

Primary Amebic Meningoencephalitis Deaths Associated With Sinus Irrigation Using Contaminated Tap Water

Jonathan S. Yoder,¹ Susanne Straif-Bourgeois,⁴ Sharon L. Roy,¹ Thomas A. Moore,⁵ Govinda S. Visvesvara,¹ Raoult C. Ratard,⁴ Vincent R. Hill,¹ Jon D. Wilson,⁶ Andrea J. Linscott,⁵ Ron Crager,⁷ Natalia A. Kozak,² Rama Sriram,¹ Jothikumar Narayanan,¹ Bonnie Mull,¹ Amy M. Kahler,¹ Chandra Schneeberger,¹ Alexandre J. da Silva,³ Mahendra Poudel,⁵ Katherine L. Baumgarten,⁵ Lihua Xiao,¹ and Michael J. Beach¹

¹National Center for Emerging and Zoonotic Infectious Diseases, ²National Center for Immunization and Respiratory Diseases, and ³Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; ⁴Louisiana Department of Health and Hospitals, and ⁵Ochsner Health System, New Orleans, ⁶Department of Pathology, Louisiana State University Health Science Center—Shreveport, and ⁷DeSoto Parish Coroner, Mansfield, Louisiana

Background. *Naegleria fowleri* is a climate-sensitive, thermophilic amoeba found in the environment, including warm, freshwater lakes and rivers. Primary amebic meningoencephalitis (PAM), which is almost universally fatal, occurs when *N. fowleri*-containing water enters the nose, typically during swimming, and *N. fowleri* migrates to the brain via the olfactory nerve. In 2011, 2 adults died in Louisiana hospitals of infectious meningoencephalitis after brief illnesses.

Methods. Clinical and environmental testing and case investigations were initiated to determine the cause of death and to identify the exposures.

Results. Both patients had diagnoses of PAM. Their only reported water exposures were tap water used for household activities, including regular sinus irrigation with neti pots. Water samples, tap swab samples, and neti pots were collected from both households and tested; *N. fowleri* were identified in water samples from both homes.

Conclusions. These are the first reported PAM cases in the United States associated with the presence of *N. fowleri* in household plumbing served by treated municipal water supplies and the first reports of PAM potentially associated with the use of a nasal irrigation device. These cases occurred in the context of an expanding geographic range for PAM beyond southern tier states with recent case reports from Minnesota, Kansas, and Virginia. These infections introduce an additional consideration for physicians recommending nasal irrigation and demonstrate the importance of using appropriate water (distilled, boiled, filtered) for nasal irrigation. Furthermore, the changing epidemiology of PAM highlights the importance of raising awareness about this disease among physicians treating persons showing meningitislike symptoms.

Many species of *Naegleria* are found in the environment [1] but only 1 species, *Naegleria fowleri*, causes a rare but serious disease called primary amebic meningoencephalitis (PAM) [2–4]. *N. fowleri* is a

thermophilic, free-living amoeba that is frequently detected in natural bodies of warm freshwater, where it feeds on bacteria. It tolerates temperatures of up to 45° C and thrives during warmer months of the year when the ambient temperature increases. This organism has been isolated from freshwater lakes, ponds, and rivers [5–7], hot springs [8], thermally polluted water [9–11], warm groundwater [12], inadequately treated swimming pools [13], sewage [14, 15] and soil [16, 17]. PAM typically occurs after exposure to warm, untreated water, frequently associated with swimming. The median age of case patients in the United States is 12

Received 20 April 2012; accepted 19 June 2012; electronically published 22 August 2012.

Correspondence: Jonathan S. Yoder, MSW, MPH, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS C-09, Atlanta, GA 30329 (jey9@cdc.gov).

Clinical Infectious Diseases 2012;55(9):e79–85

Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2012.

DOI: 10.1093/cid/cis626

years, with the majority of infections occurring in males [3]. *N. fowleri* enters the nose, and migrates along the olfactory nerve through the cribriform plate to the brain. Onset of symptoms occurs 1–7 days after exposure [18]. Signs and symptoms of infection are similar to those of bacterial or viral meningitis and include headache, fever, stiff neck, anorexia, vomiting, altered mental status, seizures, and coma. The median time from symptom onset to death typically is 5 days [18].

