# New Information on Waterborne *Cryptosporidium* and the Role of Biofilms

By Kelly A. Reynolds, MSPH, Ph.D.

Despite the multitude of improvements in source-water protection and drinking water treatment, waterborne outbreaks are still documented every year in the United States. Further endemic waterborne disease is estimated at more than 19.5 million cases per year in this country.[1] Distribution systems continue to be implemented in outbreaks and may be a significant source of the widespread incidence of waterborne illness in communities. Biofilms are thought to play an integral role in the survival and spread of disease-causing microbes in finished water supplies. New research supports this theory and provides valuable insights into the *Cryptosporidium*-biofilm interactions in drinking water.

## Distribution systems and historical waterborne disease outbreaks

Since 1971, the Centers for Disease Control and Prevention (CDC), the US EPA and the Council of State and Territorial Epidemiologists (CSTE) have maintained the Waterborne Disease Surveillance System, a database of reported drinking water outbreaks and their causes. Information on related sources, etiological agent, water system deficiencies, persons involved, illnesses associated, and other trends, is collected and reported by health departments in US states, territories and localities. In order to be included in the database, reported outbreaks must be definitively linked to water as the source of illness in a human population. The drinking water distribution system includes pumping, piping and storage facilities used to deliver finished water to consumers. Over a million miles of water distribution networks are currently in use around the US. During the last 36 years of collected survey data, distribution system deficiencies have been continual sources of outbreaks. Overall, distribution system deficiencies caused 10 percent of the 833 documented outbreaks, ranging as high as 31 percent for single years.[2] Lack of decrease in the relative proportion of distribution system-related outbreaks suggests the need for increased action for managing this source of drinking water contamination.

## Sources of distribution system contamination

During the latest published surveillance period, two drinking water-related outbreaks involved distribution system deficiencies. The first resulted after the installation of a new water main in Indiana. Following a pressure test, the main was left under pressure with non-potable water, resulting in a cross-contamination event with the drinking water supply. In the second outbreak, backflow prevention devices were absent on water distribution lines to toilet facilities in a camp in Maryland.[3] Transient low-pressure events in the distribution network provide an opportunity for contaminants to enter pipes through leaks.[4] During power outages, 90 percent of nodes have been shown to draw a negative pressure.[5] Approximately a quarter of the distribution pipelines in the US are considered to be in poor condition. Main breaks are common, and water losses during distribution are commonly higher than 10 percent, with some systems reporting losses of up to 32 percent of the finished water supply during distribution.[1] Distribution system hazards associated with cross connections, backflow events, storage integrity, main construction and repair, pressure and intrusion factors, and biofilm formation are identified as priority research issues by the US EPA. Recent studies suggest that contamination of the distribution system significantly contributes to endemic illnesses in the population.[6] Biofilm formation is common in distribution pipelines and is known to aid in the accumulation of disease-causing microbes.

## Biofilms and pathogens

Biofilms are a complex mixture of microbes and matter. They are a natural component of aquatic environments and frequently associated with adverse taste and odor, but are not necessarily harmful. Harmful microbes, however, including human infectious viruses and *Cryptosporidium*, can become trapped in natural biofilms where they persist and are protected from residual disinfectants. The source of these contaminants may be from intrusion events from microbes damaged during conventional water treatment that collect in biofilms, recover, repair themselves and regrow in the system. While human viruses and *Cryptosporidium* are present in water due to fecal contamination events or incomplete treatment, other biofilm-associated pathogens are indigenous to water environments, including *Legionella* (Legionnaire's disease), *Helicobacter pylori* (primary cause of stomach ulcers), *Mycobacterium* (causing life-threatening infections in AIDS patients), *Naegleria* (free-living amoeba causing rare but fatal infections via recreational water exposures), and various fungi. Biofilms continue to grow and eventually slough off, either spreading to new locations or dispersing into the water column. This slow release of biofilm organisms may account for persistent, low-level water contamination events. Conversely, sheering forces related to water flow result in increased sloughing of biofilm from surfaces into the water supply. This condition may account for at least some of the many contamination events and outbreaks where contributing sources could not be identified.

## Cryptosporidium-biofilm interactions

*Cryptosporidium* is a protozoan parasite, perhaps best known for the 1993 waterborne outbreak in Milwaukee, WI, causing

