Meningo-Encephalitis Due to Free Living Amebas

1) 2011 Cases

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 the *N. fowleri* migrate to the brain via the olfactory nerve. In 2011, two 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 area 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 meningitis-like symptoms.

Many species of *Naegleria* are found in the environment [1] but only one 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 one to seven days after exposure [18]. Signs and symptoms of infection are similar to those of bac-

terial 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 five days [18].

CASE REPORTS

Case 1

On June 5, 2011, a 28 year-old man from southern Louisiana abruptly developed a severe occipital headache radiating down his neck with neck stiffness, back pain, and intermittent vomiting. He had a history of migraines and thus did not seek medical attention at symptom onset. On June 6th, 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. Non-contrast 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 six centimeters 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, June 7th,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 June 8th, 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 June 10th, realtime 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 June 17th, 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 (e.g., 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*.

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

	Culture (or IFA) Result		*Naegleria fowleri* PCR[a] Result		Temperature °F (°C)		Total Chlorine Residual, mg/L[b]	
Sample Type and Source	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2
Clinical								
Patient CSF	*N. fowleri*	NT	Positive, genotype 1	NT	NA	NA	NA	NA
Autopsy specimen (brain tissue)	NT	*N. fowleri* (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	*Hartmannella*	Negative	Negative	NT	NT	NT	NT	NT
Bathtub faucet	NT	*Hartmannella*	NT	NT	NT	NT	NT	NT
Toilet tank sediment	*Hartmannella*	Negative	Negative	NT	NT	NT	NT	NT
Handheld shower nozzle	*Vannella*	NT	Negative	NT	NT	NT	NT	NT
Kitchen faucet	Negative	*Hartmannella*	Negative	NT	NT	NT	NT	NT
Kitchen spray nozzle	*Hartmannella*	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	*Hartmannella*	Amebae[c]	Negative	Positive	103 (39.3)	115 (46.1)	NT	0.02
Bathtub faucet	NT	*N. fowleri*	NT	Positive, genotype 1	NT	115 (46.3)	NT	0.02
Bathroom sink faucet	Negative	*N. fowleri*	Negative	Positive, genotype 1	107 (41.5)	99 (37.0)	NT	0
Water heater	*Hartmannella*, *Naegleria* sp.[d]	Negative	Positive	Negative	113 (44.8)	NT	NT	0.09
Neti pot	*Hartmannella*	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

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 five minutes to kill any remaining *N. fowleri* (and *Legionella*) in the water heater and premise 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 September 28th, 2011, a 51-year-old woman from northern Louisiana was admitted to the hospital with a three-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 October 2nd. An autopsy was performed by the Forensics Unit at the Louisiana State University Health Science Center-Shreveport Department of Pathology; 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 November 18th, 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 two 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 two-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 two 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 one, four, 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 four 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 two unrelated fatal infections, with similar results.

DISCUSSION

PAM cases and deaths associated with exposure to tap water within a household highlight the changing epidemiolo-

gy of *N. fowleri* in the United States. Previously, cases in the U.S. usually occurred among persons recreating in warm freshwater in southern-tier states [18]. The two Louisiana cases in 2011 represent for the first time, that disinfected tap water was implicated in *N. fowleri* infection. Although *N. fowleri* are ubiquitous in freshwater aquatic environments, tap water in the U.S. 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].