CASE REPORTS

Case 1

On 5 June 2011, a 28 year-old man from southern Louisiana abruptly developed a severe occipital headache radiating down his neck, neck stiffness, back pain, and intermittent vomiting. He had a history of migraines and thus did not seek medical attention at symptom onset. On 6 June he was brought to a New Orleans hospital emergency department with confusion. On initial examination, the patient was febrile (temperature, 38.5°C), disoriented, and combative. Further examination revealed meningismus but no gross motor deficits or cranial nerve abnormalities. No skin lesions were noted. Pending a lumbar puncture, glucocorticoids and empiric anti-infective therapy (intravenous ceftriaxone, linezolid, and acyclovir) were started. The patient was emergently intubated and sedated for airway protection.

On admission, peripheral blood examination showed an elevated white blood cell count. Rapid human immunodeficiency virus test results were negative. Noncontrast head computed tomographic findings were normal. Lumbar puncture revealed grossly turbid cerebrospinal fluid (CSF); opening pressure was not measured but appeared to be elevated because CSF projected 6 cm out of the needle on removal of the stylet. CSF results suggested acute bacterial meningitis, although the red blood cell count was elevated (343 cells/mL), and the white blood cell differential showed that 17% were “other” cells. The CSF Gram stain showed many neutrophils but no microorganisms. The negative Gram stain prompted a wet mount by laboratory personnel the next morning, 7 June which clearly identified motile ameboid trophozoites. Further review of the CSF cytospin or “cytocentrifuge” preparation from the night before revealed that the “other” cells were ameboid trophozoites. Images were sent to the Centers for Disease Control and Prevention (CDC) DPDx Web site (<http://www.dpd.cdc.gov/dpdx/Default.htm>) for telediagnosis that afternoon and the presumptive diagnosis of amebic meningoencephalitis was supported, pending testing by real-time polymerase chain reaction (PCR) [19] and culture.

Treatment with liposomal amphotericin B and rifampin began almost immediately after trophozoite identification. The

patient was urgently transferred to a neurologic critical care unit. Mannitol and dexamethasone were used to decrease intracranial pressure. Overnight, he remained intubated and hypotensive, requiring vasopressor medications to stabilize his blood pressure. Serial neurologic examinations demonstrated rapid decline in response to verbal commands followed by fixed, midrange pupils. On 8 June, computed tomography with contrast enhancement showed complete sulcal effacement and diminished ventricle size consistent with increased intracranial pressure. The patient’s neurologic condition remained unchanged, and he was declared brain dead. On 10 June, real-time PCR testing at the CDC confirmed the diagnosis of PAM due to *N. fowleri*; *N. fowleri* were also cultured from the CSF (Table 1). Amplification and bidirectional sequencing of the internal transcribed spacer and mitochondrial small subunit ribosomal RNA genes [20] identified genotype 1.

On 17 June 2011, the Louisiana Department of Health and Hospitals began an epidemiologic investigation of the first case. The patient’s mother, with whom he lived, and his co-workers knew of no recent history of recreational freshwater contact (eg, fishing, boating, swimming, diving, or tubing). The patient had chronic allergic sinusitis and irrigated his sinuses with a neti pot (Figure 1) at least once daily, using tap water to which he added a commercially available salt packet (same brand as the irrigation device). For convenience, he kept the device next to the bathroom sink.

Water samples from the municipal water treatment plant and distribution system, water samples and swabs from the patient’s household, and the irrigation device were sent to the CDC for further testing (Table 1). Water temperature and total chlorine residual measurements were made on some of the water samples at the time of collection. Water temperatures in the premise plumbing hot water system (a tankless water heater) ranged from 103°F to 113°F (39°C–45°C).

No amebae were detected in the samples from the municipal water treatment plant and distribution system serving this household (Table 1). However, multiple types of amebae (*Hartmannella*, *Vannella*, and *Naegleria* sp.) were detected in some of the samples taken at various locations within the household. Water collected from the tankless water heater was culture positive for *Naegleria* sp. and *Hartmannella* sp. and was PCR positive for *N. fowleri*; the genotype could not be identified. The sample was also culture positive for *Legionella pneumophila*. The neti pot was culture positive for *Hartmannella* sp. but PCR negative for *N. fowleri*.

