Q-1449

Identification of Amoebic Activity and *Naegleria fowleri* in Arizona Drinking Water Systems

Hodon Ryu[1], Albert Brown[2], Precious Biyela[3], Absar Alum[1], Bruce Rittmann[3], and Morteza Abbaszadegan[1]

National Science Foundation Water & Environmental Technology Center, Civil, Environmental & Sustainable Engineering[1], Environmental Technology Management[2], Center for Environmental Biotechnology, Biodesign Institute[3], Arizona State University, Tempe, AZ

Contact Information:
Hodon Ryu: ryu.hodon@epa.gov
Morteza Abbaszadegan: abbaszadegan@asu.edu

Abstract

Recent *Naegleria fowleri* outbreaks reported from Arizona were closely related to groundwater. In this study, we attempted to identify amoebic activity in water and biofilms in full-scale groundwater sourced drinking water distribution systems and to investigate the fate of *N. fowleri* in a laboratory-scale pipe-loop system. A total of 8 10-L water and 20 biofilm samples were collected from sites across two distribution systems: one carrying chlorine-treated water and the other carrying unchlorinated water. The sampling sites were chosen to represent variations in water chemistry, retention time, and biofilm-formation potential. Laboratory pilot systems consisting of a loop of PVC and cast-iron piping (35 gallon of water for each system) were spiked with a 3.5 X 107 TCID50 of live *N. fowleri* and sampled on a weekly basis for 5 months. A total of 40 50-mL water and 4 biofilm samples were analyzed for the presence or absence of *N. fowleri*. Amoebic activity was detected in 2 of the 8 (25%) water samples and 14 of 20 (70%) biofilm samples. The relatively high fraction of biofilm samples showing amoebic activity suggests that distribution system biofilms are important reservoirs for protozoan pathogens. Amoebic activity was more prevalent in samples obtained from sites where water stagnation occurs and that had lower concentrations of residual chlorine and higher concentrations of heterotrophic bacteria. Almost one-third (31%) of the sampling locations that originally had amoebic activity showed no such activity after the best operational practices such as flushing. In both pipe loops, *N. fowleri* were observed in water and biofilm samples for entire duration of the experiment, demonstrating that the biofilm habitat in the pipe loops was conducive to *N. fowleri* survival. Further study needs to be focused on the factors leading to biofilm accumulation and *N. fowleri* accumulation. Despite these findings, more comprehensive evaluations are necessary on how to improve water quality, disinfection, and distribution-system design and operation to minimize biofilm reservoirs in distribution systems.

Background & Rationale

Naegleria fowleri outbreaks

In the decade between 1998 and 2007, 33 *N. fowleri*-caused infections were reported across the US. Of these, 31 victims had contact with recreational water, and 2 victims had contact with water from a geothermal water source (CDC, public factsheet 2008). During the summer of 2007, 6 cases were reported in Arizona, Texas, and Florida.

Research motivation

The reasons why *N. fowleri* is present in certain waters are not well understood. We focus on drinking water as a possible source of *N. fowleri* infection, since 2 of the cases of infection in Arizona were associated with an inadequately managed drinking-water supply. We address the following questions.

- How can drinking water be a source of *N. fowleri*?
- What are the reservoirs for *N. fowleri* in a drinking-water system?
- How can those reservoirs be eliminated?

Research hypotheses

- Biofilms in drinking water systems provide a substrate for growth and survival of *N. fowleri*
- Factors that reduce the accumulation, survival and spread of *N. fowleri* include:
  - Chemical and biological factors
  - Plumbing construction materials
  - Operational practices

Research approach

- Test for presence of *N. fowleri* in samples from drinking water systems before and after an operational practice
- Test for survival and growth of *N. fowleri* on pilot-scale pipe loop biofilm accumulation
- Evaluation of selected chemical and biological parameters as indicators of *N. fowleri*

Materials & Methods

Pilot-scale Simulator: Cast Iron & PVC

- *N. fowleri* spiked : 3.5x10^7 ^{1}TCID$_{50}$
- Estimated number of *N. fowleri* in flowing water: 10^3 TCID$_{50}$/mL

Weekly water sampling from both pipes until no amoebic activity is observed (natural die-off, >4.5 log$_{10}$)

Biofilms from both pipes were assayed for amoebic activity at the end of simulator run

^{1} Tissue Culture Infective Dose per ml

Sampling

5-month old biofilm samples from newly installed pipe segments were collected and analyzed for amoebic activity.

