indirect immunofluorescence staining; IV, intravenous; MRI, magnetic resonance imaging; PAM, primary amebic meningoencephalitis; PCR, polymerase chain reaction test; PMN, polymorphonuclear leukocyte; RBC, red blood cell; WBC, white blood cell

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Introduction

Naegleria fowleri is a free-living ameba (FLA) inhabiting warm freshwater sites. It has also been isolated from soil (1). *N. fowleri* enters the body when contaminated water moves up the nose, such as during swimming or nasal irrigation (2). Amebae then cross the cribriform plate and reach the brain through the olfactory tract to cause primary amebic meningoencephalitis (PAM) (1) (Table 1). PAM progresses rapidly and has a mortality of approximately 99%, based on documented US cases. During

Table 1: Epidemiologic and clinical summary of central nervous system disease (CNS) caused by the free-living amebae *Naegleria fowleri* and *Balamuthia mandrillaris* in the United States[1,2]

	Naegleria fowleri	*Balamuthia mandrillaris*
Disease	Primary amebic meningoencephalitis (PAM)	Granulomatous amebic encephalitis (GAE)
Number of reported cases (years)	128 (1962–2012)	85 (1974–2012)
Age	All ages but predominantly children (median 11 years, range 8 months–66 years)	All ages (median 35 years, range 8 months–89 years)
Sex	Males >75% of reported cases	Males >65% of reported cases
Ethnicity	No pattern discerned	More common among Hispanics
Immune status	Immunocompetent	Sometimes immunosuppressed
Exposure	Warm fresh water (recreational, potable)	Soil and dust, possibly fresh water
Geographic distribution	Historically southern-tier states but changing distribution to include northern states (Minnesota)	Across the United States
Seasonality	Summer with peak in July and August (range April–October)	Not apparent
Route of entry	Entry through the nose during recreational water activities or nasal irrigation with direct spread to brain through cribriform plate and olfactory tract	Entry through soil contamination of skin wounds or cuts or through inhalation of dust with hematogenous spread to the brain
Incubation period	Median 5 days (range 1–7 days)	Unknown—believed to be weeks or months; may be more rapid in transplant-transmission cases
Signs and symptoms	Acute onset of headache, fever, nausea, vomiting, stiff neck, seizures, altered mental status, hallucinations, coma	Subacute or chronic onset of headache, stiff neck, neck pain, photophobia, nausea, vomiting, altered mental status, behavioral changes, seizures, ataxia, focal neurologic deficits, impaired speech, weight loss; CNS symptoms may be preceded by one or more chronic skin lesions on face, trunk or limbs appearing as nodules, ulcers or abscesses
Differential diagnosis	Bacterial or viral meningitis	Brain tumor, lymphoma, stroke, vasculitis, abscess, tuberculosis or fungal meningoencephalitis, neurocysticercosis, toxoplasmosis, acute disseminated encephalomyelitis
Cerebrospinal fluid (CSF) parameters	Elevated opening pressure, polymophonuclear pleocytosis, normal or low glucose, elevated protein; blood and/or motile amebae are suggestive of PAM	Normal or mildly elevated opening pressure, moderate lymphocytic pleocytosis, normal or low glucose, normal or elevated protein; generally none to a few red blood cells and amebae are rarely observed
Brain imaging results	CT and MRI often normal early in disease, then may show cerebral edema with basilar meningeal enhancement and obliteration of basilar cisterns; MRI might show one or more small enhancing round lesions in some cases[3,4,5]	CT may show single or multiple hypodense, ring-enhancing, space-occupying lesions with occasional hemorrhage within lesions; MRI similar to CT but might be abnormal when CT is unremarkable[3,4]
Laboratory confirmation[6]	Amebae may be detected in CSF or tissue specimens: • Direct visualization of amebae with Giemsa-Wright or modified trichrome stains • Polymerase chain reaction (PCR) testing for nucleic acid • Immunohistochemistry to detect antigens Serology for *N. fowleri* is currently considered a research technique and has not been evaluated for use as a routine diagnostic procedure	Amebae may be detected in tissue specimens: • Direct visualization of amebae with hematoxylin and eosin or periodic acid-Schiff stains • PCR testing for nucleic acid • Immunohistochemistry to detect antigens Serology for *B. mandrillaris* is currently considered a research technique and has not been evaluated for use as a routine diagnostic procedure