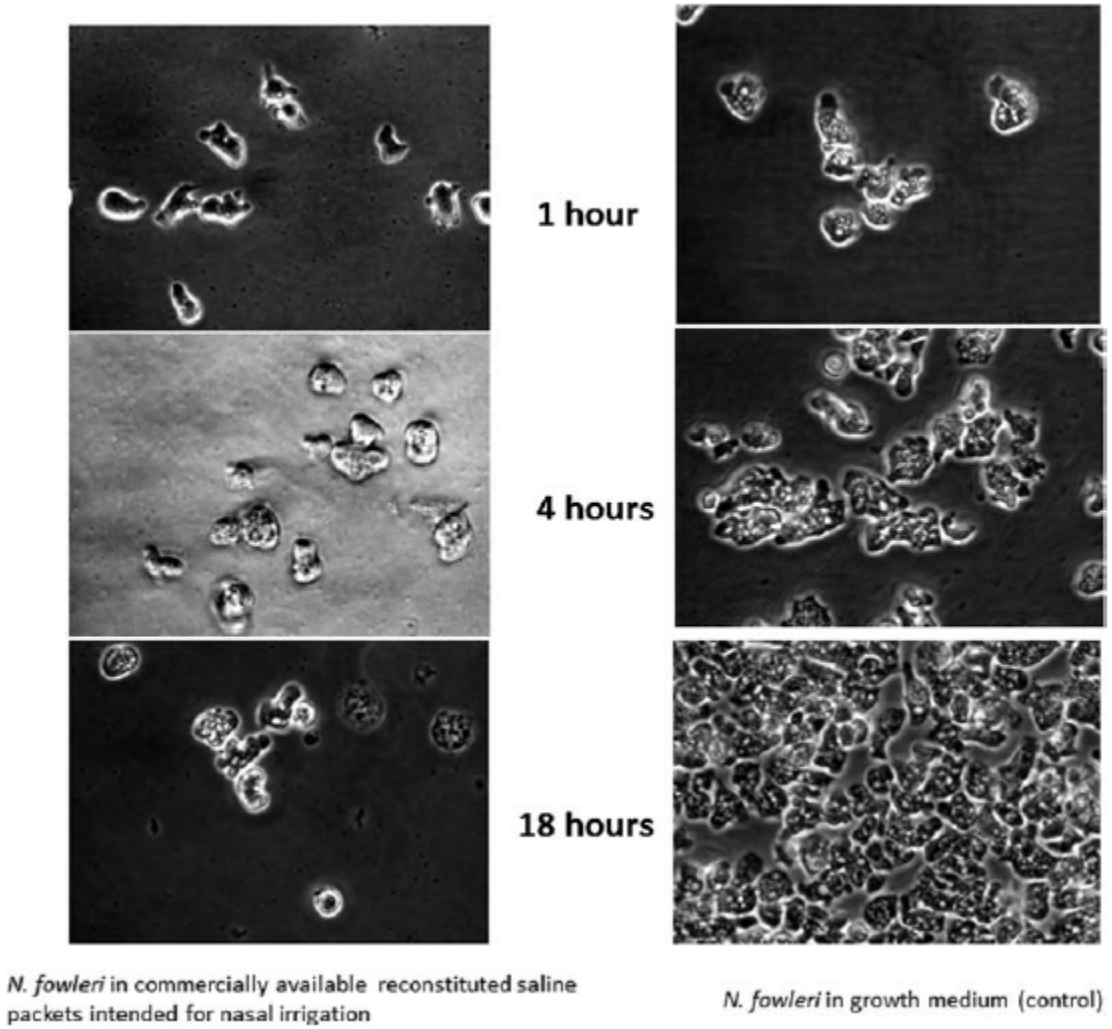
(When the cases occurred, there was no treatment of the municipal water supply by methods of chlorination, ozonation, UV irradiation, or filtration [20].) These Louisiana cases represent the first reported cases in the United States associated with treated municipal water, although other such cases have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

Environmental pathogens (eg, *Pseudomonas aeruginosa, Legionella* spp., non-tuberculous 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 U.S. 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 basis for the household premise plumbing remediation recommendations in these cases [30, 31]. Pre-remediation 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 U.S. communities.

