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Surveillance of *Naegleria fowleri* in Louisiana's public water systems

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ABSTRACT

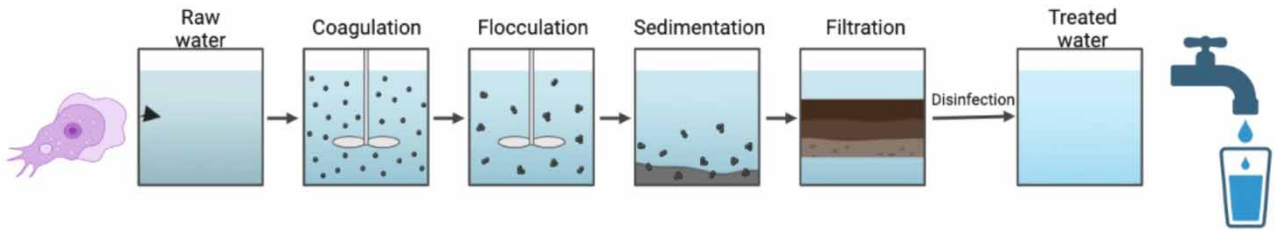
The free-living amoeba *Naegleria fowleri* (Nf) inhabits soil and natural waters worldwide: it is thermophilic and thrives at temperatures up to 45 °C and in a multitude of environments. Three deaths in Louisiana were attributed to primary amoebic meningoencephalitis (PAM) caused by Nf infection in 2011 and 2013. Following these incidents, public water systems are now monitored for the presence of Nf in Louisiana. From 2014 to 2018, 29% (27/93) of samples collected showed positive for Nf and 68% (63/93) showed all thermophilic amoeba culture. Ten raw water sources and 17 distribution water systems tested positive. The year 2017 showed the highest number of samples with Nf ($n = 10$) followed by nine samples in 2015. As climate change increases surface water temperatures, continued testing for Nf prevalence will be an important facet of water monitoring and will need to extend into locations farther north than the current most common range.

Key words: brain-eating amoeba, monitoring, potable water

HIGHLIGHTS

- The occurrence of *Naegleria fowleri* in public water systems was studied.
- About 29% (27/93) of samples collected showed positive for Nf.
- More research is warranted to determine the survival of this emerging pathogen in water systems.

GRAPHICAL ABSTRACT



INTRODUCTION

Naegleria fowleri, commonly called the 'brain-eating amoeba', is a pathogenic free-living amoeba (FLA), which is found naturally in hot springs and warm surface waters. *N. fowleri* causes primary amoebic meningoencephalitis (PAM). This infection occurs when the *N. fowleri* enters the nasal cavity, travels through the olfactory mucosa and along the olfactory nerve, and ultimately feeds on nerve tissues in the brain, resulting in tissue necrosis, bleeding, and – in nearly all cases – death within 2 weeks of initial infection. Three deaths that were attributed to PAM have been recorded in recent years in Louisiana (Isaac & Sherchan 2020).

N. fowleri is listed in the United States Environmental Protection Agency (U.S. EPA) draft Contaminant Candidate List 5 (CCL5). However, less frequently known is that *N. fowleri* amoeba can occur in treated waters such as swimming pools, water heaters, municipal systems, and other water transport systems that have been inadequately treated or negligently maintained

to prevent amoebic growth. It is possible to contract the amoeba through treated water with no or low chlorine residual. There have been several recent cases of *N. fowleri* infections via municipal water sources (Yu *et al.* 2017). In 2011, two people died of PAM caused by *N. fowleri* in DeSoto and St. Bernard Parishes in Louisiana. Both cases involved treated tap water that lacked detectable chlorine residual that was self-introduced into the sinus cavity via net-pots. In 2013, there was a second death in St. Bernard Parish (a 4-year-old boy) caused by PAM, which was confirmed to be *N. fowleri* infection. The route of exposure was inhalation of treated tap water while the child played on a home Slip n' Slide (water slide). Four out of 16 water distribution system samples collected by the CDC in St. Bernard Parish were positive for *N. fowleri*. According to Cope *et al.* (2019), the water temperature was >30 °C in three of the four positive sampling locations. To date, a total of nine Louisiana public water systems have tested positive for *N. fowleri*.