Table 1: Continued

	Naegleria fowleri	*Balamuthia mandrillaris*
Prognosis	Mortality >99%	Mortality >90%
Duration of illness from onset to death	Median 5 days (range 1–12 days)	Median 28 days (range 4–450 days)
Treatment[7]	No effective treatment has been established; multiple medications used in combination	No effective treatment has been established; multiple medications used in combination
Organ donors and outcomes	During 1995–2012, five known patients with PAM in the United States donated solid organs to 21 transplant recipients, none of whom are known to have developed PAM; further, five recipients had negative *N. fowleri* serologies and one biopsy from a donated heart tested negative for *N. fowleri*	During 2009–2012, three known patients with GAE due to *Balamuthia* in the United States donated solid organs to 13 transplant recipients: four developed GAE and died, one developed GAE and survived with treatment, and six of the remaining eight recipients who did not develop *Balamuthia* GAE developed positive *Balamuthia* serologies[8]

CT, computed tomography; MRI, magnetic resonance imaging.
[1]Data summarized from information available on the Centers for Disease Control and Prevention (CDC) website at http://www.cdc.gov/parasites/naegleria/index.html and http://www.cdc.gov/parasites/balamuthia/. Case counts and other epidemiologic data include unpublished data from the CDC.
[2]Roy S, Cope J, Yoder J, Visvesvara G. Free living ameba—What every clinician needs to know. Decision support in medicine [Internet]. 2013 June [cited 2013 July 10]: [about 9 pp.]. Available at https://www.decisionsupportinmedicine.com/DSM/a/articles/5/A/5523.
[3]Kidney DD, Kim SH. CNS infections with free-living amebas: Neuroimaging findings. Am J Roentgenol 1998; 171: 809–812.
[4]Singh P, Kochhar R, Vashishta RK, et al. Amebic meningoencephalitis: Spectrum of imaging findings. Am J Neuroradiol 2006; 27: 1217–1221.
[5]Rai R, Singh DK, Srivastava AK, Bhargava A. Primary amebic meningoencephalitis. Indian Pediatr 2008; 45: 1004–1005.
[6]Diagnostic testing is not widely available for PAM. Clinicians who suspect PAM should contact their state health department and/or the CDC (24/7 Emergency Operation Center—770-488-1700). CDC can assist with diagnosis and provide treatment recommendations. Telediagnosis can be arranged at CDC by emailing photos through DPDx, CDC's Division of Parasitic Diseases and Malaria telediagnosis tool. Instructions for submitting photos through DPDx are available at http://www.dpd.cdc.gov/dpdx/HTML/Contactus.htm.
[7]Detailed discussions of possible chemotherapeutic drug combinations can be found at http://www.cdc.gov/parasites/naegleria/treatment-hcp.html and http://www.cdc.gov/parasites/balamuthia/treatment.html. For 24/7 treatment recommendations, please contact the CDC Emergency Operation Center at 770-488-1700.
[8]Gupte AA, Hocevar SN, Lea AS, et al. Transmission of *Balamuthia mandrillaris* through solid organ transplantation: Utility of organ recipient serology to guide clinical management (submitted to the *American Journal of Transplantation*).

1962–2012, 128 PAM cases were reported in the United States ([3], CDC unpublished data); only one survived (4). Along with a second case from Mexico (5), these are the only well-documented survivors in North America through 2012.

PAM generally affects young, previously healthy individuals (3) who may be viewed as candidates for deceased-donor organ donation. Successful organ donations from three patients with PAM have been reported (6–8). However, while no cases of *N. fowleri* transplant transmission have been documented thus far, the true risk is unknown. Transplant transmission of another FLA, *Balamuthia mandrillaris*, has been reported on three occasions (9–11), prompting questions about the potential risk for transplant transmission of *N. fowleri* and about the similarities and differences between these two amebae in terms of epidemiologic and clinical characteristics and diagnostic methods that might assist in recognizing and differentiating these cases to assist with management of recipients postdonation (Table 1). Consequently, the Centers for Disease Control and Prevention (CDC) has collaborated with attending physicians and pathologists to investigate possible *Naegleria* dissemination to organs outside the central nervous system (CNS). We summarize new pathology and laboratory data obtained from previously unreported patients with PAM and new serology data from transplant recipients associated with PAM organ donors. Additionally, we review animal studies and historical human data suggestive of possible extra-CNS amebic dissemination, and discuss the potential risk for *N. fowleri* transmission through solid organ transplantation.