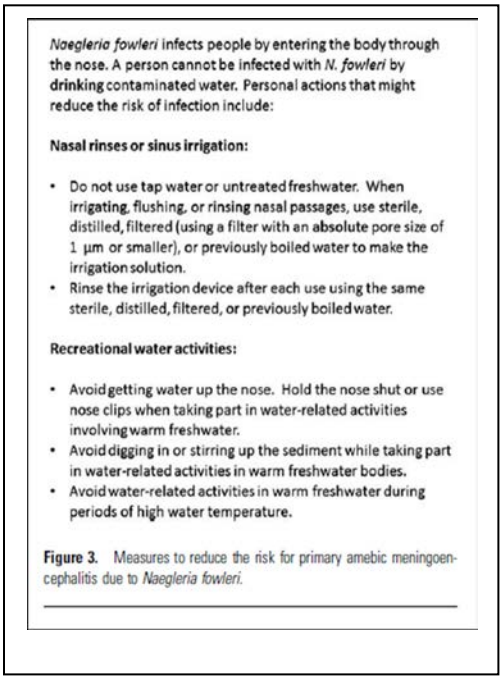
Nasal irrigation using saline solutions has been advocated as a safe, inexpensive method for managing chronic allergic rhinosinusitis [32]. However, the PAM cases reported here and others in Australia [22] and Pakistan [23] involving direct or forceful application of tap water into the nasal passages (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 (less than one 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 less than or equal to 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 U.S. 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.

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 ×600 magnification).



After one hour, there was no decrease or increase in numbers of amebae in saline or growth medium; after four hours, there was no decrease or increase in the number of amebae in saline solution but a two-fold increase in numbers in growth medium; after 18 hours, more than half of the amebae were dead in saline solution and good growth continued in growth medium.



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*.

Notes

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2-2011 Case of Granulomatous Amebic Encephalitis (GAE)

In November 2011, a 67-year-old male presented to an emergency department with complaints of dizziness, ataxia, nausea, vomiting and fever. He was transferred to another hospital where the brain MRI revealed two to three ring-enhanced lesions. A brain biopsy sent to the CDC tested positive for *Balamuthia mandrillaris* by Polymerase chain reaction (PCR). The patient was treated with Miltefosine (a treatment normally used for visceral Leishmaniasis and a new investigational drug for amebic encephalitis), but the patient's health declined and he died after 40 days of hospitalization.

The patient had immigrated to the U.S. in 1979, and had a history of sinusitis and chronic kidney disease, but seemed to be immune-competent. He lived in Central Louisiana where he owned a plant nursery for the previous five years. After notification from the CDC about the GAE diagnosis, staff from the Infectious Disease Epidemiology Section (IDEpi) and the Office of Public Health (OPH) Region 5 office collected environmental samples (water and soil) from the patient's residence and adjacent nursery. All water samples were found to be negative. However,

two of the soil samples, one from the work station and one from the greenhouse (Figure 3), were found to be positive for *Balamuthia mandrillaris* by PCR.

Figure 3: Greenhouse with Positive Soil Sample. (Courtesy of Steven Joubert)

Balamuthia is the third and latest discovered genera of free-living ameba which can cause Granulomatous Amebic Encephalitis (GAE) in humans and animals. *Balamuthia mandrillaris* was first isolated in 1986 from a from a mandrill baboon that had died of encephalitis in a zoo in the United States. In 1991, the first human infection of *Balamuthia* was diagnosed and since then more than 200 cases worldwide have been identified. In the U.S., at least 70 cases have been diagnosed to date, with more cases occurring in warmer climates. The incubation period ranges usually from weeks to months but can be two years or longer. Prognosis is very poor if diagnosis occurs after the amebas have invaded the brain. The case fatality rate is greater than 95%. Both healthy and immunocompromised persons can develop the disease; exposure to soil through gardening, playing with dirt or inhaling dirt increases the risk for this fatal infection.

