Project Profile

Project Title: Cryptosporidium parvum Viability Assay
Project Number: 351
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Objectives:
The main objective of this study was to further develop and potentially automate an existing nucleic acid staining methodology for determination of the viability of C. parvum oocysts in water. The specific aims of the research were: 1) To develop an automated procedure for staining that would indicate oocyst viability; 2) To determine the potential use of nucleic acid stains for determining the viability of C. parvum oocysts after exposure to different chemical disinfectants; and 3) To establish an automated immunofluorescence-nucleic acid staining technique for detection of viable parasites in natural water.

Background:
An ongoing challenge in the detection and disinfection of Cryptosporidium spp. is the difficulty in determining whether a parasite is viable. The ideal test for defining viability is the ability to cause disease in the human population. Clearly, this is not currently feasible and animal models have been used as surrogates. However, animal infectivity methodologies are tedious, difficult, expensive and are not readily amenable to normal laboratory analysis. This study examined nucleic acid staining of C. parvum oocysts as an indicator of parasite viability.

Highlights:
This research:
- Demonstrated a relationship between nucleic acid staining and animal infectivity
- Developed an automated, flow cytometry-based viability assay
- Improved the sensitivity of the animal infectivity assay

Approach:
To determine the potential use of nucleic acid stains for viability determinations after exposure to chemical disinfectants the researchers used microscopical examinations after disinfection with chlorine, monochloramine, chlorine dioxide, or ozone using the fluorogenic dyes SYTO®-9 and SYTO®-59. Animal infectivity was done on spilt samples of C. parvum oocysts to determine whether nucleic acid staining correlated with animal infectivity.
Two approaches were employed for the automation of nucleic acid staining and viability determinations. The first approach used a microtitreplate fluorimeter to determine the proportion of viable organisms in heat- or chemically-inactivated oocyst populations stained with SYTO®-9 or SYTO®-59. The second method involved the use of flow cytometry for estimating the number of viable parasites in heat- or chemically-inactivated oocyst populations.

**Results/Findings:**
This work demonstrated a relationship between nucleic acid staining of *C. parvum* oocysts with either SYTO®-9 or SYTO®-59 and animal infectivity. An automated flow cytometry-based nucleic acid staining viability assay was developed and was found to be highly reproducible, more reliable, and significantly less time consuming than the standard epi-fluorescence microscopy assays.

Flow cytometry assays for the detection of *C. parvum* oocysts in intestinal homogenates of neonatal mice inoculated with the parasites was developed and found to be superior to phase contrast and epi-fluorescence microscopy thus improving the animal infectivity assay. This study also developed a combined flow cytometry-epi-fluorescence method for the detection of viable *C. parvum* in natural water.

The analysis of nucleic acid staining after chemical inactivation indicated the increase in oocyst fluorescence intensity was not only a function of the level of treatment (e.g., CT) and corresponding reduction in infectivity, but was also dependent on the disinfectant and the experimental conditions (e.g., pH and temperature). Nucleic acid staining predictive models were developed on a chemical disinfectant-specific basis because changes to oocyst cell wall permeability and to SYTO®-9 dye were found to be disinfectant dependent.

**Impact:**
The application for the analytical advances made in this research will be useful in conducting chemical disinfection studies for *C. parvum*. Further optimization of the in vitro viability assay based on nucleic acid staining of chemically inactivated *C. parvum* oocysts must await the results of studies aimed at understanding the physiology of the oocyst cell wall, particularly changes in permeability following chemical disinfection.

**Participating Utilities** Aqualta Ltd., City of Calgary Water Works