The household occupants were advised to remediate the premise plumbing by setting the tankless water heater thermostat at a level at which each of the distal taps would have a water temperature of 160°F (71°C) and to initially run all taps (one faucet at a time) for at least 5 minutes to kill any remaining *N. fowleri* (and *Legionella*) in the water heater and premise

Table 1 Clinical Specimen and Environmental Sample Test Results for Cases of Primary Amebic Meningoencephalitis, Louisiana, 2011

Sample Type and Source	Culture (or IFA) Result		<i>Naegleria fowleri</i> PCR ^a Result		Temperature °F (°C)		Total Chlorine Residual, mg/L ^b	
	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2
Clinical								
Patient CSF	<i>N. fowleri</i>	NT	Positive, genotype 1	NT	NA	NA	NA	NA
Autopsy specimen (brain tissue)	NT	<i>N. fowleri</i> (by IFA)	NT	Positive	NA	NA	NA	NA
Water treatment plant								
Reservoir	Negative	NT	Negative	Negative	77 (25)	51 (10)	2.99	3.9
Clear well	Negative	NT	Negative	NT	77 (25)	NT	3.83	NT
Distribution system								
Tower	Negative	NT	Negative	Negative	NT	53 (11)	0.2	1.1
Water main serving case patient's neighborhood	Negative	NT	Negative	NT	NT	NT	0.16	NT
Point of entry of municipal water into case patient's residence	Negative	NT	Negative	Negative	NT	99 (37)	0.22	0.61
Household								
Swab samples								
Bathroom tap	Negative	Negative	Negative	NT	NT	NT	NT	NT
Main shower nozzle	<i>Hartmannella</i>	Negative	Negative	NT	NT	NT	NT	NT
Bathtub faucet	NT	<i>Hartmannella</i>	NT	NT	NT	NT	NT	NT
Toilet tank sediment	<i>Hartmannella</i>	Negative	Negative	NT	NT	NT	NT	NT
Handheld shower nozzle	<i>Vannella</i>	NT	Negative	NT	NT	NT	NT	NT
Kitchen faucet	Negative	<i>Hartmannella</i>	Negative	NT	NT	NT	NT	NT
Kitchen spray nozzle	<i>Hartmannella</i>	NT	Negative	NT	NT	NT	NT	NT
Dishwasher nozzle	Negative	Negative	Negative	NT	NT	NT	NT	NT
Dishwasher drain	Negative	Negative	Negative	NT	NT	NT	NT	NT
Water samples								
Handheld shower nozzle	Negative	NT	Negative	NT	110 (43.3)	NT	NT	NT
Kitchen faucet	Negative	Amebae ^c	Negative	Positive	112 (44.5)	91 (32.5)	NT	0
Main shower nozzle	<i>Hartmannella</i>	Amebae ^c	Negative	Positive	103 (39.3)	115 (46.1)	NT	0.02
Bathtub faucet	NT	<i>N. fowleri</i>	NT	Positive, genotype 1	NT	115 (46.3)	NT	0.02
Bathroom sink faucet	Negative	<i>N. fowleri</i>	Negative	Positive, genotype 1	107 (41.5)	99 (37.0)	NT	0
Water heater	<i>Hartmannella</i> , <i>Naegleria</i> sp. ^d	Negative	Positive	Negative	113 (44.8)	NT	NT	0.09
Neti pot	<i>Hartmannella</i>	Negative	Negative	NT	NT	NT	NT	NT

Abbreviations: CSF, cerebrospinal fluid; IFA, immunofluorescence assay; NA, not available; NT, not tested; PCR, polymerase chain reaction.

^a Real-time PCR analysis was used for detection of *N. fowleri*. Molecular characterization was attempted on available samples through amplification and bidirectional sequencing of the internal transcribed spacer and mitochondrial small subunit ribosomal RNA genes; where available, genotype information is provided.

^b The municipal water systems used monochloramine for residual disinfection. The expected range for total chlorine residual in distribution systems is 0.2–4 mg/L.

