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Quantitative assessment of *Naegleria fowleri* and fecal indicator bacteria in brackish water of Lake Pontchartrain, Louisiana

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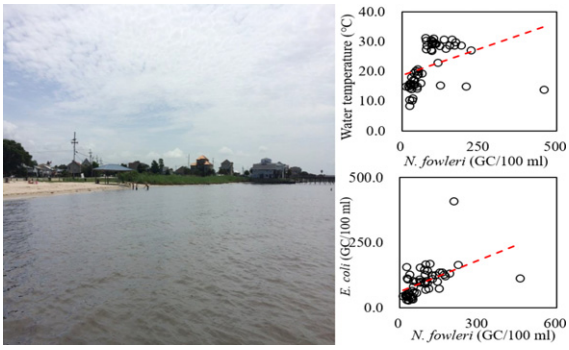
HIGHLIGHTS

- Quantitative PCR assays were conducted to quantify *N. fowleri*, *E. coli* and enterococci.
- Significant positive relationship between *E. coli* and enterococci results was observed.
- *N. fowleri* was widespread at all sampling sites during our study period.
- Water temperature and *E. coli* concentration (qPCR) were indicative of *N. fowleri* concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Brackish water samples from Lake Pontchartrain in Louisiana were assessed for the presence of pathogenic amoeba *Naegleria fowleri*, which causes primary amoebic meningoencephalitis (PAM). In our study, quantitative polymerase chain reaction (qPCR) methods were used to determine *N. fowleri*, *E. coli*, and enterococci in water collected from Lake Pontchartrain. *N. fowleri* target sequence was detected in 35.4% (56/158) of the water samples from ten sites around the lake. Statistically significant positive correlations between *N. fowleri* concentration and water temperature as well as *E. coli* (qPCR) were observed. Multiple linear regression (MLR) model shows seasonal factor (summer or winter) has significant effect on the concentration of *N. fowleri*, *E. coli* and enterococci (qPCR) concentration. Significant positive relationships between *E. coli* and enterococci was observed from both qPCR ($r = 0.25$) and culture based method ($r = 0.54$). Meanwhile, significant positive correlation between qPCR and culture based methods for enterococci concentration was observed ($r = 0.33$). In our study, water temperature and *E. coli* concentration were indicative of *N. fowleri* concentrations in brackish water environment. Future research is needed to determine whether sediment is a source of *N. fowleri* found in the water column.

1. Introduction

Naegleria fowleri is a free-living, single-cell amoeba which is the causative agent of the primary amoebic meningoencephalitis (PAM), a rare and typically fatal disease (over a 97% case fatality rate) in young adults following exposure to contaminated fresh water (Carter, 1972; Marciano-Cabral, 1988; Schuster and Visvesvara, 2004; Visvesvara et al., 2007). Infection occurs when *N. fowleri*, in its trophozoite stage, entering the central nervous system via the nose to olfactory neurophilia resulting in hemorrhaging, strong inflammation, vomiting, seizures, and eventually death due to swelling of the brain

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(Marciano-Cabral and Cline, 1987; Siddiqui et al., 2016; Visvesvara et al., 2007). The onset of symptoms occurs between 5 and 7 days after exposure to the polluted water, with death following an average of 6.4 days later (Bright and Gerba, 2017; Linam et al., 2015). Previous studies have shown the presence of *N. fowleri* in soil, tap water, swimming pools, environmental water, thermal effluents, and natural hot springs worldwide (Ahmad et al., 2010; Bonilla-Lemus et al., 2014; Di Filippo et al., 2015; Edagawa et al., 2009; Gianinazzi et al., 2009; Jamerson et al., 2009; Niyyati et al., 2012; Painter et al., 2013; Winck et al., 2011). Even though PAM is a rare disease, there has been 143 cases reported in 19 states of America from 1962 to 2016 (https://www.cdc.gov/parasites/naegleria/). In 2003, *N. fowleri* was detected in the residual water pipes and sinks that resulted in the death of two children in Arizona (Marciano-Cabral et al., 2003), and most recently, two deaths in Louisiana associated with public drinking water systems and *N. fowleri* infection raised the question of why this amoeba resides in some water sources and not in others. Additionally, the role of environmental factors, such as temperature and water physicochemical parameters, to expand the occurrence of the organism are still unclear. (Yoder et al., 2012).

The laboratory identification of PAM is usually based on the demonstration of *N. fowleri* trophozoites in cerebrospinal fluid, microscopic examination of biopsy or autopsy specimens, or culture methods (Siddiqui et al., 2016). However, it is often misdiagnosed due to the lack of expertise among laboratories in recognizing these pathogens morphologically (McCool et al., 1983; Murakawa et al., 1995). Researchers in Japan have also observed 50% of *Naegleria* amoebae failed to form flagellates (Edagawa et al., 2009). In such cases, *N. fowleri* could be misdiagnosed when researchers use enflagellation experiment to differentiate from other pathogenic amoebae. Increasing interest is now directed toward the use of cultivation-independent methods based on polymerase chain reaction (PCR) assays that target *N. fowleri* due to its sensitive, rapid, and precise identification in clinical samples and natural environmental samples (Kilvington and Beeching, 1995; Marciano-Cabral et al., 2003; Pélandakis et al., 2000, p. 8; Qvarnstrom et al., 2006; Réveiller et al., 2002).