The Louisiana Department of Health (LDH) also confirmed the presence of *N. fowleri* in South Bossier Parish on 28 September 2018 (Wooten 2019). *N. fowleri* is thermophilic and able to proliferate in temperatures up to 45 °C. Warmer surface water temperatures due to global climate change will provide new environmental niches and higher risks of *N. fowleri* exposure (Bright & Gerba 2017; Xue *et al.* 2018). The annual number of *N. fowleri* cases associated with recreational and tap water exposure is increasing globally (Yu *et al.* 2017; Xue *et al.* 2018; Cope *et al.* 2019). However, water utilities only test for indicator organisms and are currently not required to test for the presence of *N. fowleri* due to limitations related to federal regulations, cost, time, and labor. In this study, we summarized *N. fowleri* surveillance in potable water systems in the State of Louisiana.

METHODS

Ultrafilters that had processed 100 L of water samples were immediately transported to the laboratory in a cooler. These ultrafilters were backlashed as described by Cope *et al.* (2019) using WB saline with 0.1% Tween 80 and eluates processed by centrifugation at 1,500 g for 15 min at room temperature. Supernatants were removed and then pellets that were mixed with an overnight culture of *Escherichia coli* were plated on Nelson's agar and incubated at 42 °C for up to 7 days. Then, plates were examined using an inverted microscope to observe amoeba activity. At 7 days of incubation, the entire surface of the plate was harvested and centrifuged to produce a pellet, and DNA was extracted for quantitative polymerase chain reaction (qPCR) confirmation (Cope *et al.* 2019).

The TaqMan-based assay was performed with 5 μL of template DNA, 250 nM of the forward (JBVF, 5′-AGG TAC TTA CGT TAG AGT GCT AGT-3′) and reverse primers (JBVR, 5′-ATG GGA CAA TCC GGT TTT CTC A-3′), 100 nM of the FAM-labeled probe (JBVP, 5′-FAM-AC GCC CTA GCT GGT TAT GCC GGA TT-BHQ1-3′), and nanopure water (Mull *et al.* 2013). The thermal cycling conditions of the qPCR assay were as follows: (i) 95 °C for 15 min (activation of Taq DNA polymerase) and (ii) 45 cycles of 95 °C for 15 s and 63 °C for 33 s (Mull *et al.* 2013). No genotyping was performed in this study and organisms obtained by culture were confirmed only by qPCR for *N. fowleri* as described by Cope *et al.* (2015).

Sample location sites

A total of 93 samples were collected during the period 2014–2018 and consisted of raw source water; point of entry (POE) – treated water delivered to the distribution; total coliform rule (TCR) – distribution system lines; additional chlorine residual (ACR) – distribution system lines; maximum residence time (MRT) – oldest water in the distribution system.

RESULTS AND DISCUSSION

The presence of *N. fowleri* was detected in 27 samples (29%) out of 93 collected. Positive samples were collected from 11 parishes – St. Bernard, St. John, Sabine, Ascension, Ouachita, Terrebonne, Caldwell, Tensas, St. Mary, St. James, and Bossier – geographically distributed throughout Louisiana (Figure 1).

However, the results presented need to be interpreted with care. First, not all water systems have been sampled in each of those parishes. Second, most water systems only serve water to certain sections of the parish (not the whole parish). For example, St. John WD1, Ebarb WD1, Ascension CUD1, Schriever, North Monroe, and the City of Bossier only serve water to a portion of the parish, unlike St. Bernard that serves the whole parish. Of the parishes with *N. fowleri*-positive water samples, Ouachita Parish had the greatest number, with eight positive samples (two raw water samples and six distribution system samples). Five positive samples were from St. Bernard Parish, including four distribution system samples and one raw water sample. In Terrebonne Parish, three positive samples were two from distribution systems and one raw water sample. Two positive samples were also in Sabine Parish – one of these was raw water samples, while the other was from a