^c Amebae observed but not characteristic of *N. fowleri*.

^d The water sample from the tankless water heater for patient 1 was also positive by culture for non-serogroup 1 *Legionella pneumophila*.



Figure 1. Neti pot.

plumbing, subsequently lowering the thermostat to about 120°F to reduce the risk of scalding. To reduce the risk of recolonization, the occupants were further advised to repeat this procedure every few weeks.

Case 2

On 28 September 2011, a 51-year-old woman from northern Louisiana was admitted to the hospital with a 3-day history of altered mental status, nausea, vomiting, poor appetite, listlessness, fatigue, and high fever. On examination, she was febrile (temperature, 38.6°C) and lethargic with neck stiffness and thyromegaly. Meningitis was suspected based on complete blood cell count, CSF findings, and physical examination, although tests for specific viral, bacterial, and fungal pathogens were negative, as were bacterial culture. No gross motor deficits or cranial nerve abnormalities were found. The patient died on 2 October. An autopsy was performed by the Forensics Unit at the Louisiana State University Health Science Center–Shreveport Department of Pathology, and amebic meningoencephalitis was identified. Based on the distribution of gross alterations affecting the brain, and absence of obvious cyst forms, *Naegleria* sp. was suspected. On 18 November, brain tissue forwarded to the CDC for confirmatory testing tested positive for *N. fowleri* by immunohistochemistry, prompting an epidemiologic investigation. The genotype could not be identified.

The patient lived by herself; her parents lived on the same street block. According to her parents, the patient did not have any recreational freshwater exposure in the 2 weeks before illness onset. Because the patient had sinus problems, she regularly used a neti pot, especially after working in the yard, to remove dust from her nasal area. Her parents doubted that she used distilled, filtered, or previously boiled water for nasal irrigation. Water samples and swab samples from the patient's residence were collected (Table 1). Water temperature

and total chlorine residual measurements were taken on water samples at the time of collection. Water temperatures in the system, including the water heater tank, ranged from 91°F to 115°F (33°C–46°C). Water samples collected from the kitchen faucet, shower, bathtub faucet, and bathroom sink faucet tested positive by direct PCR for *N. fowleri*; further identified as genotype 1. No amebae were cultured from the neti pot. The ability to recover organisms from the neti pot might have been diminished by the 2-month delay between the last usage of the device and testing; furthermore, the neti pot was dry when tested. The family was advised to remediate the hot water system, as directed for the first case.

N. fowleri Inactivation Testing Using Neti Pot Saline Solutions

The ability of commercially available reconstituted salt packets in the neti pot to inactivate *N. fowleri* was tested at the CDC laboratory. Saline solution packets from 2 companies were prepared according to the manufacturers' directions. A saline solution packet was dissolved in 240 mL of distilled water to achieve a solution with 0.9% sodium chloride. Experiments were set up in duplicates in Costar 24-well Cluster flat-bottom plates with lids. *N. fowleri* amebae from the first case patient actively growing in modified Nelson medium were adjusted to obtain 500 amebae per milliliter of the saline solutions, and 2 mL of this solution was dispensed into each well and incubated in moist chambers at 37°C for 1, 4, and 18 hours; 2 mL of *N. fowleri* amebae (500 amebae per milliliter) in growth medium were set up as controls. At the end of each time period the amebae in the wells were photographed with an Olympus IMT-2 inverted microscope equipped with a digital camera. Test results revealed that the number of *N. fowleri* organisms did not appreciably decrease or degrade after 4 hours; observation at 18 hours revealed that more than half of the amebae had died (cells rounding up or lifting off plate; Figure 2). The experiment was replicated with *N. fowleri* amebae isolated from 2 unrelated fatal infections, with similar results.

