Advancing Cryptosporidium parvum Detection Methodologies
[Project #2502]

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OBJECTIVES:
The goals of this project were to
• develop a continuous flow centrifugation method for concentrating large volumes of source and finished water to recover C. parvum oocysts
• optimize a polymerase chain reaction method for detecting low numbers of C. parvum oocysts
• compare various molecular-based assays to mouse infection for estimating the viability of C. parvum oocysts stored in water
• apply viability assays to C. parvum oocysts that had been recovered from large volumes of source and finished water

BACKGROUND:
Cryptosporidiosis has emerged as a significant health threat worldwide due in part to the consumption of drinking water contaminated with the causative organism, C. parvum. Current techniques for detecting C. parvum in water often rely on microscopic examination of oocysts. Because these techniques are rather insensitive, molecular techniques as well as concentration methods are needed to not only detect C. parvum, but also determine whether the oocysts are viable and possibly infectious.

HIGHLIGHTS:
The following are highlights from this project:
• Continuous flow centrifugation appears to be a promising method for concentrating C. parvum oocysts from source and finished water.
• The results from cell culture-PCR and fluorescence in-situ hybridization appear to correlate well with the “gold standard” mouse infectivity as measures of viable C. parvum oocysts.
• The sensitivity of molecular techniques should be improved if these methods are to be used by the water industry to detect low concentrations of C. parvum oocysts in water.
• C. parvum oocysts remain infectious for up to seven months when stored in water at 15°C.

APPROACH:
In the first year of the project, the technique for concentrating C. parvum oocysts, namely continuous flow centrifugation (CFC), as well as methods for detecting viable oocysts, were refined. These techniques included polymerase chain reaction (PCR), reverse transcriptase-PCR, mouse infection-PCR, cell culture-PCR, amylopectin assay, and fluorescence in-situ hybridization (FISH). In the second year of the study, these techniques were applied to C. parvum oocysts that had been stored in water at constant temperature. A subsample of oocysts were removed at monthly intervals and subjected to viability assays described above.

RESULTS/FINDINGS:
Real-time PCR using probes based on a gene sequence for a highly abundant ribosomal protein showed sensitivity down to 50 C. parvum oocysts. Applying nested PCR followed improved sensitivity to five oocysts. Cell culture-PCR was capable of discriminating between live and dead C. parvum oocysts. The FISH assay using probes directed to ribosomal RNA followed by immunostaining with fluorescent-tagged monoclonal antibodies reacted with both type 1 and type 2 C. parvum oocysts.

C. parvum oocysts stored for 1 to 7 months at 15°C were found to be infectious for mice and cell cultures and were viable as determined by FISH analysis. Mouse infection and cell culture PCR showed C. parvum oocysts stored beyond 7 months were non-viable. While low FISH reaction was observed with 8 month-old oocysts, no reaction was observed at 9 months. Oocyst amylopectin concentrations showed a two-fold decrease between 1 and 2 months storage. Amylopectin decreased another two-fold between 2 and 3 months and remained at this level for the remainder of the storage experiment. Similar findings were observed with the RT-PCR signal directed to mRNA coding for amyloglucosidase. Oocyst recoveries using CFC of source water always exceeded recoveries from finished water. Source water recoveries ranged from 83% to 106%, while recoveries from finished water ranged from 25% to 92%. Oocysts recovered from source and finished water were detectable by PCR and were infectious for cell culture and viable as measured by FISH staining.

IMPACT:
Continuous flow centrifugation (CFC) is a useful method for concentrating high numbers of C. parvum oocysts from source and finished water. This method has a number of advantages over cartridge and membrane filtration or flocculation. Research is needed to improve the percentage recovery of low numbers of C. parvum oocysts. Real-time PCR using fluorescent-tagged probes may become a standard method for detecting C. parvum oocysts in...
particulate matter concentrated from source or finished water.
FISH coupled to immunostaining with fluorescent-tagged antibodies should be considered by water quality laboratories as a useful method for detecting *C. parvum* oocysts. This assay has the added advantage that all of the equipment needed to measure FISH reaction is presently being used in IMS and FITC anti-*Cryptosporidium* staining protocols.

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