Improving *Cryptosporidium* testing methods: a public health perspective

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ABSTRACT

Cryptosporidium is a protozoan parasite found in surface waters throughout the United States. Waterborne cryptosporidiosis outbreaks may be associated with contaminated drinking water supplies. The approved method, USEPA (United States Environmental Protection Agency) Method 1623, for testing for the presence of *Cryptosporidium* in United States surface waters has several limitations. Firstly, recovery efficiency varies widely. Secondly, Method 1623 does not specify a mechanism for assessing the viability and infectivity of oocysts detected, or the *Cryptosporidium* species of the oocysts. Lastly, there are logistical limitations which are relevant to Method 1623 in particular, and to the state of the science of *Cryptosporidium* testing in general. Methods that give specific results more quickly, with higher recoveries and better consistency must be developed and made accessible for utilities to use. Improved *Cryptosporidium* testing methods can minimize uncertainty; this, in turn, will simplify the risk communication task, and the level of trust which the public has in the water utility can be maintained and improved. This paper reviews the current and ongoing research on analytical, monitoring, and sampling methods for *Cryptosporidium*, and identifies the needs that should be considered in future research.

Key words | *Cryptosporidium*, drinking water, Method 1623

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INTRODUCTION

Cryptosporidium is a protozoan parasite found in surface waters throughout the United States. The eggs, or oocysts, of the protozoa are shed in the faeces of infected humans or animals. Infection with the parasite may result in diarrhoea and other symptoms of gastrointestinal illness. Children, the elderly, and people with compromised immune systems are particularly susceptible to illness after exposure to *Cryptosporidium*. Waterborne cryptosporidiosis outbreaks have been associated with contaminated drinking water supplies. Since *Cryptosporidium* is resistant to disinfection by chlorine or chloramine, it can only be removed from a contaminated water source by filtration and inactivated by alternative disinfectants such as ozone, ultraviolet radiation, and chlorine dioxide (Chen *et al.* 2002).

The San Francisco Public Utilities Commission (SFPUC) water system serves approximately 2.4 million customers in the San Francisco Bay area. Although there are two filtration treatment plants that treat water from local reservoirs, 85% of the water comes from the Hetch Hetchy Reservoir, 160 miles east of the city in the Sierra Mountains. The water from this protected source is unfiltered, although it does undergo some treatment, including chloramination and fluoridation.

Even though this pristine water source is not at high risk of *Cryptosporidium* contamination, the utility has an ongoing monitoring and surveillance program for *Cryptosporidium*. The aim of these activities is to detect contamination early and prevent an outbreak of disease in the event that *Cryptosporidium* occurs in the water. In 2004, the *Cryptosporidium* Detection Action Plan was completed. The plan describes what would be done in the event that elevated levels of *Cryptosporidium* are discovered in a

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routine water sample. Developing the action plan revealed how future research could be focused to meet the practical requirements of a water utility in trying to protect public health and communicate risk information to users.

The foundation of the *Cryptosporidium* Detection Action Plan is the presumption that there is some level of *Cryptosporidium* above which there is an unacceptable level of risk of illness. At this hypothetical level, some critical proportion of the population is at an unacceptable and increased risk of developing disease. Determining what that level is, in theory, and how it translates into practice, raised a number of issues about the approved method used to detect *Cryptosporidium* in the drinking water.

CRYPTOSPORIDIUM TESTING: LIMITATIONS AND AREAS FOR FUTURE RESEARCH

The San Francisco Public Utilities Commission (SFPUC) laboratory tests for *Cryptosporidium*, using a method approved by the US Environmental Protection Agency (USEPA), USEPA Method 1623. (Method 1623 is also used for *Giardia* detection.) The method first requires filtration of a water sample on to a filter medium. For *Cryptosporidium* detection, the material that remains on the filter media is eluted and any oocysts are separated using magnetic beads conjugated to anti-*Cryptosporidium* antibodies. The oocysts are stained with fluorescently labeled monoclonal antibodies, and the sample is then examined microscopically and compared to specified criteria for size, shape, color and morphology (USEPA 2001). Although the method only requires 10 litre samples, the SFPUC uses 100 litre samples to improve accuracy and recovery.

Method 1623 has several limitations. Firstly, recovery efficiency varies widely. Secondly, Method 1623 does not specify a mechanism for assessing the viability and infectivity of the oocysts detected, or the *Cryptosporidium* species of the oocysts. Lastly, there are logistical limitations which are relevant to Method 1623 in particular, and to the state of the science of *Cryptosporidium* testing in general.

Recovery efficiency

The recovery efficiency for Method 1623 is variable: one study found efficiencies in stream water ranged from 2% to 60% (Francy *et al.* 2004). DiGiorgio and colleagues found recovery efficiencies in ambient waters ranged from 36% to 75% (DiGiorgio *et al.* 2002). LeChevallier and colleagues (2003) conducted a large study of rivers and reservoirs in North America, finding average efficiency of 72% ± 22%, with a range of 5.9% to 110.2%.

