Detection of Infectious Cryptosporidium in Drinking Water

Waterborne transmission of the protozoan parasite Cryptosporidium remains a significant threat of disease with severe consequences for persons with weakened immune systems. New drinking water standards under the U.S. Environmental Protection Agency (USEPA) Long Term 2 Enhanced Surface Water Treatment Rule are aimed at reducing the risk of cryptosporidiosis. Utilities that have poor quality source water will be required to provide additional treatment to minimize the risk of Cryptosporidium on public health.

Properly operating drinking water treatment plants that utilize conventional filtration methods usually remove Cryptosporidium from source water efficiently. However, a recent study by the American Water Company (Aboytes, Di Giovanni, Abrams et al., 2004) evaluated finished drinking water samples from 80 surface water treatment plants across the U.S. for the presence of infectious Cryptosporidium using a cell culture-polymerase chain reaction (CC-PCR) technique (Di Giovanni, Hashemi, Shaw et al., 1999). This study concluded that nearly all conventional treatment plants are at risk for passing infectious oocysts. Based on this study, the overall risk of Cryptosporidium infection for conventionally treated drinking water was 52 infections/10,000 people/year, and that an additional treatment barrier, such as ultraviolet light disinfection, is needed to meet the USEPA risk goal of 1 infection/10,000 people/year.

Properly operating drinking water treatment plants that utilize conventional filtration methods usually remove Cryptosporidium from source water efficiently. However, a recent study by the American Water Company (Aboytes, Di Giovanni, Abrams et al., 2004) evaluated finished drinking water samples from 80 surface water treatment plants across the U.S. for the presence of infectious Cryptosporidium using a cell culture-polymerase chain reaction (CC-PCR) technique (Di Giovanni, Hashemi, Shaw et al., 1999). This study concluded that nearly all conventional treatment plants are at risk for passing infectious oocysts. Based on this study, the overall risk of Cryptosporidium infection for conventionally treated drinking water was 52 infections/10,000 people/year, and that an additional treatment barrier, such as ultraviolet light disinfection, is needed to meet the USEPA risk goal of 1 infection/10,000 people/year.

Comparison of the CC-PCR method to two additional cell culture methods that have been used for disinfection studies and correlated with animal infectivity is needed to better understand the human health significance of the CC-PCR results. The additional two cell culture techniques for the detection of infectious Cryptosporidium are the cell culture reverse transcription polymerase chain reaction (CC-RT-PCR), and the cell culture focus detection (FDM) microscopy method. Additional drinking water sample analysis is needed to corroborate the results of this earlier study.

Objectives

- Phase I: Determine the sensitivity, specificity, and accuracy of the CC-PCR (benchmark method), CC-RT-PCR, and FDM assays for the detection of low numbers of infectious Cryptosporidium. For this objective, flow cytometry will be used to prepare precise numbers of Cryptosporidium oocyst inocula, and several different strains of Cryptosporidium were evaluated.

- Phase II: Determine the prevalence of infectious Cryptosporidium in finished drinking water using the most robust cell culture method determined in Phase I. A total of 260,000 liters of drinking water from different drinking water systems across the U.S. will be analyzed over the course of the study.

Findings and Benefits

After extensive testing, the FDM method followed by PCR-based genotyping of Cryptosporidium clusters of cell culture infection was selected for Phase II. Over the 22-month field study, 184 paired field samples from 14 different treatment plants were processed by each lab (368 samples total), for a total analysis of 342,853 L of drinking water. No field samples tested positive, while controls have provided consistent and appropriate results. The lack of positive samples may be due to the selection of utilities that participated in the study (on a volunteer basis). Most of these utilities have historically very low levels of Cryptosporidium in their source water. In contrast to the American Water study, results indicated that treatment plans with quality source water are unlikely to pass infectious Cryptosporidium.

Distribution of Cryptosporidium annual risk of infection based on monthly data from the Aboytes, Di Giovanni, Abrams et al. (2004) 2-year study of 82 treatment plants in 14 states. Dark shaded bars represent the limit of risk detection (i.e., no oocysts were detected). The USEPA goal for annual risk of infection is 1/10,000 people.