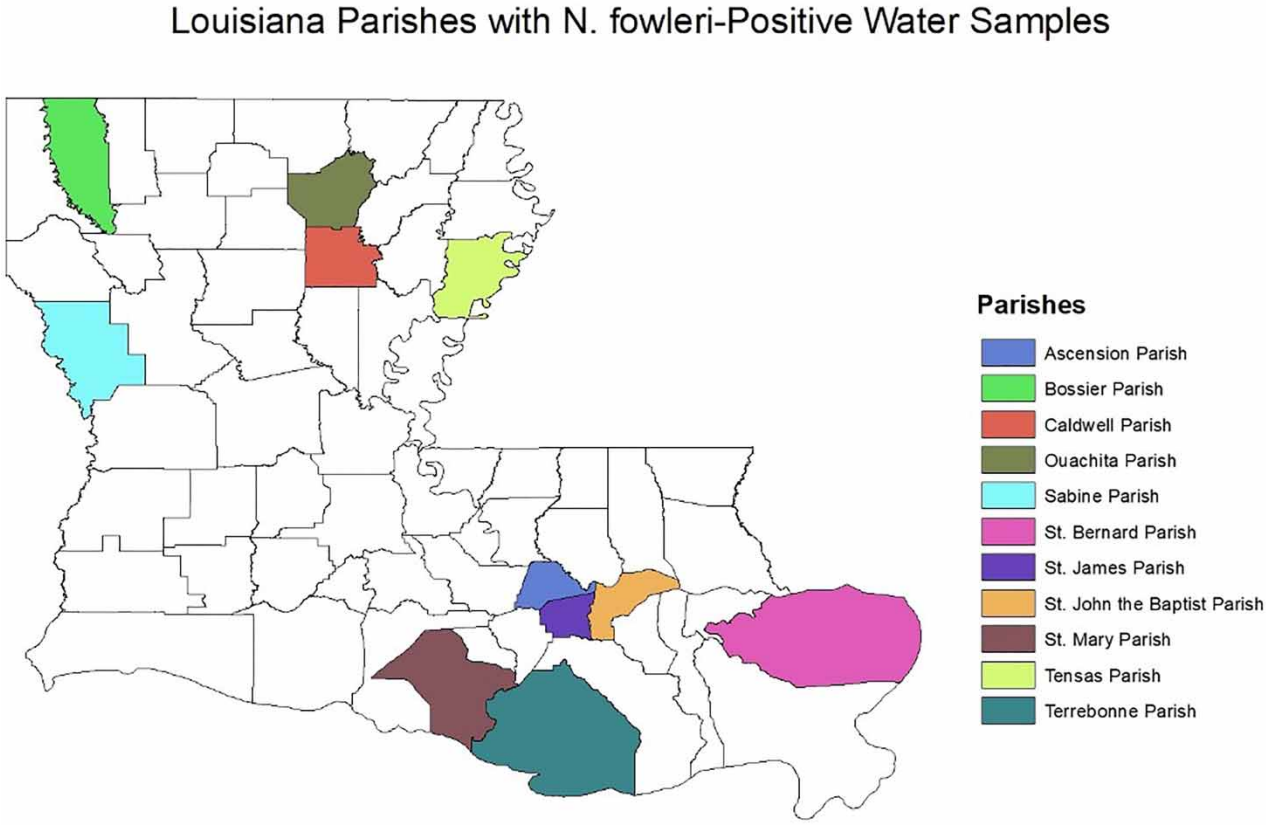


Figure 1 | Louisiana parishes in which water tested positive for the presence of *N. fowleri.*

distribution system. Ascension Parish had two positive samples, including one raw water sample and one distribution system sample, while Bossier Parish also had two positive samples, both from distribution systems. The remaining five parishes each had one positive sample – from a distribution system in St. John Parish and from raw water in St. Mary, Tensas, Caldwell, and St. James parishes. Of all positive samples, 10 raw source water samples tested positive for the presence of *N. fowleri* and 17 distribution system samples tested positive (Table 1).

Deaths from PAM contracted through drinking water distribution systems have occurred in many countries, including Australia, the United States, and Pakistan (Miller *et al.* 2018). Generally, drinking water sources are treated with chlorine, and in the case of Australia, the target concentration was 0.5 mg/L, which was constantly maintained throughout the entire system and appears to control the growth of *N. fowleri* (Morgan *et al.* 2016). However, a study found that *N. fowleri* (presumably as cysts) can survive short durations of higher concentrations of chlorine, even up to 20 mg/L (Miller *et al.* 2015). In Louisiana, following the St. Bernard 2013 PAM infection, LDH issued a new rule requiring public water systems to maintain a minimum of 0.5 mg/L of free or total chlorine throughout the entire drinking water system constantly and must be monitored regularly. However, seven out of 27 water systems tested in Louisiana were found to contain *N. fowleri* mostly at locations with low (0.5 mg/L) to no chlorine (LDHH 2017).