Still little is known about the epidemiology of *Balamuthia mandrillaris*. In contrast to other GAE causing ameba such as *Naegleria fowleri* and *Acanthamoeba* sp., *Balamuthia* is difficult to isolate from environmental samples. To date, the organism has been isolated from rich, fertile soil (such as compost or potting soil), but never from water samples. Nutrient rich soil is a perfect environment for bacterial growth and therefore also a good environment for amebas which feed on bacteria. It is also unclear why it is such a rare disease even though the exposure to soil seems to be prevalent in the general population. It might be that there are still undiscovered specific individual factors that make some people more susceptible to *Balamuthia* infection and disease.

Most likely *Balamuthia* enters the body when soil containing these free living amebas gets into contact with skin wounds or cuts, or when dust containing these amebas is inhaled. Unlike *Naegleria fowleri*, which has a preference for invasion of the CNS via the olfactory nerves, it is suggested that *Balamuthia* ameba travel through disrupted skin or through lung membranes into the vasculature and subsequently enter the CNS.

Persons who have regular contact with soil such as agricultural workers or hobby gardeners should cover skin lesions and wear protective clothing while working with soil to prevent *Balamuthia* infection.

3-2013 Case of *Naegleria foweleri* Meningo-encephalitis

Clinical History

On July 27, 2013 a four-year-old boy was admitted into a hospital for meningoencephalitis. The child's condition got worse; he died in early August in spite of treatment. No definite etiology was determined as the cause of death. Specimens were sent to the CDC for additional testing of brain tissue. In mid-August, the etiologic diagnosis of primary amebic meningoencephalitis (PAM) due to *Naegleria fowleri* (Nf) was confirmed.

Epidemiological Investigation

The epidemiologic investigation focused on soil and water contact during the prior two weeks, when the boy spent visiting a relative in Violet, Louisiana (St. Bernard Parish). It appeared that this child had no contact with surface water (pond, river, ditch or puddle) during the entire period. Besides contact with tap water while inside the home, he played in the yard adjacent to the house. On July 18, he had spent almost the entire day playing on a backyard water slide; as the water slide sprayed water, the child slipped both head first and feet first into the water. Given an exposure on July 18 with the onset on July 25, the incubation period of seven days was consistent with that of PAM. Two garden hoses were used to connect an outside faucet to the water slide. This faucet was located between the

municipal water connection and the home. After consultation with the CDC, it was decided to collect water samples from the hoses, the water slide and several locations inside the residence (Table 2).

Table 2: Results of Soil and Water Sample Testing – Violet, Louisiana, 2013

Sample ID	Quantity Collected (Liter or Kg)	Residual Chlorine (mg/L)	Direct PCR Results for *N. fowleri*	Culture Observations- Amebas Present?	Culture PCR Results for *N. fowleri*	Flagellation?	Genotype Results
Soil #1 (contains grass, yellow tie)	~1kg	---	Pos	Y	Neg		G I
Soil #2 (no grass, orange tie)	~1kg	---	Neg	Y	Neg		
Garden Hose #1 (green)	1.0L	---	Pos	Y	Pos	Neg	G III
Garden Hose #2 (orange)	1.2L	---	Pos	Y	Pos	Pos	G III
Water Slide	1.9L	---	Neg	Y	Neg		
Outside Hose Bib	158.0L	0.0	Pos	Y	Pos	Pos	G III
Kitchen Sink Hot Water	0.7L	0.0	Neg	Y	Neg	Neg	
Bathtub Faucet	0.7L	0.0	Neg	Y	Neg	Neg	
Bathtub Faucet, Sink, Showerhead	Swab	---	Neg	Y	Neg		
Toilet Tank	0.7	0.0	Neg	Y	Pos	Pos	G III
Hot Water Heater	0.7	---	Neg	Y	Pos	Pos	G III

One of the soil samples was positive for *N.fowleri* -Genotype I. Numerous samples from inside the residence hot and cold water system, the hoses, and the outside faucet located between the street water line and the home were positive for *Nf*-Genotype III, similar to the type encountered in the child's brain tissue, leaving no doubt as to the exposure of the child. Concerns about the drinking water system were immediately raised for the following reasons:

1-There was a PAM case due to *Nf* in 2011 in the same public water system*. The water source was tap water from the residence, used during a sinus irrigation with a neti pot.