Materials and Methods

PAM diagnosis requires specialized laboratory testing only available in a few laboratories including CDC, which is consulted for diagnostic, clinical and epidemiologic assistance by health professionals in the majority of reported PAM cases. The patients described herein represent all PAM cases during 2009–2012 for whom CDC provided laboratory testing and who either (1) donated organs or (2) underwent expanded autopsies with extra-CNS tissue testing. Clinical descriptions were compiled from reports from healthcare personnel; hospital, laboratory and autopsy record reviews; and, in one patient, published literature. Laboratory tests performed at CDC are also summarized. Various methodologies are available at CDC to identify FLA,

including immunohistochemical techniques, culture and a triplex real-time polymerase chain reaction (PCR) test. Immunohistochemical techniques, such as indirect immunofluorescence (IIF) (12) and immune alkaline phosphatase (IHC) (13), use antibody specific for *N. fowleri* followed by microscopic examination to identify *N. fowleri* in tissue, culture or cerebrospinal fluid (CSF). PCR testing can simultaneously identify and differentiate three meningoencephalitis-causing FLA (*N. fowleri*, *Acanthamoeba* spp. and *B. mandrillaris*) (14) and is used at CDC to diagnose and confirm PAM cases. An experimental immunofluorescence antibody assay can detect serum antibodies to *N. fowleri* using an antibody competition assay. A positive serologic response is considered to be a titer of 1:128 or greater and indicates exposure to *N. fowleri* (4).

Results

Case #1

On August 14, 2009, a 10-year-old male was hospitalized in Florida with a 1-day history of meningitis-like symptoms (Table 2). He was given ceftriaxone, rifampin and vancomycin for presumptive bacterial meningitis but later developed cough, shortness of breath, confusion, disorientation with hallucinations and at least one grand mal seizure. A computed tomography (CT) brain scan without contrast on August 16 showed diffuse edema. Multiple organ system failure occurred and he died on August 17. Both kidneys were recovered and transplanted into two recipients.

On August 19, a postmortem diagnosis of PAM was made when amebic trophozoites were observed in brain tissue at autopsy. CDC confirmed *N. fowleri* in the brain tissue by IIF; histopathology revealed deep parenchymal arterioles cuffed with trophozoites in the perivascular spaces. Autopsy specimens, including hematoxylin and eosin-stained (H&E) slides of pretransplant kidney biopsies, were sent to CDC; no amebae were found in the kidneys. However, trophozoites were observed in H&E-stained sections of spleen, thyroid, lung and heart; these tissues were also positive by IIF and IHC (Figure 1). Amebae observed in these organs were sparse; mostly in gaps, spaces, lumens or along the periphery of tissues; frequently degraded; and generally not associated with tissue reaction, either inflammation or necrosis. The spleen showed fibro-congestive changes including polymorphonuclear leukocyte (PMN) infiltrates with numerous amebic antigens and degraded amebae along with a few whole amebae in the parenchyma on IIF, although only a few amebic particles and one whole ameba were seen on the periphery of the spleen by IHC. Amebae were also observed on the edge and inside the parenchymal thyroid tissue by IIF and IHC. Some amebae in the lung sections were phagocytized by PMNs. Liver tissue tested negative for *N. fowleri* by IIF and IHC. All postmortem fresh tissue specimens obtained during autopsy were placed in cassettes and submerged in the same container of formalin before sectioning, potentially resulting in cross-contamination from the brain.

The kidney recipients were informed about these findings and followed closely for potential signs of organ rejection and PAM. One recipient had a kidney biopsy 1 year posttransplant per protocol, showing only mild fibrosis. The other recipient had diminished renal function with two biopsies to evaluate for possible rejection, at 9 months and 2.5 years posttransplant, both of which showed moderate fibrosis. Neither of the grafted kidneys showed evidence of *Naegleria* and, in August 2012, both recipients were well. Neither were tested for *N. fowleri* exposure or given anti-amebic chemotherapy.

Case #2

On September 18, 2009, a previously described 22-year-old male patient with PAM in Florida, diagnosed premortem, donated his kidneys, lungs, liver, heart, pancreas and bowel to seven recipients (8). The pancreas/bowel recipient was alive and well in September 2011, and five other recipients were alive and well in March 2012. The right lung recipient died in August 2010 from recurrent disease unrelated to his transplant without evidence of PAM. None of the recipients received anti-amebic chemotherapy for *N. fowleri* exposure.

