**Watershed Monitoring to Prevent the Transmission of Waterborne Cryptosporidiosis**

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*Cryptosporidium* is a protozoan parasite, transmitted via ingestion of fecally-contaminated food and water, responsible for a gastrointestinal disease that is generally self-limiting in otherwise healthy people but potentially fatal for immunocompromised individuals. Currently available medications for the treatment of cryptosporidiosis have not proven effective for immunocompromised patients. Furthermore, removal/inactivation of *Cryptosporidium* oocysts in water treatment plants is not guaranteed because the oocysts are small enough (4-8 μm) to pass through sand filters under suboptimal coagulation/flocculation regimes and are resistant to chlorine disinfection. Monitoring *Cryptosporidium* contamination in watersheds that serve as sources of municipal drinking water is necessary to identify public health risks and determine where limited budgets and resources should most effectively be targeted to protect consumers from waterborne exposure to pathogenic oocysts.

Environmental monitoring of waterborne pathogens can inform watershed management strategies which will have the largest impacts on public health. In a watershed monitoring study spanning May 2005-April 2008, we were able to show that oocysts were present in an urban watershed year-round, independent of wet weather events, in approximately 20% of water samples, 5% of wastewater treatment plant (WWTP) effluents, and 7% of fecal samples. Of the *Cryptosporidium* spp. genotypes detected, 67% were human-infectious, and similar genotype profiles were detected in the watershed each year. Phylogenetic analysis suggested that deer, geese, and WWTPs were sources of oocysts in the watershed and thus critical targets upon which to focus watershed protection strategies.

Water utilities using surface water (or ground water under the direct influence of surface water) are required by the U.S. Environmental Protection Agency (EPA) to monitor for *Cryptosporidium* in their water supplies under the Long Term 2 Enhanced Surface Water Treatment Rule (i.e., LT2). Systems were required to monitor their water sources monthly for two years (monitoring start dates began in October 2006 and were staggered by system size), and results of this monitoring determined what level of additional treatment or source water protection (if any) was required to reduce *Cryptosporidium* loads in the water supply. Systems are required to complete a second round of *Cryptosporidium* monitoring six years after completing the initial round to determine if source water conditions have changed significantly. The Philadelphia Water Department (PWD; Philadelphia, Pennsylvania, USA) will begin its second round of LT2 monitoring in spring 2015.

Current sample methodology for *Cryptosporidium* monitoring in water supplies (EPA Method 1623.1) relies on filtering and processing 10 L of water, providing a "snapshot" of river conditions at the time of filtration. Briefly, the method involves filtration, elution of filtered material from the filter capsule, immunomagnetic separation (IMS) of the oocysts from the eluted material, and an immunofluorescent assay (IFA) for quantification of oocyst concentrations based on microscopy counts. This method has been tested by various laboratories using various waters, filters, and IMS systems, and reported *Cryptosporidium* recoveries have been generally low but variable, ranging from 12-85% under different experimental conditions. It must be noted that, because oocysts are discrete particles in water, a sample deemed negative by Method 1623.1 does not mean that oocysts are not (or have not recently been) present in the

water supply, only that no oocysts were captured/detected in that 10-L water sample. Previous work by the PI, in collaboration with the PWD, has shown a lack of correlation in oocyst detection among replicate filters analyzed by the same assay at the same site on the same day. Given the high cost of the filters (in excess of $100 each), it is not reasonable to expect utilities to analyze multiple filters at a single sample location; thus, new sampling methodologies are warranted.

We have shown that oocyst contamination is detected as often with the use of substrates deployed in waterways as with filtration-based methods. Biofilms grow on solid substrates (e.g., glass slides) submerged in water, and we have been examining how environmental biofilms are able to adsorb waterborne contaminants at a rate consistent with traditional EPA methodologies. In the case of *Cryptosporidium*, oocysts traveling along water flow paths that intersect biofilm surfaces will attach to the biofilm; monitoring biofilms for oocyst contamination thus provides an integrated look at stream conditions over time. We have shown that biofilm sampling can identify oocyst contamination in water supplies at least as often as the conventional filtration-based approach, and in similar quantities per sample. Collaborating with the PWD, we monitored for *Cryptosporidium* in the city's water supply at two distinct stream locations (QL and MC, respectively) from July 2013 through August 2014 using both filtration-based and biofilm sampling protocols (water was filtered every two weeks; glass slides were deployed in the water for the entire two-week period between sample dates). *Cryptosporidium* were detected at QL in 32% and 36% of filter and biofilm samples, respectively, and at MC in 45% and 53% of filter and biofilm samples, respectively. Numbers of oocysts per sample were similar among filtered (0 to 3 oocysts at QL; 0 to 4 oocysts at MC) and biofilm (0 to 8 oocysts at QL; 0 to 3 oocysts at MC) samples as well. Furthermore, biofilm collection is significantly cheaper than filtration ($3 per set of six slides compared to $110 per filter) and could thus be performed more frequently, at more locations, and by more utilities to monitor for oocyst contamination in drinking water supplies.

While the potential for using naturally grown biofilms as capture materials is significant, the use of biofilms for standardized detection of *Cryptosporidium* oocysts in water supplies is contraindicated due to the natural variation in biofilm composition and development (based on season, geography, and other natural watershed differences). Therefore, we are currently working to engineer a substrate that will permit oocyst attachment similar to what we observe in environmental biofilms, and to deploy this substrate in surface waters to obtain an integrated view of water contamination over that time period. The engineered substrates have a few minimum requirements: they must be more cost effective than current methods, with a robust *Cryptosporidium* capture efficiency and sufficient resistance to biofilm growth as to not damage the sorptive capacity. Furthermore, they must have the ability to be stored and handled prior to deployment without special conditions or equipment.

An engineered substrate with these features will enable water utilities to sample water supplies more frequently, and at more locations, than is currently possible given limited operating budgets. Water sampling with a substrate designed to adsorb oocysts could replace or supplement filtration-based *Cryptosporidium* monitoring required by the EPA, and these manufactured substrates could be strategically placed along the length of a complex watershed to identify point sources of oocyst contamination requiring intervention. Sampling with the substrates could also enable local and state agencies to quickly identify contaminated watersheds and the associated utilities at risk for increased oocyst loads in the water supply.