DISCUSSION

PAM cases and deaths associated with exposure to tap water within a household highlight the changing epidemiology of *N. fowleri* in the United States. Previously, cases in the United States usually occurred among persons recreating in warm freshwater in southern-tier states [18]. The 2 cases in Louisiana in 2011 represent the first time disinfected tap water has been implicated in *N. fowleri* infection. Although *N. fowleri* are ubiquitous in freshwater aquatic environments, tap water in the United States has previously been implicated only as an uncommon exposure source. Two PAM cases in Arizona (2002) were associated with untreated geothermal municipal well water [21]

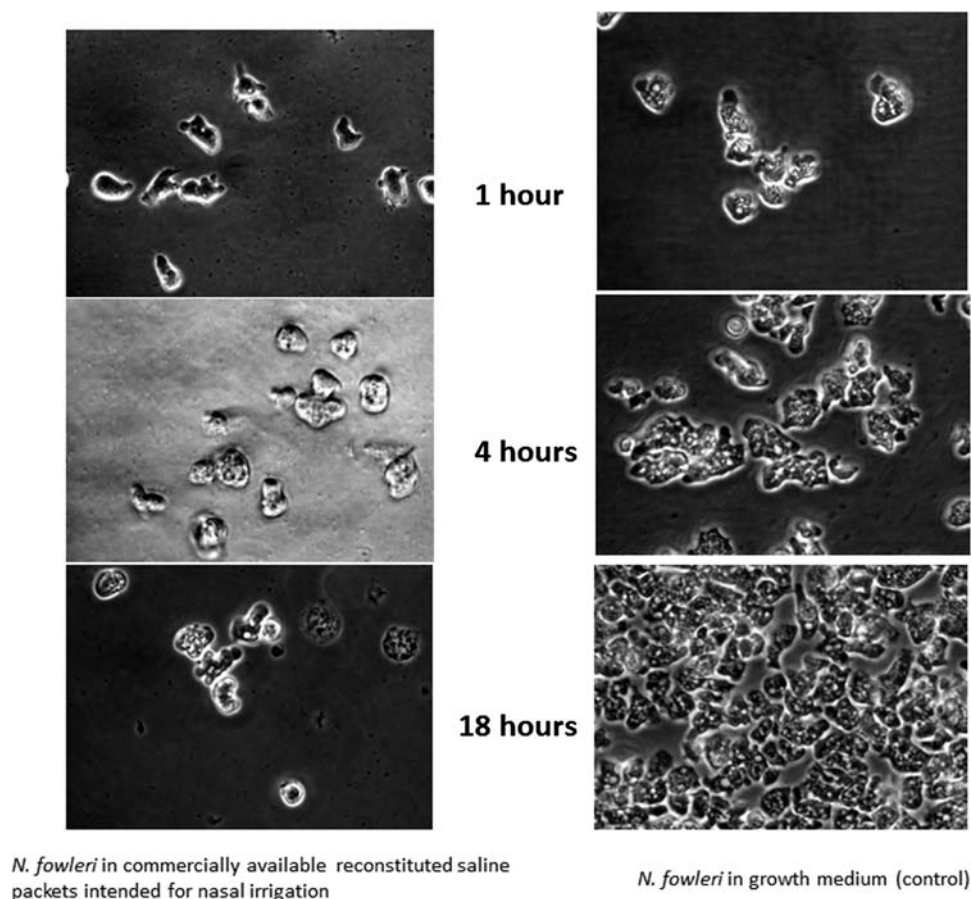


Figure 2. *Naegleria fowleri* exposure to a commercial saline solution designed for nasal irrigation, by length of exposure (left) and *N. fowleri* in growth medium (control; right; viewed at $\times 600$ magnification). After 1 hour, there was no decrease or increase in numbers of amoebae in saline or growth medium; after 4 hours, there was no decrease or increase in the number of amoebae in saline solution but a twofold increase in numbers in growth medium; after 18 hours, more than half of the amoebae were dead in saline solution and good growth continued in growth medium.

[When the cases occurred, there was no treatment of the municipal water supply by methods of chlorination, ozonation, UV irradiation, or filtration (20)]. These Louisiana cases represent the first reported cases in the United States associated with treated municipal water, although other such cases have been reported in Australia [22] and Pakistan [23]. The municipal water distribution systems for both Louisiana cases was chloraminated and total chlorine residual levels ranged from 0.2 to 1.1 mg/L in the distribution systems (Table 1), thereby meeting US Environmental Protection Agency (EPA) regulations for treated drinking water from surface water supplies [24] (*N. fowleri* is not among the >90 contaminants under national drinking water regulation by the EPA but has been on EPA's Contaminant Candidate List 3 [25]).

