United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract

Government Performance Results Act (GPRA) Goal # 2 - Clean Water
Annual Performance Measure # FY04

Significant Research Findings:

Detecting CCL-related, Emerging and Regulated Waterborne Human Protozoa

Scientific Problem and Policy Issues

Many outbreaks of disease linked to drinking water contaminated with *Cryptosporidium* have been described, including the Milwaukee outbreak in 1993. In most cases, infection with this pathogen causes self-limiting gastroenteritis of varying severity. However, in some individuals, especially the immuno-compromised, infection can sometimes lead to death. The Office of Water has set a goal of zero cases of cryptosporidiosis due to waterborne transmission. In an effort to reach this goal and establish appropriate drinking water regulations that protect public health, the Office of Water has conducted a number of data gathering efforts to determine the extent to which surface source water, and ground water under the direct influence of surface water, are contaminated by *Cryptosporidium* oocysts. Because the recovery efficiency of the methods used to detect and identify *Cryptosporidium* oocysts vary significantly from one water matrix to another, there is a need for the Office of Water to more accurately determine and/or estimate the actual number of oocysts present in samples on a routine basis. The present research provides an approach, using an internal standard in each sample, for the *Cryptosporidium* oocysts percent recovery to be estimated in each sample. This internal standard recovery value allows the true number of environmental oocysts present in a sample to be more accurately calculated from the number of recovered oocysts. Using this improved monitoring technique, the need for duplicate sample analysis is eliminated while the data quality is improved. Knowing the true *Cryptosporidium* oocyst level in source water should allow the Office of Water to regulate water utilities and protect public health more effectively.

Research Approach

In this study, the recovery efficiencies of *Cryptosporidium* oocysts by Method 1623, using both modified and conventional seeding procedures, were compared using 30 stream water samples collected at 20 sites from throughout the United States. For each sample, one unseeded and two seeded subsamples were analyzed. One of the samples was seeded with live unmodified oocysts while the other sample was seeded with color modified oocysts (using ColorSeed) for accurate recovery measurements. The collection and processing of these samples afforded the opportunity to address other issues regarding the use of Method 1623 for monitoring waters. These issues included determining whether water quality factors affected recoveries of oocysts and whether fecal indicators (*Escherichia coli*, *Clostridium perfingens*, and somatic and F-specific coliphage) could be used

	as surrogates for the presence of *Cryptosporidium* in stream waters. This study was subjected to both internal as well as external peer review.
Results and Impact	Method 1623 is widely used to monitor source waters and drinking water supplies for *Cryptosporidium* oocysts. Matrix spikes, used to determine the effect of the water matrix on the method recovery efficiency, require the collection and analysis of two environmental samples, one for the analysis of endemic oocysts, and the other for analysis of recovery efficiency. Method 1623 specifies that a matrix spike is required every time a new water matrix is analyzed by the laboratory and every 20th time that matrix is analyzed in the laboratory. A new product, ColorSeed, enables the analyst to determine the recovery efficiency using colored oocysts that can be differentiated from endemic organisms in a single sample. Twenty-nine stream water samples and one untreated effluent sample from a cattle feedlot were collected in triplicate to compare the modified seeding procedures to conventional seeding procedures using live unmodified oocysts. Significant negative correlations were found between the average oocyst recovery and turbidity. This was especially apparent in samples with turbidity values greater than 100 nepholmetric turbidity units and also in samples with suspended sediment concentrations greater than 100 mg/liter. *Cryptosporidium* oocysts were found in 16.7% of the unseeded samples, and concentrations (adjusted for recoveries) ranged from 4 to 80 oocysts/10 liters. Determining recovery efficiency also provided data to calculate detection limits which ranged from <2 to <215 oocysts/10 liters. Oocyst recoveries ranged from 2.0 to 61% for live unmodified oocysts and from 3.0 to 59% for modified colored oocysts. These recovery results between the two seeding procedures were highly correlated and were not significantly different. By using ColorSeed as an internal control, each and every sample can now be seeded and the recovery efficiency can be determined. No longer are duplicate samples required to determine the recovery efficiency for various water matrices. Not only should the quality of the data improve, but the time and cost required to obtain results should decrease.
Research Collaboration and Research Products	This project was done as part of an interagency agreement with the U.S. Geological Survey (DW 1493929501) and the U.S. Environmental Protection Agency. D.S. Francy[1], O.D. Simmons III[2], M.W. Ware[3], E.J. Granger[1], M.D. Sobsey[2], and F.W. Schaefer, III[3]. 2004. Effects of seeding procedures and water quality on recovery of *Cryptosporidium* oocysts from stream water by using U.S. Environmental Protection Agency Method 1623. Applied and Environmental Microbiology 70(7):4118-4128. [1]U.S. Geological Survey, Columbus, Ohio 43229 [2]School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27599 [3]U.S. Environmental Protection Agency, Cincinnati, Ohio 45268
Future Research	The Office of Water is interested in determining how well the color in modified *Cryptosporidium* oocysts (ColorSeed) resists exposure to various water treatment plant disinfectants like chlorine, chlorine dioxide, chloramine, and ozone.
Contacts for	Questions and inquiries can be directed to:

Additional Information

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