Approximately 2 years posttransplant, the liver recipient tested negative for *N. fowleri* antibodies with titers of 1:8 and lower. At this time, CDC obtained a small piece of banked heart tissue biopsied from the heart recipient approximately 1 month posttransplant; it tested negative for amebae by culture and Giemsa staining. Since then, multiple protocol biopsies of the heart have shown no evidence of rejection or other pathology and the heart recipient was alive and well in June 2012.

Case #3

On August 9, 2011, a 16-year-old female in Florida presented to an emergency room with a 3-day history of headache, fever and vomiting. Electrolytes and blood counts were normal. She was treated with an intravenous (IV) fluid bolus and ceftriaxone, and discharged. On August 10, she was hospitalized with high fever, neck pain and stiffness, fatigue and altered mental status. PAM was presumptively diagnosed because of motile amebae observed in her CSF and later confirmed by PCR and culture. PAM treatment was initiated with IV amphotericin B, azithromycin, fluconazole and oral rifampin with dexamethasone for anticipated cerebral edema. Subsequently, she developed unequal pupils and progressive lethargy. On August 11, she was intubated and given hypertonic saline and mannitol. Intrathecal amphotericin B was administered through a lumbar spinal needle and a second CSF sample demonstrated the continued presence of motile amebae and progressive pleocytosis. On August 12, an external ventricular drain was placed and showed normal intracranial pressure (ICP). Intraventricular amphotericin B and IV chlorpromazine were administered. She deteriorated and died on August 13.

On August 14, her kidneys, lungs, liver and pancreas were recovered and transplanted into four recipients. All recipients were alive and well in March 2012. One recipient

Table 2: Case descriptions of referenced patients with primary amebic meningoencephalitis (PAM) due to *Naegleria fowleri*

Case number (year and state)	Sex (age in years)	Exposure history	Presenting symptoms and signs[1]	¶nitial cerebrospinal fluid (CSF) test results (illness day of specimen collection)	Diagnosis of PAM	Premortem *Naegleria fowleri*-specific treatment	Expanded autopsy	Organ donation (number of recipients)
1 (2009 Florida)	Male (10)	Swimming, jumping, inner tubing in a lake 6 days before symptom onset	Headache, vomiting, fever, neck stiffness, general malaise	WBC 7950 cells/mm^3 RBC 40 cells/mm^3 Protein 622 mg/dL Glucose 1 mg/dL Gram-negative diplococci Bacterial cultures negative (collected on day 2 of 5)	Postmortem diagnosis following transplant surgeries based on brain tissue IIF[2]	No	Yes	Kidneys (2)
2[3] (2009 Florida)	Male (22)	Wakeboarding at a water sports arena 3 days before symptom onset	Headache, fever, neck pain, photosensitivity, positive Brudzinski and Kernig signs	WBC 1680 cells/mm^3 RBC 263 cells/mm^3 Protein 450 mg/dL Glucose 42 mg/dL Gram stain negative Bacterial cultures negative Amebae observed (collected on day 2 of 5)	Premortem diagnosis based on motile amebae seen on CSF wet mount; diagnosis confirmed postmortem by CSF PCR[4] and culture	Yes[5]	No	Kidneys, liver, lungs, pancreas, heart, bowel (7)
3 (2011 Florida)	Female (16)	Swimming and diving in a river 2 days before symptom onset	Headache, vomiting, fever, neck pain and stiffness, fatigue, altered mental status, a Glasgow Coma Scale score of 15, and nonfocal findings on neurologic examination	WBC 4180 cells/mm^3 RBC 220 cells/mm^3 Protein 362 mg/dL Glucose 24 mg/dL Cloudy Amebae observed (collected on day 4 of 7)	Premortem diagnosis based on motile amebae seen on CSF wet mount; diagnosis confirmed postmortem by CSF PCR and culture	Yes[6]	No	Kidneys, liver, lungs, pancreas (4)
4 (2011 Kansas)	Male (14)	Swimming and splashing in a lake 4 and 5 days before symptom onset	Headache, nausea, decreased oral intake, fever, neck stiffness, photophobia, confusion	WBC 1000 cells/mm^3 RBC 200 cells/mm^3 Protein 151 mg/dL Glucose 46 mg/dL (collected on day 2 of 7)	Postmortem diagnosis based on CSF PCR testing and brain tissue IIF	No	Yes	No organ donation
5 (2012 South Carolina)	Male (8)	Diving, swimming and tubing in a lake, pond, ocean and pool on multiple occasions within the 7 days before symptom onset	Headache, vomiting, abdominal pain, fever, lethargy	WBC 10 113 cells/mm^3 RBC 27 cells/mm^3 Protein 390 mg/dL Glucose 8 mg/dL (collected on day 4 of 5)	Postmortem diagnosis based on CSF wet mount, culture and PCR testing	No	Yes	No organ donation
6 (2012 Minnesota)	Male (9)	Swam in multiple fresh water sites, including lake 1 day before symptom onset	Headache, vomiting, fever, lethargy, confusion, blurred vision, diplopia	WBC 1463 cells/mm^3 RBC 228 cells/mm^3 Protein 411 mg/dL Glucose 68 mg/dL Bacterial cultures positive for coagulase-negative *Staphylococcus* species Amebae observed (collected on day 4 of 6)	Postmortem diagnosis based on CSF wet mount and PCR testing	No	Yes	No organ donation