Environmental pathogens (eg, *Pseudomonas aeruginosa*, *Legionella* spp., nontuberculous mycobacteria) have emerged as well-documented biofilm colonizers within pipes found in manmade, engineered environmental habitats where they

amplify in warm water niches such as water heaters, and shower heads [26, 27]. *Naegleria* spp. also seem to be able to colonize biofilms and have been isolated from premise plumbing served by both treated and untreated municipal water supplies [21, 28]. *N. fowleri* cysts and trophozoites are fairly resistant to chlorine disinfection [29, 30]. Although *N. fowleri* were not isolated from the municipal water system in the Louisiana cases, *Naegleria* spp. were isolated from the premise plumbing, along with other amoebae. It is unclear how *N. fowleri* were introduced into the premise plumbing of these patients' houses. However, once introduced, they were able to colonize the hot water systems of these homes. Because these were the first US PAM cases associated with residential disinfected drinking water supplies, no formalized *N. fowleri* remediation and prevention guidelines exist for this setting. Temperatures used for *Legionella* remediation in nonresidential building systems and centralized systems in multifamily residential buildings should also kill *N. fowleri* and formed the

Naegleria fowleri infects people by entering the body through the nose. A person cannot be infected with *N. fowleri* by drinking contaminated water. Personal actions that might reduce the risk of infection include:

Nasal rinses or sinus irrigation:

- Do not use tap water or untreated freshwater. When irrigating, flushing, or rinsing nasal passages, use sterile, distilled, filtered (using a filter with an absolute pore size of 1 μm or smaller), or previously boiled water to make the irrigation solution.
- Rinse the irrigation device after each use using the same sterile, distilled, filtered, or previously boiled water.

Recreational water activities:

- Avoid getting water up the nose. Hold the nose shut or use nose clips when taking part in water-related activities involving warm freshwater.
- Avoid digging in or stirring up the sediment while taking part in water-related activities in warm freshwater bodies.
- Avoid water-related activities in warm freshwater during periods of high water temperature.

Figure 3. Measures to reduce the risk for primary amebic meningoencephalitis due to *Naegleria fowleri*.

basis for the household premise plumbing remediation recommendations in these cases [30, 31]. Preremediation water temperatures measured in these households were far below these levels. Remediation recommendations were specific to these households and situations and should not be seen as general recommendations for all homeowners. No data are available on the prevalence of *N. fowleri* in other residential units served by the same water utilities or in other Louisiana and US communities.

Nasal irrigation using saline solutions has been advocated as a safe, inexpensive method for managing chronic allergic rhinosinusitis [32]. However, the PAM cases reported here and others in Australia [22] and Pakistan [23] involving direct or forceful application of tap water into the nasal passages (eg, directing shower water up the nose, religious ablutions) indicate that there is a small risk associated with this practice when tap water is used. Although *N. fowleri* cannot survive in the level of salinity found in marine environments [33], adding salt mixtures to tap water to prepare and rapidly use nasal irrigation solutions does not seem to inactivate *N. fowleri* fast enough. The length of contact time found in real world conditions (<1 minute) would probably not effectively inactivate *N. fowleri*, which probably requires hours for full inactivation (Figure 2). Persons practicing nasal irrigation should be aware of the likely small risk for *N. fowleri*

contamination of tap water and the ability of *N. fowleri* to survive for short times in nasal irrigation salt solutions made with contaminated tap water. As a result, these persons should ensure that water used to create nasal irrigation solutions is distilled, filtered (using a filter with an absolute pore size of $\leq 1 \mu\text{m}$), or previously boiled (Figure 3).