2-There was no chlorine residual in the water from the faucet.

To address these concerns, the Department of Health and Hospitals' OPH, after consultation with the CDC, collected samples of water from the town water supply focusing on areas of low chlorine residual (Table 3).

Table 3: Results of St. Bernard Water Supply Testing Prior to Remediation – Louisiana, 2013

Sample ID	Volume Collected (L)	Total Chlorine (mg/L)	Direct PCR Results for *N. fowleri*	Culture Observations- Amebas Present?	Culture PCR Results for *N. fowleri*	Flagellation?	Genotype Results
Water Tower Ultrafilter, Violet, LA.	119.0	1.7	Neg	No	Neg		
Flushing Station Packenham Rd. - Grab Sample, Violet, LA.	0.7	1.2	Neg	No	Neg		
Water Tower Grab Sample, Violet, LA.	0.7		Neg	No	Neg		
Water Plant Grab Sample, St. Bernard Reservoir, LA.	0.7	3.8	Neg	No	Neg		
Water Plant Ultrafilter, St. Bernard Reservoir, LA.	119.0		Neg	No	Neg		
Location #1-Angelique Dr. Outside Hose Bib Ultrafilter, Violet, LA.	510.3	0.24	Neg				
Location #1-Hot Water Heater Grab Sample	0.5		Neg				
Location #2-Meraux Ln.Outside Hose Bib Ultrafilter, Meraux, LA.	340.2	0.53	Neg				
Location #3-Beachhead Ln. Outside Hose Bib Ultrafilter, Violet, LA.	418	0	Neg				
Location #4-Angela Ave.Outside Hose Bib Ultrafilter, Arabi, LA.	350	0	Pos	Y	Pos	Pos	G III
Location #5-Mehle Ave. Outside Hose Bib Ultrafilter, Arabi, LA.	302.4	0	Pos	Y	Pos	Pos	G III
Franke Pl. and St. Bernard Hwy. Ultrafilter, Violet, LA.	146.1	0.1	Neg				
W. Smith Jr. Elementary School Ultrafilter, Violet, LA.	116.2	0.3	Neg				
Cougar Dr. Ultrafilter, Arabi, LA.	136.3	0.2	Neg				
Bridgehead St. Fire hydrant Ultrafilter, Violet, LA.	235.5	trace	Pos	Y	Pos	Pos	G III
Mehle Ave. Fire hydrant Ultrafilter, Arabi, LA.	200.6	0	Pos	Y	Pos	Pos	G III

It became obvious that the source of the *Naegleri fowleri* was the municipal water supply.

Nf is a free-living ameba commonly found in soil and water throughout the world. It can also become a human parasite under very special circumstances. It has to penetrate the human body (and in other animals) through the paper thin bone layer (the "cribriform plate") that separates the ceiling of the nasal cavity and the base of the brain. Then *Nf* proliferates in the brain tissue causing a nearly always fatal meningoencephalitis. The majority of exposures come from submersion of the head under surface waters (diving in ponds, swimming in lakes and rivers, playing in ditches or puddles). Cases linked to domestic water supplies have been less common. Most of these have occurred when water supplies were not treated, or did not have suffi-

cient residual chlorine. It is estimated that a residual chlorine level of 0.5 mg/L is sufficient to prevent the multiplication of *Nf*. Following this case, the parish switched from chloramine to free chlorine disinfection of its water distribution system. Chlorine levels were increased and a parish-wide residual of not less than 1.0 ppm free chlorine was achieved.

For a more detailed discussion of free-living ameba parasitology, modes of exposure, case histories and prevention, visit the Infectious Disease Epidemiology Section, Epidemiology
al *http://dhh.louisiana.gov/index.cfm/page/531*.