RBC, red blood cell; WBC, white blood cell.

[1]All patients were previously healthy and had no significant past medical history.

[2]IIF = indirect immunofluorescence specific for *N. fowleri*.

[3]Tuppeny M. Primary amoebic meningoencephalitis with subsequent organ procurement: a case study. J Neurosci Nurs 2011; 43:274–279.

[4]PCR = triplex real-time polymerase chain reaction specific for *Naegleria fowleri*.

[5]Premortem treatment for *N. fowleri* included amphotericin B (intravenous and intraventricular), rifampin, azithromycin and fluconazole.

[6]Premortem treatment for *N. fowleri* included amphotericin B (intravenous and intraventricular), rifampin, azithromycin, fluconazole and chlorpromazine. Dexamethasone was also administered for anticipated cerebral edema.

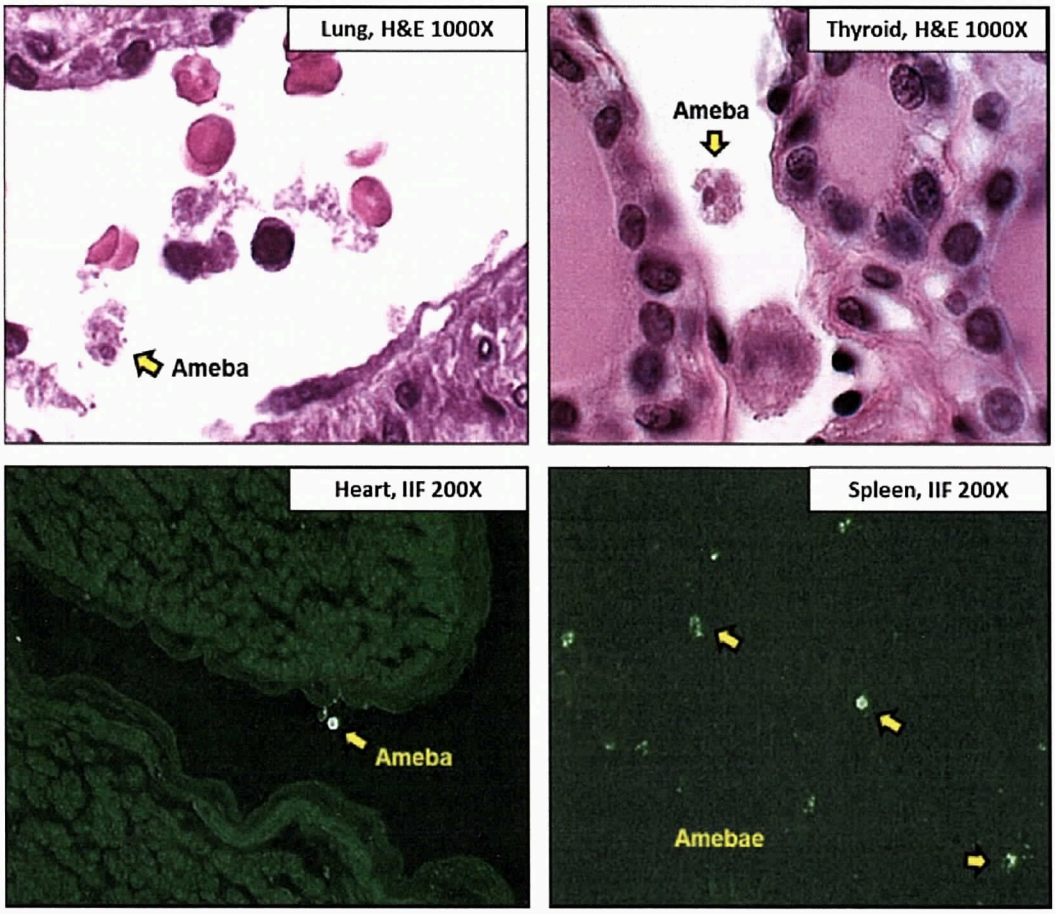

Figure 1: Postmortem examination of lung, thyroid, heart and spleen tissue from a 10-year-old male organ donor in Florida (Case #1) who died from primary amebic meningoencephalitis due to *Naegleria fowleri* in 2009. *N. fowleri* trophozoites in lung (upper left) and thyroid (upper right) sections by hematoxylin and eosin (H&E) staining at 1000× magnification. *N. fowleri* trophozoites fluorescing in heart (lower left) and spleen (lower right) sections by indirect immunofluorescence (IIF) at 200× magnification.

with a bilateral lung transplant received treatment for possible *Naegleria* exposure with 2 days of IV Ambisome (August 14–16, 2011), which was stopped because of renal dysfunction; no further anti-amebic treatment was given. Approximately 7 weeks posttransplant, the four recipients provided serum for serologic testing of *N. fowleri* antibodies at CDC. All four tested negative with titers of 1:8 or lower.

Case #4

On August 19, 2011, a 14-year-old male in Kansas presented to hospital 1 day after illness onset. He was presumptively treated for meningitis with ceftriaxone, vancomycin, acyclovir and mannitol. A magnetic resonance imaging (MRI) of the brain on August 20 was unremarkable. His symptoms progressed and he developed increased confusion, agitation, tonic–clonic activity and had a seizure. He subsequently desaturated and was intubated. Tonsilar herniation of the brain occurred and he was pronounced dead on August 24. He was not an organ donor.