N. fowleri has a three-stage lifecycle. *N. fowleri* exists in soil in a cystic form and excysts to a flagellate stage when in contact with warm water and a source of food (bacteria). The trophozoite stage feeds on bacteria at the air– or biofilm–water interface in hot springs, surface waters, and other water systems that may remain still for prolonged periods (Mull *et al.* 2013; Cope & Ali 2016; Lu *et al.* 2016). To date, a total of 145 positive cases of PAM caused by *N. fowleri* have been reported in the United States (CDC 2019; Gharpure *et al.* 2021). The majority of *N. fowleri* cases (over >50%) in the United States have occurred in Texas and Florida from 1962 to 2008 (Yoder *et al.* 2010). However, recent cases have also occurred in north-tier states like Minnesota (2012), Virginia, Kansas, and North Carolina (2016) (Gharpure *et al.* 2021). Moreover, as changes in the global climate result in warmer surface temperatures, infections by *N. fowleri* are expected to increase (Gharpure *et al.* 2021).

Table 1 | Collected samples positive for the presence of *N. fowleri*

Field sample description	Matrix type	Collection type	Date of collection	*Amoebae culture**	*N. fowleri* PCR*
Raw water – Little Flock	Raw source	Ultrafilter	**8/25/2014**	P	P
Raw	Raw source	Ultrafilter	**6/24/2015**	P	P
Raw	Raw source	Ultrafilter	**8/20/2015**	P	P
Raw – Franklin	Raw source	Ultrafilter	**8/31/2016**	P	P
Raw	Raw source	Ultrafilter	**9/21/2016**	P	P
Raw	Raw source	Ultrafilter	**6/20/2017**	P	P
Raw-001	Raw source	Ultrafilter	**7/3/2017**	P	P
Raw POE002	Raw source	Ultrafilter	**7/18/2017**	P	P
RAW-PUA	Raw source	Ultrafilter	**8/8/2017**	P	P
Raw	Raw source	Ultrafilter	**8/22/2018**	P	P
POE-015	Distribution system water	Ultrafilter	**7/3/2017**	P	P
948 Angela St-AME001	Distribution system water	Ultrafilter	**7/24/2015**	P	P
AME-002	Distribution system water	Ultrafilter	**8/5/2015**	P	P
TCR-003	Distribution system water	Ultrafilter	**7/24/2015**	P	P
TCR-010	Distribution system water	Ultrafilter	**7/24/2015**	P	P
TCR-002	Distribution system water	Ultrafilter	**6/20/2017**	P	P
TCR-074 hwy 165 Century Link	Distribution system water	Ultrafilter	**7/3/2017**	P	P
TCR-045 Oauchita Parish High School	Distribution system water	Ultrafilter	**7/3/2017**	P	P
TCR-002	Distribution system water	Ultrafilter	**9/19/2018**	P	P
TCR-088	Distribution system water	Ultrafilter	**10/2/2018**	P	P
ACR011	Distribution system water	Ultrafilter	**8/25/2014**	P	P
ACR-005	Distribution system water	Ultrafilter	**6/24/2015**	P	P
ACR-182	Distribution system water	Ultrafilter	**6/20/2017**	P	P
MRT	Distribution system water	Ultrafilter	**8/12/2014**	P	P
MRT-009	Distribution system water	Ultrafilter	**7/14/2015**	P	P
MRT-034	Distribution system water	Ultrafilter	**6/20/2017**	P	P
MRT-034	Distribution system water	Ultrafilter	**8/20/2015**	P	P

*P, positive.

Further studies would generate a robust dataset on the impact of changing climate on the proliferation of *N. fowleri* in natural and engineered water systems.

Our research demonstrates that *N. fowleri* was detected in 27 samples out of 93 (29%) during the period 2014–2018. More studies are needed to find a better indicator for *N. fowleri.* Since 10 raw source water and 17 distribution system water samples tested positive, it is important to manage PAM risk by monitoring residual chlorine at the end of distribution system lines, as required by the LDH.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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