403,000 illnesses, 4,400 hospitalizations and 50 deaths. Spread by the fecal-oral route in humans and commonly found in animal reservoirs (i.e., cattle and sheep), *Cryptosporidium* is frequently isolated from surface, and sometimes groundwater, sources. Due to their resistance to chlorine, alternative disinfectants and/or filtration treatments have been mandated in federal regulations aimed at reducing the risks of *Cryptosporidium* in drinking water. Although billions of oocysts are shed in the stool of infected persons or animals, less than 10 are capable of causing an infection. The organism produces an environmentally stable oocyst enabling its survival for months in surface waters. Association with biofilms aids in the accumulation of *Cryptosporidium* in the distribution system, where it's eventual release could represent concentrations high enough to cause infections in persons exposed. In a recent outbreak, *Cryptosporidium* was identified as the causative agent where the source water was contaminated. To manage the outbreak, the water supply was changed to eliminate the affected area. Risk managers expected the outbreak to subside immediately but instead, oocysts were detectable in the system for up to 19 days. Increased concentrations were associated with flushing operations, likely due to the stripping of biofilms embedded with *Cryptosporidium*.[7]

Very few scientific papers have been published evaluating the interaction of *Cryptosporidium* and biofilms.[8] In a controlled laboratory study, a pipeline biofilm constructed from tap water was inoculated with infectious viruses and parasites, including *Cryptosporidium*. Within one hour, the seeded organisms attached to the biofilm where the viruses and parasites remained viable for six and more than 34 days, respectively. Once flow velocity was increased in the pipe, the microbes were readily transferred from the surface to the water phase. This study suggested biofilms be considered a potential secondary source of water contamination.[9] More recently, researchers have documented seasonal differences with biofilm formation and the retention or release of *Cryptosporidium*. Biofilms isolated from a Pennsylvania stream in the spring retained the highest percentage of oocysts followed by the winter, summer and fall, in decreasing order.[10] This trend did not statistically correlate with physical parameters of the water (i.e., temperature, pH, etc.) but was replicated over a second season. These results suggest that the seasonal variation in the microbial communities that make up the biofilm affects the attachment and detachment potentials of the pathogen.

## Conclusion

Drinking water biofilms are complex communities that are only vaguely understood by researchers. An earlier *On Tap* article (November 2008) focused on the ability of biofilm bacteria to communicate on a cellular level via signaling molecules. These signals between bacterial communities control how and when biofilms are formed. Understanding such signaling mechanisms and the relationship to the attachment or detachment of pathogens could have important implications for the water industry and public health.

## References


1. Reynolds, K.A.; Mena, K.D. ; Gerba, C.P. Risk of waterborne illness via drinking water in the United States. *Reviews in Environmental Contamination and Toxicology*. 192:117-158.

2. Craun, G.F.; Brunkard, J.M.; Yoder, J.S. *et al.* (2010). Causes of outbreaks associated with drinking water in the United States from 1971-2006. *Clinical Microbiology Reviews*. 23:507-528.

3. CDC (2008). Surveillance for waterborne-disease and outbreaks associated with drinking water and water not intended for drinking—United States, 2005-2006. Centers for Disease Control and Prevention MMWR *57* (SS09):39-62.

4. LeChevallier, M.W.; Gullick, R.W.; Karim, M.R.; Friedman, M.; Funk, J.E. (2003). The potential for health risks from intrusion of contaminants into the distribution system from pressure transients. *Journal of Water and Health.* 1:3-14.

5. LeChevallier, M.W.; Gullick, R.W.; Karim, M. (2003). *The Potential for Health Risks from Intrusion of Contaminants into the Distribution System from Pressure Transients.* Distribution System White Paper. http://www.epa.gov/safewater/tcr/pdf/intrusion.pdf

6. Nygard, K.; Wahl, E.; Krogh, T. *et al.* 2007. Breaks and maintenance work in the water distribution systems and gastrointestinal illness: a cohort study. *International Journal of Epidemiology*. 36:873-880.

7. Howe, A.D.; Forster, S.; Morton, S. *et al.* 2002. *Cryptosporidium* oocysts in a water supply: Discussion. Emerging Infectious Diseases. 8: 619-624.

8. Angles, M.L.; Chandy, J.P.; Cox, P.T. *et al.* 2007. Implications of biofilm-associated waterborne *Cryptosporidium* oocysts for the water industry. *Trends in Parasitology*. 23:352-356.

9. Helmi, K.; Skraber, S.; Gantzer, C. *et al.* 2008. Interactions of *Cryptosporidium parvum, Giardia lamblia,* vaccinal poliovirus type 1 and bacteriophages ΦX174 and MS2 with a drinking water biofilm and a wastewater biofilm. *Applied and Environmental Microbiology*. 74: 2079-2088.

10. Wolyniak, E.A.; Hargreaves, B.R.; Jellison, K.L. Seasonal retention and release of *Cryptosporidium parvum* oocysts by environmental biofilms in the laboratory. *Applied Environmental Microbiology*. 76: 1021-1027.


## About the author


♦ *Dr. Kelly A. Reynolds is an Associate Professor at the University of Arizona College of Public Health. She holds a Master of Science Degree in public health (MSPH) from the University of South Florida and a doctorate in microbiology from the University of Arizona. Reynolds has been a member of the WC&P Technical Review Committee since 1997. She can be reached via email at reynolds@u.arizona.edu*