Several efforts are underway to better assess recovery and to improve recovery efficiencies. For instance, some researchers are looking at ways to enumerate recovery efficiency using known quantities of labelled oocysts seeded into samples (Warnecke *et al.* 2003). Since low recovery is due either to losses during the filtration, centrifugation and clarification steps (LeChevallier *et al.* 2003), improving recovery efficiency requires reduction of the losses in each of those steps of the testing process. Hu and colleagues (2004) reported that recovery efficiency could be improved from 18.1% in seeded tap water to over 80% with the addition of silica particles. Continuous channel separation centrifugation is another technique which shows promise for improving recovery efficiencies (Borchardt & Spencer 2002).

Viability, infectivity and species identification

Information about whether a detected oocyst is viable or infective is important for determining the level of risk to the population. However, Method 1623 does not include determination of viability or infectivity. Techniques to determine viability and infectivity include dye techniques (e.g. propidium iodide (PI) or 4′-6-diamidino-2-phenlyindole (DAPI)), fluorescence in situ hybridization (FISH), or PCR techniques with animal models or cell cultures. Viability may also be determined by *in vitro* excystation, which involves mimicry of the conditions of the human intestinal tract and determines the number of sporozoites released, compared to the number of oocysts which remain intact (Campbell *et al.* 1992). However, recent studies suggest that excystation may not be an accurate technique for determining viability (Hou *et al.* 2004).

Many of the techniques for determining viability and infectivity are resource intensive, and methods such as mouse infectivity studies are not appropriate for implementation in a utility environment. However, the cell culture-polymerase chain reaction (CC-PCR) technique was successfully used to detect live and infectious oocysts in finished water from

conventional surface water treatment plants (Aboytes *et al.* 2004). Future research needs to determine resource efficient ways of evaluating viability and infectivity in the utility laboratory setting. Other barriers to implementation of these methods in a utility environment need to be identified, and ways to overcome obstacles delineated, so that these techniques can be implemented more widely.

Method 1623 does not identify the species of *Cryptosporidium* or the host origin. There are several different *Cryptosporidium* species (Xiao *et al.* 2004). Most human infections are due to only two: *C. parvum* and *C. hominis* (Hlavsa *et al.* 2005). The ability to determine the species would greatly enhance the ability to respond appropriately to oocyst detection in a water sample. If, for example, the species detected was one not believed to be responsible for disease in humans, the response to the detection might not emphasize outbreak prevention. Species identification can also help in determining the source of contamination. For instance, Glaberman *et al.* (2002) analyzed the genotypes present in three cryptosporidiosis outbreaks which occurred in the Belfast area between April 2000 and April 2001. The results of the analysis showed that the outbreaks did not share a common source and that the sub-genotype responsible for two of the outbreaks was probably endemic in the community. Thus, these two outbreaks were probably caused by contamination with sewage or wastewater.

Logistics

Managing the logistics of transporting large quantities of water back to the laboratory for filtration is prohibitive, so the San Francisco Public Utilities Commission, like many other utilities, filters the water samples in the field. It takes 50 minutes to collect a 100 litre sample at the specified rate of 2 litres per minute, which can be challenging in severe weather conditions or when time schedules are tight. However, transporting such a large volume of water back to the laboratory for processing is cumbersome. Therefore, research needs to identify ways to expedite field filtration, or ways to produce reliable results with smaller samples which can be more easily transported.

In practice, it may take up to one week from the time a sample is collected and the time the results are observed in the laboratory. By this time, the water from which the sample was drawn could already be at someone's tap. This directly undermines the outbreak detection and public health goals of *Cryptosporidium* testing. Methods which can produce results more quickly are needed. As these technologies develop and more timely methods become available, results from water samples collected at the tap may become more relevant to outbreak prevention. As a result, the water industry and regulatory agencies may begin to promote testing of finished waters over testing of source waters.

In the event of an outbreak, it would be beneficial to have methods which allow confirmatory testing. This may be accomplished either through the development of analytical methods which are amenable to confirmatory testing or through the development of techniques for concentrating and storing small samples of water which can be looked at later, if necessary, to confirm a result.

Finally, although *Cryptosporidium* is not well correlated with turbidity or bacterial indicators (Bonadonna *et al.* 2002), research should continue to support development and validation of potential proxy measures.

CONCLUSION

The approved testing method in use by many utilities in the United States is limited by speed, accuracy, and complexity. Methods which give specific results more quickly, with higher recoveries and better consistency must be developed and made accessible for utilities to use. In the event of exceeding a given level of oocysts detected in the drinking water, it will be necessary to communicate the level of risk to the public. In this risk communication, the uncertainty in the test method used to determine the oocyst concentration will need to be translated to the public. Improved *Cryptosporidium* testing methods can minimize uncertainty; this in turn will simplify the risk communication task, and the level of trust which the public has in the water utility can be maintained and improved.

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