Recently, the geographic pattern of this climate-sensitive, thermophilic ameba seems to be changing, with single cases recently reported for the first time in Minnesota (2010) and Kansas (2011), and for the first time since 1969 in Virginia (2011) [18, 34] (CDC unpublished data). These cases occurred in warm freshwater locations after localized heat waves and might reflect an expansion of the geographic range of PAM or increased *N. fowleri* activity in northern climates. It is unclear whether the increased temperature and heat waves projected in climate change models will lead to further expansion of the geographic range. To better address this, improved and systematic environmental sampling of water bodies and systems in the United States is needed to create a baseline for *N. fowleri* occurrence, followed by systematic monitoring over time to better interpret, understand, and predict potential changes in *N. fowleri* ecology that could lead to improved prevention activities. In addition, developing efficacious therapies and raising the level of clinical awareness about PAM infections may improve the prognosis for future patients.

Notes

Acknowledgments. The following persons assisted with the clinical, epidemiological, and laboratory portions of this investigation: Mahendra Poudel, MD, Asher Shahzad, MD, Katherine L. Baumgarten, MD, Lourdes Lago, MD, Vivek Sabharwal, MD, Erik T. Sundell, MD, Ochsner Health Systems; Nancy Hartwell, Louisiana State University Health Sciences Center, Shreveport; Gary Balsamo, DVM, Keasha Henson, MPH, Louisiana Department of Health and Hospitals; Ellen Brown, AS, Lauri Hicks, DO, Claressa Lucas, PhD, Jonas Winchell, PhD, National Center for Immunization and Respiratory Diseases; Dawn M. Roellig, MS, PhD, Rebecca Bandea, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

Financial support. The authors completed this work in the course of their work for their affiliated institutions and received no additional funding.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. De Jonckheere JF. Molecular definition and the ubiquity of species in the genus *Naegleria*. *Protist* **2004**; 155:89–103.
2. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immun Med Microbiol* **2007**; 50:1–26.
3. Marciano-Cabral F, Cabral GA. The immune response to *Naegleria fowleri* amebae and pathogenesis of infection. *FEMS Immun Med Microbiol* **2007**; 51:243–59.
4. Visvesvara GS, Roy SL, Maguire JH. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*,

