Genotyping Cryptosporidium Recovered From Water Regulatory Slides

Dr. George D. Di Giovanni, Texas A&M AgriLife Research
Rebecca M. Hoffman, Wisconsin State Laboratory of Hygiene, University of Wisconsin– Madison
Gregory D. Sturbaum, CH Diagnostic & Consulting Service, Inc. and ALS Laboratory Group

BACKGROUND

Current drinking water quality regulations in the US, United Kingdom, Australia and other parts of the world require monitoring for the parasite *Cryptosporidium* due to continued waterborne disease outbreaks and its risk to human health. There are currently over 20 recognized species and 60 genotypes of *Cryptosporidium*, most of which are frequently found in water. Of these, only three species (*C. parvum*, *C. hominis*, and *C. meleagridis*) are responsible for the vast majority of human disease. The current regulatory methods for the detection of *Cryptosporidium* in water rely upon immunofluorescent assay microscopy. Due to cross-reactivity of antibodies used in the assay, both human and animal associated *Cryptosporidium* oocysts are detected. Further, the microscopy methods are not capable of determining the species or genotype of the *Cryptosporidium* detected. This is a significant limitation when gauging the public health risk posed by this parasite. Development and application of a polymerase chain reaction (PCR) assay for genotyping *Cryptosporidium* oocysts recovered from water regulatory slides is greatly needed to help identify human and animal sources of waterborne *Cryptosporidium* for watershed management and to refine risk assessment models.

An international technology transfer workshop was held at the Texas AgriLife Research Center at El Paso

OBJECTIVES

The first phase of this research involved method development and was completed under Water Research Foundation Project 4099. The overall objective of Project 4099 was to develop a simple and reliable method for genotyping single *Cryptosporidium* oocysts recovered from water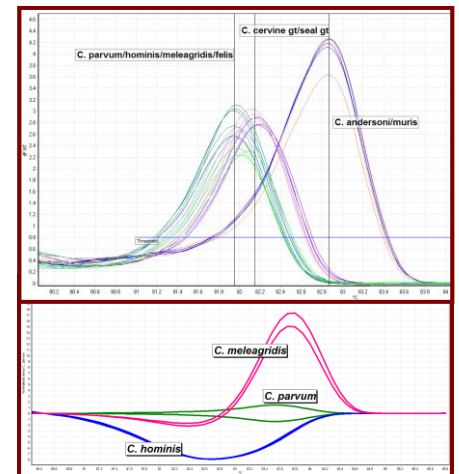 regulatory slides that could be readily transferred to water quality testing laboratories with minimal or no molecular biology experience. The second phase of research is currently being conducted under Water Research Foundation Project 4284. Project 4284 includes an international technology transfer workshop and international round-robin method evaluation to help transfer this technology to end users.

Genotyping of human pathogenic and animal associated Cryptosporidium by real-time PCR high resolution melt analysis

FINDINGS AND BENEFITS

- The developed method includes removal of oocysts from slides, DNA extraction, and genotyping using conventional PCR or real-time PCR with high resolution melt analysis. Preliminary single lab evaluation revealed an 80% detection rate for field slides seeded with single flow cytometry sorted oocysts.
- The published Water Research Foundation Project 4099 report includes a detailed sample processing protocol, reagent preparation protocols, electronic worksheets, and a method demonstration/training DVD.
- An international technology transfer workshop was held at the Texas AgriLife Research Center at El Paso. The workshop included participants from ten partner laboratories located in the US, Canada, Scotland, England, Wales, and Australia.
- An international round-robin method evaluation is currently underway with the participation of 26 analysts from 13 partner laboratories located in 7 countries.
- Application of the developed method will bring added value to regulatory monitoring, aid the development of effective watershed management strategies, and help refine *Cryptosporidium* human health risk assessment models.

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USDA United States Department of Agriculture National Institute of Food and Agriculture