Postmortem CSF tested positive for *N. fowleri* by PCR at CDC. CDC also received slides of brain, spinal cord, kidney, lung, liver and heart from an expanded autopsy. Only CNS tissues tested positive for *N. fowleri* by IHC whereas CNS, kidney and lung tissues tested positive by IIF, with many trophozoites and much antigenic debris observed in the kidney, and a few trophozoites with a little debris observed in both lungs. Six slides of kidney, lung and liver tissues taken from patients with diseases other than PAM were also sent to CDC as controls—all tested negative by IIF. As with Case #1, cross-tissue contamination possibly occurred because all organs were sectioned using the same tools (rinsed between organs) and cassettes containing all organ specimens were placed in the same formalin-filled container prior to processing. However, in Case #4, the liver and heart, which were negative for *N. fowleri* by IHC and IIF, were *also* in this same container and placed in the same cassettes as extra-CNS tissues that tested positive (left lung and right lung, respectively).

Case #5

On July 15, 2012, an 8-year-old male from South Carolina was admitted to hospital 2 days after illness onset with presumptive meningitis and started on ceftriaxone and vancomycin. A head CT scan on July 16 showed diffuse subarachnoid hemorrhage within the basilar cisterns and layering along the tentorium with accompanying left parietal soft tissue swelling. The patient's condition deteriorated requiring placement of an ICP monitor. He became comatose with fixed, dilated pupils and was pronounced dead on July 17. He was not an organ donor.

A postmortem diagnosis of PAM was made based on testing at CDC, including wet-mount observation of amebae in the CSF and positive CSF PCR and cultures. The patient underwent an expanded autopsy and tested positive for *N. fowleri* in extra-CNS tissues by PCR. As with Cases #1 and 4, histopathology results were difficult to interpret because autopsy tissues were potentially cross-contaminated when CNS and extra-CNS tissue specimens were preserved in the same formalin container. However, in this case, organs of the chest and abdomen were completely dissected and fresh unpreserved tissue specimens from these organs were collected and stored before the cranium was opened. These unpreserved tissues, collected before cross-contamination could occur, were sent to CDC for PCR. The brain, right lung, spleen and intestine tested positive for *N. fowleri* by PCR while the kidney, heart, pancreas and liver tested negative. Whether these positive PCR findings represented viable intact organisms in extra-CNS tissues or leakage of *Naegleria* debris across a compromised blood–brain barrier is unknown, but neither scenario could be ruled out. Further, it is unknown whether such leakage across the blood–brain barrier occurred premortem or postmortem.