Environmental studies have shown that human activities as well as climatic factors may contribute to the distribution of *N. fowleri*. For example, *N. fowleri* shows seasonal variations and could be influenced by water quality and microbial parameters (Niyyati et al., 2012; Siddiqui et al., 2016). Thermal pollution from industrial plants and cooling systems of nuclear power generating plant facilitates the growth of thermophilic *Naegleria* (Jamerson et al., 2009; Tyndall et al., 1989). Many of the studies have reported that *Naegleria* grow better in the presence of *E. coli* over several other bacterial species (Ahmad et al., 2010; Jamerson et al., 2009; Painter et al., 2013). However, very few studies have considered a possible link between populations of *N. fowleri* and *E. coli* due to variable physical parameters in environmental waters. Brown et al. (1983) reported that the pathogenic free-living amoeba (FLA) were isolated from those thermal pools with a high coliform count. Research conducted by Hsu et al. (2009) showed *Naegleria*-positive samples associated with higher total coliform contents. Given the paucity of research in this area, this study investigated the relationship between *N. fowleri* and *E. coli* and enterococci in a brackish water environment.

Lake Pontchartrain is an inland bay located in the southeast portion of Louisiana and its southern shore abuts the city of New Orleans. It is part of a large and productive estuarine system that supports agriculture and aquiculture, a vital shipping route, and one of the most important fisheries in the United States. The lake provides essential habitat for countless species of fish, birds, mammals, reptiles, and plants, as well as generating a multi-million-dollar fishing, tourism, sailing, and recreation industry. Throughout much of the 20th Century, the lake experienced mass environmental degradation from urban and agricultural runoff, shellfish dredging, overfishing, artificial saltwater and freshwater inputs, shoreline alteration, and industrial discharges (Hou et al., 2006). These disturbances led to increased microbial loading and decreased water quality in the lake (Jin et al., 2004).

The aim of this study was to investigate the distribution of *N. fowleri*, *E. coli*, and enterococci in Lake Pontchartrain; to determine the seasonal differences of *N. fowleri*; and to determine if its presence is dependent upon specific environmental conditions. Since residents of Southeast Louisiana use the lake extensively, it is important to identify areas of concern, if any, for human health. To the best of our knowledge, there is no current information on the occurrence of *N. fowleri* in Lake Pontchartrain, and this thermophilic amoeba is an emerging public health concern given *N. fowleri* infections occurred in Louisiana recently (Cope et al., 2015; Yoder et al., 2012). The significant findings of this study will lead to a better understanding of the ecology of *N. fowleri* in brackish water environments as well as further elucidation of its potential risk to human health.

2. Materials and methods

2.1. Sampling sites information

A total of 160 surface water samples were collected from ten sites along Lake Pontchartrain from May 2016 to February 2017 (Fig. 1). These ten sites were selected for weekly monitoring by the Lake Pontchartrain Basin Foundation (LPBF) due to a history of being recreational sites. GPS coordinates were used to ensure the same locations were sampled each time. Lake Pontchartrain covers 630 mile^2, services 1.5 million people, and spans across six parishes: St. Tammany, Orleans, Jefferson, St. John the Baptist, St. Charles, and Tangipahoa (https://www.epa.gov/urbanwaterspartners/lake-pontchartrain-areanew-orleans-louisiana).

2.2. Water sample collection

Surface water samples were collected from each sampling site with sterile one-liter polypropylene bottles and kept on ice immediately. Water samples were transported to the laboratory within 2 h for analysis. Physical and chemical water quality parameters, such as pH, temperature, dissolved oxygen, salinity, and specific conductance were measured in situ by using YSI Pro2030 Meter.

2.3. Quantification of fecal indicator bacteria (FIB)

Microbial analyses were performed for *E. coli* and enterococci immediately upon returning to the laboratory via the IDEXX Colilert (Kinzelman et al., 2005) and Enterolert (Ferguson et al., 2013) Defined Substrate Technology® tests, respectively (www.idexx.com, IDEXX Laboratories, Westbrook, ME, USA). Briefly, 100 ml of water sample or 1:10 dilution made with sterile deionized water was each mixed with reagent and placed in Quantitray/2000 then sealed using Quanti-Tray Sealer according to the manufacturer's instructions. After incubation, the wells that fluoresced under Ultraviolet (UV) light were quantified as positive for *E. coli* (IDEXX Colilert) and enterococci (IDEXX Enterolert). The number of positive wells were compared to the manufacturer-provided MPN table to enumerate *E. coli* and enterococci in terms of MPN/100 ml.

2.4. DNA extraction from water samples

On the day of sample arrival, 1000 ml of each sample was filtered through a 0.45 μm-pore-size, 90-mm-diameter nitrocellulose membranes (MF™ Millipore Membrane Filters, Merck KGaA, Darmstadt, Germany). After filtration, sterile forceps were used to aseptically fold each of the membrane filters and placed in separate sterile Petri Dish plate and stored at −20 °C until DNA extraction. Genomic DNA was isolated from membrane filters using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's