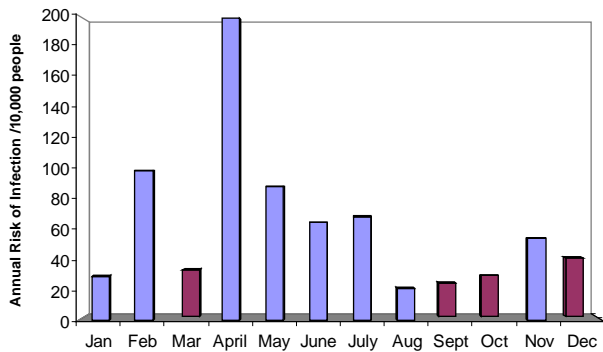


Detection of Infectious *Cryptosporidium* in Drinking Water

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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.

BACKGROUND

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 US Environmental Protection Agency (USEPA) Long Term 2 Enhanced Surface Water Treatment Rule are aimed at reducing the risk of cryptosporidiosis.

Properly operating drinking water treatment plants that utilize conventional filtration methods usually remove *Cryptosporidium* from source water efficiently. However, a 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 US 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 better understand the results of this earlier study.

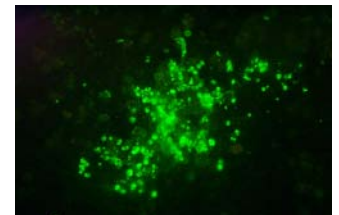
OBJECTIVES

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*.

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 US 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. However, the lack of positive samples may have been due to the selection of utilities that participated in the study, since most had historically very low levels of *Cryptosporidium* in their source water. In any case, in contrast to the American Water study, results of the current study indicated that treatment plants with quality source water are unlikely to pass infectious *Cryptosporidium*. Additional analysis of drinking water from plants with lower quality source water using the standardized cell culture infectivity assay developed in this study is needed to better understand the risk posed by *Cryptosporidium*.



Detection of *Cryptosporidium* infected lab-grown human intestinal cells using the FDM immunofluorescent microscopy method