- Naegleria fowleri*, and *Sappinia pedata*. In: Guerrant RL, Walker DH, Weller PF, eds. Tropical infectious diseases—principles, pathogens and practice. 3rd ed. Edinburgh, UK: Saunders Elsevier, 2011: 707–13.
5. Wellings FM, Amuso PT, Chang SL, Lewis AL. Isolation and identification of pathogenic *Naegleria* from Florida lakes. *Appl Environ Microbiol* 1977; 34:661–7.
 6. John DT, Howard MJ. Seasonal distribution of pathogenic free-living amoebae in Oklahoma waters. *Parasitol Res* 1995; 81:193–201.
 7. Ettinger MR, Webb SR, Harris SA, McIninch SP, Garman GC, Brown BL. Distribution of free-living amoebae in James River, Virginia, USA. *Parasitol Res* 2003; 89:6–15.
 8. Sheehan KB, Fagg JA, Ferris MJ, Henson JM. PCR detection and analysis of the free-living amoeba *Naegleria* in hot springs in Yellowstone and Grand Teton National Parks. *Appl Environ Microbiol* 2003; 69:5914–8.
 9. De Jonckheere J, Van Dijck P, Van de Voorde H. The effect of thermal pollution on the distribution of *Naegleria fowleri*. *J Hygiene* 1975; 75:7–13.
 10. Behets J, Declerck P, Delaet Y, Verelst L, Ollevier F. Survey for the presence of specific free-living amoebae in cooling waters from Belgian power plants. *Parasitol Res* 2007; 100:1249–56.
 11. Tyndall RL, Ironside KS, Metler PL, Tan EL, Hazen TC, Fliermans CB. Effect of thermal additions on the density and distribution of thermophilic amoebae and pathogenic *Naegleria fowleri* in a newly created cooling lake. *Appl Environ Microbiol* 1989; 55:722–32.
 12. Blair B, Sarkar P, Bright KR, Marciano-Cabral F, Gerba CP. *Naegleria fowleri* in well water. *Emerg Infect Dis* 2008; 14:1499–501.
 13. Kadlec V, Skvářová J, Cerva L, Nebáznivá D. Virulent *Naegleria fowleri* in indoor swimming pool. *Folia Parasitol (Praha)* 1980; 27:11–7.
 14. Griffin JL. The pathogenic amoeboflagellate *Naegleria fowleri*: environmental isolations, competitors, ecologic interactions, and the flagellate-empty habitat hypothesis. *J Protozool* 1983; 30:403–9.
 15. Bose K, Ghosh DK, Ghosh KN, Bhattacharya A, Das SR. Characterization of potentially pathogenic free-living amoebae in sewage samples of Calcutta, India. *Braz J Med Biol Res* 1990; 23:1271–8.
 16. Maclean RC, Richardson DJ, LePardo R, Marciano-Cabral F. The identification of *Naegleria fowleri* from water and soil samples by nested PCR. *Parasitol Res* 2004; 93:211–7.
 17. Anderson K, Jamieson A. Primary amoebic meningoencephalitis. *Lancet* 1972; 2:379.
 18. Yoder JS, Eddy BA, Visvesvara GS, Capewell L, Beach MJ. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962–2008. *Epidemiol Infect* 2010; 138:968–75.
 19. Qvarnstrom Y, Visvesvara GS, Sriram R, da Silva AJ. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J Clin Microbiol* 2006; 44:3589–95.
 20. Zhou L, Sriram R, Visvesvara GS, Xiao L. Genetic variations in the internal transcribed spacer and mitochondrial small subunit rRNA gene of *Naegleria* spp. *J Eukaryot Microbiol* 2003; 50(Suppl):522–6.
 21. Marciano-Cabral F, MacLean R, Mensah A, LaPat-Polasko L. Identification of *Naegleria fowleri* in domestic water sources by nested PCR. *Appl Environ Microbiol* 2003; 69:5864–9.
 22. Dorsch MM, Cameron AS, Robinson BS. The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia. *Trans R Soc Trop Med Hyg* 1983; 77:372–7.
 23. Shakoor S, Beg MA, Mahmood SF, et al. Primary amoebic meningoencephalitis caused by *Naegleria fowleri*, Karachi, Pakistan. *Emerg Infect Dis* 2011; 17:258–61.
 24. United States Environmental Protection Agency. Comprehensive surface water treatment rules quick reference guide: systems using conventional or direct filtration 2010. Report 816-F-10-074. Available at: http://www.epa.gov/ogwdw/mdbp/pdfs/qrg_mdbp_surfacewatertreatment_convent_direct.pdf. Accessed 24 July 2012.
 25. United States Environmental Protection Agency. Contaminant candidate list 3 - CCL. Available at: <http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm>. Accessed 24 July 2012.
 26. Falkinham JO III. Mycobacterial aerosols and respiratory disease. *Emerg Infect Dis* 2003; 9:763–7.
 27. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis* 2002; 8:881–90.
 28. Marciano-Cabral F, Jamerson M, Kaneshiro ES. Free-living amoebae, *Legionella* and *Mycobacterium* in tap water supplies by a municipal drinking water utility in the USA. *J Water and Health* 2010; 8:71–82.
 29. Gerba CP, Blair BL, Sarkar P, Bright KR, MassLean RC, Marciano-Cabral F. Occurrence of *Naegleria fowleri* in drinking water. In: Ortega- Pierres , et al., eds. *Giardia and Cryptosporidium: from molecules to disease*. CAB International, Oxfordshire, UK 2008:238–47.
 30. Chang SL. Resistance of pathogenic *Naegleria* to some common physical and chemical agents. *Appl Environ Microbiol* 1978; 35:368–75.
 31. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc, ASHRAE Standards Committee 1999–2000. Minimizing the risk of legionellosis associated with building water systems. Atlanta, GA: ASHRAE; 2000. Available at: <http://spxcooling.com/pdf/guide12.pdf>. Accessed 24 July 2012.
 32. Tomooka LT, Murphy C, Davidson TM. Clinical study and literature review of nasal irrigation. *Laryngoscope* 2000; 110:1189–93.
 33. Rodríguez-Zaragoza S. Ecology of free-living amoebae. *Crit Rev Microbiol* 1994; 20:225–41.
 34. Kemble SK, Lynfield R, Devries AS, et al. Fatal *Naegleria fowleri* infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism. *Clin Infect Dis* 2012; 54:805–9.