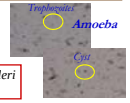
Processing of water and biofilm samples

Samples were were processed within 1 week of sample collection. Approximately 10 liters of the collected water were passed through a 1-µm-pore-size polypropylene disk filter (Cuno, Incorporated, Meriden, CT), and the filter was eluted in steril Page's amoeba saline. Biofilm samples were concentrated using centrifugation at 5,000 x g for 10 min and resuspended in steril Page's amoeba saline. All samples were dispensed into individual 75-cm2 tissue culture flasks in 10-ml volumes in duplicate and incubated either at 44 or 37°C.

N. fowleri propagation

N. fowleri (ATCC 30894) was maintained with regular subculturing (every 4-5 days) in Cline medium which is based on Balamuth medium and Nelson medium supplemented with hemin and calf serum (Cline et al., 1983; Marciano-Cabral and Fulford, 1986).

Viability assay for amoebic activity (Marciano-Cabral et al., 2003)



Nested PCR for confirmation of *N. fowleri* (Reveiller et al., 2002)

Full-scale Water System Selection

- *Chlorinated groundwater source (pop. 145,000): City of Peoria*
- *Unchlorinated groundwater source (pop. 100): U of A Maricopa Agricultural Center*

Hydrant Flush Operational Practice

Field Sampling

A total of 28 (8 40-L water and 20 biofilm) samples were collected and processed.

Results & Discussion

Pilot-scale Simulator: Cast Iron & PVC

- **Amoebic activity was observed in both water and biofilm samples after 5-month simulator run.**
- ***N. fowleri* was observed in biofilm after 5 months**
- **This supports the hypothesis that biofilm can sustain *N. fowleri***

Physicochemcial parameters

Cast Iron	PVC
DOC lower over time	*DOC lower over time*
BDOC lower over time	*BDOC lower over time*
HPC lower	*HPC higher*
NO3 higher	*NO3 lower*
Biofilm thickness greater/uneven	*Biofilm thickness less/even*
Biofilm bacteria lower/unit area	*Biofilm bacteria higher/unit area*

Field sampling results

- **All samples were analyzed for amoebic activities using a viability assay.**
- **Amoebic activity was observed in two of 8 water samples and 14 of 20 biofilm samples.**
- **31% of the sampling locations that originally had amoebic activity showed no such activity after the best operational practices such as flushing**
- **For confirmation, DNA has been extracted from the positive samples and stored at -80 C for a molecular analysis. Preliminary results are inconclusive.**

Physicochemcial parameters

HPC
- ✓ Higher in unchlorinated system
- ✓ Higher with residence time/stagnation
- ✓ Lower after flushing

Cl residual
- ✓ Lower with residence time/stagnation

BDOC
- ✓ Lower in unchlorinated system

DOC
- ✓ Lower in unchlorinated system
- ✓ Lower with more residence time/stagnation
- ✓ Operational practices had insignificant effect

NO_3
- ✓ Lower in unchlorinated system
- ✓ Lower after flushing in both systems

Summary

- *N. fowleri* colonized biofilms in the laboratory setting.
- Amoebic activity was present in 70% and 25% of biofilm and water samples from water distribution systems, respectively.
- Amoebic activity increases with:
  - Lack of chlorine, higher HPC, Stagnation/lack of flushing, and Low DOC & BDOC

References

Cline, M., F. Marciano-Cabral, and S. G. Bradley. 1983. Comparison of *Naegleria fowleri* and *Naegleria giruibeni* cultivated in the same nutrient medium. J. Protozool. 30:387-391.

Marciano-Cabral, F., and D. E. Fulford. 1986. Cytopathology of pathogenic and nonpathogenic *Naegleria* species for cultured rat neuroblastoma cells. Appl. Environ. Microbiol. 51: 1133-1137.

Marciano-Cabral, F., R. MacLean, A. Mensah, and L. LaPat-Polasko. 2003. Identification of *Naegleria fowleri* in domestic water sources by nested PCR. Appl. Environ. Microbiol. 69:5864-5869.

Reveiller, F. L., P. A. Cabanes, and F. Marciano-Cabral. 2002. Development of a nested PCR assay to detect the pathogenic free-living amoeba Naegleria fowleri. Parasitol. Res. 88:443-450.

Acknowledgments

This research was supported by Arizona Water Institute and National Science Foundation Water Environmental Technology Center at Arizona State University. The authors acknowledge the help of personnel at the city of Peoria and Maricopa Agricultural Center for water sampling. We specially thank the late Mr. Daniel Kennedy for arranging field work and sample collection.