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

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Background. Naegleria fowleri is a climate-sensitive, thermophilic ameba 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 ameba 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.
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 coworkers 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

<table>
<thead>
<tr>
<th>Sample Type and Source</th>
<th>Culture (or IFA) Result</th>
<th>Naegleria fowleri PCR&lt;sup&gt;a&lt;/sup&gt; Result</th>
<th>Temperature °F (°C)</th>
<th>Total Chlorine Residual, mg/L&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient 1</td>
<td>Patient 2</td>
<td>Patient 1</td>
<td>Patient 2</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Patient CSF</td>
<td>N. fowleri</td>
<td>NT</td>
<td>Positive</td>
<td>NT</td>
</tr>
<tr>
<td>Autopsy specimen (brain tissue)</td>
<td>NT</td>
<td>N. fowleri (by IFA)</td>
<td>NT</td>
<td>Positive</td>
</tr>
<tr>
<td>Water treatment plant</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Reservoir</td>
<td>Negative</td>
<td>NT</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>Clear well</td>
<td>Negative</td>
<td>NT</td>
<td>Negative</td>
<td>NT</td>
</tr>
<tr>
<td>Distribution system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tower</td>
<td>Negative</td>
<td>NT</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>Water main serving case patient’s neighborhood</td>
<td>Negative</td>
<td>NT</td>
<td>Negative</td>
<td>NT</td>
</tr>
<tr>
<td>Point of entry of municipal water into case patient’s residence</td>
<td>Negative</td>
<td>NT</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>Household</td>
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<tr>
<td>Swab samples</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Bathroom tap</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>NT</td>
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<tr>
<td>Main shower nozzle</td>
<td>Hartmannella</td>
<td>Negative</td>
<td>Negative</td>
<td>NT</td>
</tr>
<tr>
<td>Bathtub faucet</td>
<td>NT</td>
<td>Hartmannella</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Toilet tank sediment</td>
<td>Hartmannella</td>
<td>Negative</td>
<td>Negative</td>
<td>NT</td>
</tr>
<tr>
<td>Handheld shower nozzle</td>
<td>Vannella</td>
<td>NT</td>
<td>Negative</td>
<td>NT</td>
</tr>
<tr>
<td>Kitchen faucet</td>
<td>Negative</td>
<td>Hartmannella</td>
<td>Negative</td>
<td>NT</td>
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<tr>
<td>Kitchen spray nozzle</td>
<td>Hartmannella</td>
<td>NT</td>
<td>Negative</td>
<td>NT</td>
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<td>Dishwasher nozzle</td>
<td>Negative</td>
<td>Negative</td>
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<td>Dishwasher drain</td>
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<td>Negative</td>
<td>Negative</td>
<td>NT</td>
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<tr>
<td>Water samples</td>
<td></td>
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<td></td>
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<tr>
<td>Handheld shower nozzle</td>
<td>Negative</td>
<td>NT</td>
<td>Negative</td>
<td>NT</td>
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<tr>
<td>Kitchen faucet</td>
<td>Negative</td>
<td>Amebae&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Main shower nozzle</td>
<td>Hartmannella</td>
<td>Amebae&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Bathtub faucet</td>
<td>NT</td>
<td>N. fowleri</td>
<td>NT</td>
<td>Positive</td>
</tr>
<tr>
<td>Bathroom sink faucet</td>
<td>Negative</td>
<td>N. fowleri</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Water heater</td>
<td>Hartmannella, Naegleria sp.&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

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

<sup>a</sup> 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.

<sup>b</sup> 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.

<sup>c</sup> Amebae observed but not characteristic of N. fowleri.

<sup>d</sup> The water sample from the tankless water heater for patient 1 was also positive by culture for non–serogroup 1 Legionella pneumophila.
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 diluted 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]
[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]. *Naegleria 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 amebae. 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 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 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

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References

4. Visvesvara GS, Roy SL, Maguire JH. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, 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 ≤1 μm), 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.