Case #6

On August 4, 2012, a 9-year-old male from Minnesota was admitted to hospital 2 days after illness onset with apparent meningitis and started on ceftriaxone, vancomycin, acyclovir, dexamethasone and mannitol. An unenhanced head CT on August 5 was unremarkable. The patient had progressive neurologic decline requiring intubation and ICP monitor placement. A presumptive premortem diagnosis of PAM was made based on air-dried Wright-stained CSF cytospin preparations. The patient was pronounced brain dead on August 6. He was not an organ donor.

An expanded autopsy was performed on August 7. The extra-CNS organs were removed and specimens were prepared for PCR testing at CDC before the cranium was opened, thereby ruling out possible cross-contamination from the CNS. The extra-CNS organs were grossly and microscopically normal. Intraparenchymal tissues were sampled by avoiding organ surfaces, such as the pleura and liver capsule, and by using a visibly clean scalpel for tissue sectioning. The organ surfaces were not cauterized before sectioning and sterile scalpels and forceps were not used for handling tissue samples. CSF and olfactory and auditory nerve tissue tested positive for *N. fowleri* at CDC by PCR and culture, although serum antibody tests were negative at 1:2. Tissue specimens from the liver, heart, kidney and pancreas tested negative for *N. fowleri* by PCR and culture. While lung tissue was also culture negative, it was reproducibly PCR positive. As with Case #5, it is unknown whether this PCR finding represented viable intact organisms or amebic debris in the lung. Further, it is unknown whether such leakage across the blood–brain barrier occurred premortem or postmortem.

Discussion

During 1995–2012, five known patients with PAM in the United States donated solid organs to 21 transplant recipients; three of the cases are described above (Cases #1, 2, 3) and two more have been described previously (6,7). None of these recipients are known to have developed PAM. Further, five recipients had negative *N. fowleri* serologies 7 weeks to 2 years posttransplant and one biopsy taken 1 month posttransplant from a donated heart tested negative for *N. fowleri*. However, there are data from human PAM cases and animal studies that challenge the assumption that *N. fowleri* is confined to the CNS. Since 2009, CDC has examined extra-CNS tissues from five patients with PAM (Cases #1, 2, 4–6) and found evidence of varying strength to suggest that extra-CNS dissemination of *Naegleria* might have occurred in four (Cases #1, 4–6) of the five cases.

In addition to these four cases, two patients with PAM with possible extra-CNS dissemination of *Naegleria* have been previously reported. In 1943, an emaciated 22-year-old Japanese prisoner of war was treated premortem for malaria and dysentery. On autopsy, amebae were observed by histology in his brain, lungs, stomach, small intestine, cecum, and mesenteric and gastric lymph nodes (1,15–17). In 1969, a 15-year-old female was diagnosed with PAM premortem based motile amebae observed in her CSF (18,19). She received anti-amebic chemotherapy but developed acute diffuse myocarditis and pulmonary edema and died 5 days after symptom onset. On autopsy, tissues from multiple organs were obtained under aseptic conditions and cultured. Spleen, liver and lung cultures were positive for *N. fowleri*, and an ameba was observed in the heart blood.

Extra-CNS dissemination of *Naegleria* has also been observed in multiple experiments involving mice (20–24). For example, histological examinations of mice given intranasal *Naegleria* inoculations demonstrated amebae invading the nasal mucosa by 36 h postinoculation, the olfactory nerve and anterior olfactory lobes by 72 h, the cerebral hemispheres by 96 h, and the lungs, liver, spleen and renal capillaries by 108 h (4.5 days) with death by 132 h in most mice (20). In a similar experiment, occasional amebae were histologically observed in blood vessels in

and around the olfactory bulbs and within the bone marrow and venous sinusoids of mice skulls at 96 h after intranasal inoculation (21). At the same time, amebae were also cultured from blood and lung tissue. The researchers noted that *Naegleria* entered the blood stream of the CNS and bone marrow very late in the disease when tissue destruction was advanced, thin-walled veins were compromised and mice were moribund.

