Washington County, Minnesota Lake Surveillance for *Naegleria fowleri* from 2011-2014

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ABSTRACT

The presence of the thermophilic free-living ameba, *Naegleria fowleri*, was investigated in lakes in Washington County, Minnesota, including one lake that was associated with the deaths of two children (in 2010 and 2012) from primary amebic meningoencephalitis (PAM). Samples of lake water and sediment were collected annually from 10 lakes in summer months from 2011 to 2014. In addition to culture and PCR analyses for *N. fowleri*, samples were analyzed for a suite of physical, chemical and biological parameters potentially related to *N. fowleri* presence and growth. Environmental parameters such as rainfall and air temperature were also investigated as potential factors related to *N. fowleri* detection.

N. fowleri was detected in 5 of 10 lakes in 2011 and in one lake in 2012, but was not detected in 2013 or 2014. *N. fowleri* was detected in the same lake (in 2011 and 2012) that was associated with the two cases of PAM. Average air temperatures prior to sample collection were higher in 2011 and 2012 than in 2013 and 2014, suggesting that the hot weather in these summer periods might have been associated with the growth and detection of *N. fowleri*. The amount of precipitation within 13 days of sample collection was positively associated with detection of *N. fowleri*. The results of this study provide environmental and water quality data that will help increase understanding of *N. fowleri* ecology and potential environmental and water quality factors that may be associated with the detection of this pathogen in recreational water bodies.

METHODS

- The study lakes were distributed throughout Washington County, MN and all had a recreational bathing area (Figure 1). The lakes varied in size, depth, trophic index, and soil class (**Table 1**)
- In July and August each year (2011-2014), Washington County Public Health & Environment staff collected a total of 1-L water and sediment samples at each lake (in 4, 250-mL composites)
- Water samples were analyzed for total coliforms and *E. coli* using Colilert in IDEXX Quanti-Tray/2000 format. Heterotrophic plate count bacteria were cultured on membrane filters and R2A agar (Standard Methods). Hach equipment & reagents were used for other water quality parameters.
- Water and sediment samples were tested per Mull et al. (2013)
- Thermophilic ameba cultures were assays for *N. fowleri* by real-time PCR per Mull et al. (2013).

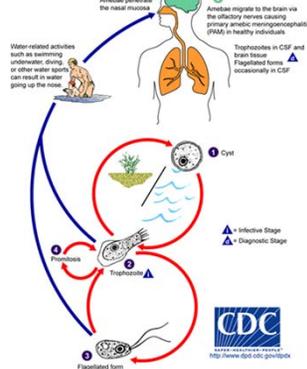## RESULTS

Life Cycle of *Naegleria fowleri*

Figure 1. Summer 2015 photo of Lily Lake, associated with deaths of two children in 2010 and 2012 from primary amebic meningoencephalitis (PAM) after swimming

Table 1. Characteristics of lakes sampled for presence of *Naegleria fowleri*, 2011-2014

Sample site	Surface area (acres)	Maximum Depth (feet)	Littoral Proportion	Trophic Index	Soil Class
Big Carnelian	457	66	28.7 %	Mesotrophic	Udifluvent
Big Marine	1799	60	64.0 %	Mesotrophic	Udifluvent
Bone	221	30	56.1 %	Eutrophic	Udifluvent
Demontreville	157	24	82.2 %	Mesotrophic	Bluffton
Elmo	281	140	16.0 %	Mesotrophic	Rosholt
Forest	2271	37	67.4 %	Eutrophic	Udifluvent
Goose	74	25	55.4 %	Eutrophic	Demontreville
Lily	43	51	46.5 %	Eutrophic	Udorthents
Little Carnelian	155	68	Unk	Oligotrophic	Mahtomedi
Square	203	68	32.0 %	Oligotrophic	Udifluvent

Provided by: Minnesota Department of Natural Resources "LakeFinder"

Table 2. All *N. fowleri* detections in Washington County lakes by Year

Sample site	Sample type	2011	2012	2013	2014
Big Carnelian	Sediment	+	-	-	-
	Water	+	-	-	-
Big Marine	Sediment	-	-	-	-
	Water	-	-	-	-
Bone	Sediment	-	-	-	-
	Water	-	-	-	-
Demontreville	Sediment	+	-	-	-
	Water	-	-	-	-
Elmo	Sediment	-	-	-	-
	Water	+	-	-	-
Forest	Sediment	-	-	-	-
	Water	-	-	-	-
Goose	Sediment	-	-	-	-
	Water	-	-	-	-
Lily	Sediment	+	+	-	-
	Water	-	-	-	-
Little Carnelian	Sediment	+	-	-	-
	Water	-	-	-	-
Square	Sediment	-	-	-	-
	Water	-	-	-	-

Table 3. Logistic regression analysis for association between water quality parameters and detection rates of *N. fowleri*

Water quality parameter (units)	Odds ratio (95% CI)	P value
Temperature (°C)	1.13 (0.77-1.64)	0.53
Dissolved O₂ (mg/L)	1.00 (0.55-1.85)	0.98
Turbidity (NTU)	0.81 (0.61-1.07)	0.14
Specific conductance (µS/cm)	1.00 (0.99-1.01)	0.43
Total Organic Carbon (mg/L)	0.93 (0.80-1.08)	0.34
Nitrogen (mg/L)	0.93 (0.58-1.50)	0.76
Phosphorus (mg/L)	0.14 (0.01-3.28)	0.22
Total coliforms (Log MPN/100 mL)	0.16 (0.03-0.95)	0.04
E. coli (Log MPN/100 mL)	0.51 (0.14-1.83)	0.29
HPC (Log CFU/1 mL)	0.27 (0.09-0.82)	0.02

Table 4. Association between precipitation and air temperature for the 12 days prior to sample collection and detection rates of *N. fowleri*

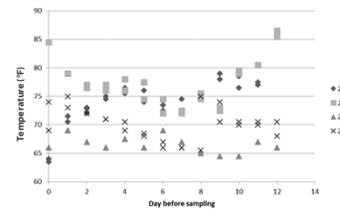| Water quality parameter (units)	Odds ratio (95% CI)	P value
Precipitation (inches)	1.85 (1.04-3.29)	0.04
Air temperature- Maximum (°C)	1.03 (0.98-1.07)	0.22
Air temperature- Minimum (°C)	1.18 (1.12-1.24)	<0.0001

Figure 2. Average air temperature at Washington County, MN study sites within 12 days of sample collection

CONCLUSIONS

- The one lake to yield detections of *N. fowleri* in 2011 and 2012 was Lily Lake (**Figure 1, Table 2**). *N. fowleri* was also detected in water and sediment samples collected from Lily Lake in 2010 during the investigation of a PAM infection related to swimming in Lily Lake (Kemble 2012).
- The only water quality parameters to be significantly associated with *N. fowleri* detection were total coliforms and HPC bacteria, but the associations were unexpected—lower concentrations of these bacteria were associated with *N. fowleri* detections (**Table 3**). It is not clear why this was observed.
- Precipitation and average air temperature in the 12 days before sampling were significantly associated with *N. fowleri* detection (**Table 4**). Warmer air and water temperatures are a well established factor for detection of *N. fowleri* and occurrence of PAM cases (Yoder et al, 2010).
- Average overall air temperatures for July 2011 and July 2012 were 5 and 7 °F higher than in 2013 and 2014, respectively (**Figure 2**). Air temperatures were reported as being elevated in the days before the 2010 case patient swam in Lily lake and precipitation was reported the day before the child's second swimming exposure at the lake (Kemble 2012).

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