Susceptibility of Multiple Strains of *C. parvum* to UV Light

[Project #2721]

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OBJECTIVES:
The objectives of this project are to
- characterize the comparative UV disinfection sensitivity of five *Cryptosporidium parvum* strains drawn from a variety of different sources
- characterize the response of prototype Iowa strain at UV doses substantially below those reported in the literature
- use bacterial and viral surrogates and radiometry to verify UV dose
- evaluate the applicability of UV-sensitive mutant strains of *B. subtilis* as surrogates for modeling the response of *Cryptosporidium*

BACKGROUND:
The threat of cryptosporidiosis remains a national concern due to the inadequacies of traditional disinfection methods. The recent validation of UV-based technologies for *Cryptosporidium* control has resulted in a fundamental shift from chemical to physical disinfection. However, previous studies have focused on a single prototype strain of the species known to be responsible for most human infections. Hence, there is a need to characterize the response of different *C. parvum* strains to UV before widespread implementation of UV-based technologies takes place in the industry.

HIGHLIGHTS:
All five strains of genotype 2 *C. parvum* oocysts were shown to be highly susceptible to UV inactivation according to neonatal mouse model infectivity studies, with comparable UV doses required for inactivation levels of 3 log10 or more (less than 5 mJ/cm²). The sensitivity of *C. parvum* oocysts to inactivation by UV was much greater than reference microorganisms coliphage MS-2 and two prototype *B. subtilis* strains; however, the UV response of *Cryptosporidium* was comparable to that observed for a UV-sensitive mutant, *B. subtilis* WN626, suggesting that this spore-forming bacterium can serve as a robust surrogate for *C. parvum* oocysts in future UV inactivation studies.

APPROACH:
The UV susceptibility of five *C. parvum* strains was determined using a bench scale collimated beam apparatus under low-pressure UV conditions. Previous studies were conducted using the Iowa strain at doses ranging from 10 to 40 mJ/cm², so very low doses (2 and 4 mJ/cm²) were included in this study. An important consideration in disinfection studies involves accurate measurement of UV dose. In addition to radiometric measurements, the UV dose responses of several surrogate microorganisms were measured. Two mutants of *B. subtilis* were examined to determine their sensitivity to UV with respect to *C. parvum*, in order to identify a surrogate with similar UV response characteristics for future UV inactivation studies. The *B. subtilis* mutants exhibited greater UV sensitivity than the prototype ‘wild’ strain.

RESULTS/FINDINGS:
Significant findings from this project included the following:
- Five strains of genotype 2 *C. parvum* oocysts were shown to be highly susceptible to UV inactivation when assayed using the neonatal mouse model. Using a bench scale, collimated beam apparatus, controlled doses of monochromatic (254 nm) UV light at 10 mJ/cm² or less provided at least 4 log10 inactivation.
- Appreciable reductions (3 to 4 log10) were observed when the Iowa stain was subjected to very low (< 5 mJ/cm²) UV doses.
- The UV responses of all test strains were comparable, and UV inactivation of the Iowa strain should be considered representative of *C. parvum* strains.
- UV inactivation of *C. parvum* (Iowa) was compared to UV-sensitive mutant strains of *B. subtilis*. A correlation was observed between one of the strains (WN626), where slightly more than 1 log10 inactivation was demonstrated per mJ/cm², suggesting its appropriateness as a surrogate for modeling the UV response of *Cryptosporidium*. This observation corresponds closely with the response of the Iowa oocysts under a UV dose range of 2 to 4 mJ/cm². Spores of *B. subtilis* WN626 can serve as robust surrogates for *C. parvum* oocysts in UV inactivation studies. *B. subtilis* spores are also easy to propagate and manipulate, are stable over time, and grow on standard bacteriological media.

IMPACT:
Previous research on the human dose response of *C. parvum* strains showed a 100-fold range in infectiousness. The possibility that these different strains might exhibit a range of sensitivities to UV, maybe extending beyond the low doses demonstrated for the prototype isolate, provided the basis for this study. These investigations showed that all five test strains were equally susceptible to low doses of UV light (<10 mJ/cm²), with greater than 3 log10 reductions. These findings indicate that strain infectivity does not impact its sensitivity to UV. The water industry
can feel confident that UV is a highly effective method for control of *Cryptosporidium* in water supplies as we move forward with implementation of this technology.