Taken together, human and animal data suggest that hematogenous spread of *N. fowleri* to extra-CNS organs might be possible. The infectious dose of *N. fowleri* has not been established in humans but may be low; in one mouse experiment, the LD50 was 300 amebae but the lowest intranasal lethal dose was only 39 amebae (20). Although the infectious dose via solid organ transplantation is unknown, these data suggest that a relatively few viable amebae may need to escape the CNS and contaminate an organ for transplant transmission to be possible. Experimental data from mouse models indicated that *Naegleria* escape the CNS late in the course of disease when tissue destruction is greatest and the blood–brain barrier is compromised. Therefore, although no cases of donor-derived organ transplant transmission of *N. fowleri* have been reported so far, the risk of such an occurrence is likely not zero.

Since 2009, solid organ transplant transmission of *Balamuthia*, a related FLA, has been reported on three occasions (9–11). It is not fully understood why transplant transmission of *Balamuthia* has been documented but not that of *Naegleria* to date. One theory has been a difference in temperature sensitivities. Unlike *Balamuthia*, *Naegleria* are known to be thermophilic (1). However, although *N. fowleri* trophozoites degenerate within hours at temperatures <10°C, the trophozoites encyst under adverse conditions and cysts capable of reforming viable trophozoites under more favorable conditions can survive for days to months at temperatures used for organ storage and transportation (25). Therefore, temperature sensitivities may not explain the difference in transplant transmission incidence between the two organisms. Another theory is that amebic transplant transmission may relate to length of infection in the donor, as implied by the animal experiments previously described. Unlike *Naegleria*, *Balamuthia* causes a chronic infection and hence has the opportunity to hematogenously spread to other organs. Also unlike *Naegleria*, *Balamuthia* is known to spread hematogenously from extra-CNS sites to the CNS (26). Moreover, the recent clusters of transplant transmission confirm that hematogenous spread of *Balamuthia* occurs from the CNS to other organs as well. However, the data summarized in this report suggest that hematogenous spread from the CNS to other organs might also occur with *Naegleria*. Further exploration of the risk for hematogenous spread of *Naegleria* is required to better understand the risk and risk factors for such extra-CNS dissemination. Additionally, greater awareness of PAM among healthcare professionals involved in transplantation and inclusion of PAM in differential diagnoses during evaluations of potential donors with meningoencephalitis are needed. According to CDC surveillance data, only 25% of US PAM cases received a premortem PAM diagnosis. Therefore, most PAM donors will be undiagnosed at the time of transplant.

To further define the risk of *N. fowleri* extra-CNS dissemination, full autopsies should be performed on patients with PAM and any potential organ donor who dies with clinical signs or symptoms suggestive of meningitis or encephalitis. This practice would also help assess the risk of extra-CNS dissemination of other encephalitis-causing pathogens known to be transplant-transmitted, such as *B. mandrillaris*, rabies and lymphocytic choriomeningitis virus (9–11,24,27). Full autopsies should include microscopic examinations of both CNS and extra-CNS tissues, particularly heart, lungs, liver and kidneys. To minimize cross-contamination between CNS and extra-CNS tissues, examination and sampling of extra-CNS tissues and organs should be completed before removal of the brain. Ideally, separate workspaces and dissecting tools should be used to obtain CNS and extra-CNS tissues. Additionally, CNS and extra-CNS tissues should be placed in separate formalin containers and processed separately for paraffin embedding.

For guidance in recognizing CNS infections in potential deceased organ donors and for considerations during donor evaluation and organ offers, the Organ Procurement and Transplantation Network has provided information on their website at http://optn.transplant.hrsa.gov/ContentDocuments/Guidance_DTAC_CNS_Infections_07-2012.pdf (28). For assistance in diagnosing PAM, recommendations for *N. fowleri*-specific chemotherapy, and questions regarding transplant transmission of infectious diseases, CDC is available 24/7 by contacting the CDC Emergency Operations Center at 770-488-7100. Additionally, more information about the clinical features of PAM and its basic work-up is available on the CDC website at http://www.cdc.gov/parasites/naegleria/health_professionals.html.

At present, no test is feasible for screening potential organ donors, no test has been approved for diagnostic use to detect organ recipient exposure or infection, no prophylactic chemotherapeutic regimen has been established and no effective treatment regimen has been identified for amebic encephalitis (6). Although to date there is no evidence of *Naegleria* transplant transmission, the risk of transplantation with an organ possibly harboring *N. fowleri*, or other nontreatable agents of infectious encephalitis, should be carefully weighed for each individual recipient against the risk of delaying transplantation while waiting for another suitable organ.

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