Paper 4.2

Capture, recovery and isolation of *Cryptosporidium* and advice as to how pool operators could implement this in practice

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Synopsis

Cryptosporidium is a pathogenic protozoan parasite causing severe gastroenteritis that is potentially fatal in susceptible individuals. Transmitted via a faecal oral route as an environmentally stable oocyst, this organism is particularly important due to its resistance to commonly used disinfection procedures.

For over a decade several methods have been successfully employed to analyse raw water and finished water samples for the presence of *Cryptosporidium*. These methods typically involve capture and recovery of target organisms from a water sample followed by purification, identification and enumeration of the captured targets.

This paper describes the techniques and methods used routinely by the water industry for the analysis of water for the presence of *Cryptosporidium* and potential means by which these techniques may be employed by swimming pool operators.

Introduction

Cryptosporidium is a coccidian protozoan parasite that is almost ubiquitous in aquatic environments. In the environment, the organism exists as an environmentally stable oocyst. This parasite has a number of, generally species specific, hosts and is known to infect mammals, including humans, birds, reptiles and fish. Ingestion of a single oocyst can potentially result in infection of the host although there is clearly a dose response. Once inside the host, specific conditions within the small intestine result in excystation and release of sporozoites. These sporozoites subsequently infect the cells lining the small intestine, passing through several complex life cycle stages before ultimately being expelled in the faeces of the infected host. Several million oocysts may be expelled per gram of faecal matter (1, 5, 9).

Transmission of *Cryptosporidium* from host to host proceeds via a faecal-oral route typically by drinking water containing oocysts, or by ingesting fomites associated with oocysts (5, 6).

Cryptosporidiosis, the condition caused by *Cryptosporidium* infection, is a severe form of gastroenteritis. Cryptosporidiosis is typified by profuse watery diarrhoea, stomach cramps, nausea and vomiting. Although, in the majority of cases, this infection is self-limiting and resolved within two to three weeks, in those hosts with a compromised immune system, such as the young, elderly or those with AIDS, the condition may be fatal (5, 6).

Cryptosporidium and the Water Industry

Cryptosporidium has long been recognised by the water industry as an important waterborne pathogen and the World Health Organisation (WHO) currently rates *Cryptosporidium* as one of the most commonly identified intestinal pathogens throughout the world.

The organism's small size and its resistance to commonly employed disinfection procedures such as chlorination (9), require stringent treatment practices involving physical removal by filtration, treatment with ozone or UV irradiation. The efficacy of ozone and practicality of UV is still a subject of some debate within the water industry.

In 1993, the United States, and possibly the water industry as a whole, suffered what is likely the worst recorded outbreak of cryptosporidiosis. A water treatment plant in Milwaukee Wisconsin serving some 900,000 people suffered several failures resulting in a water supply contaminated with *Cryptosporidium*. In the region of 400,000 people were infected and approximately 100 died, mostly people with AIDS (7).

Up until the Milwaukee outbreak the methods employed to detect *Cryptosporidium* in water were laborious and inefficient. Following the Information Collection Rule (ICR), the EPA developed Method 1622. The efficacy of Method 1622 was subsequently determined for some 3000 samples in the ICR supplemental surveys (ICRSS). Method 1622, now revised, details the methods currently recommended by USEPA for the analysis of water for *Cryptosporidium* (10).

During the 1980s and 1990s outbreaks of cryptosporidiosis were also frequent in the United Kingdom with 25 recorded outbreaks from 1988 to 1998 (3). In 1997, a notable outbreak in North London and Hertfordshire resulted in the reformation of the expert group on *Cryptosporidium*. Following this, in 1999, the United Kingdom Drinking Water Inspectorate (DWI) instituted the most rigorous regulation related to analysis of water for the presence of *Cryptosporidium* in the world. These regulations required water utilities to continually monitor the finished water for the presence of *Cryptosporidium* at sites deemed at risk. This involved sampling 1,000L volumes of finished water over a 24-hour period, 365 days of the year. The presence of one *Cryptosporidium* oocyst per 10L sample volume was sufficient to result in legal action against the offending utility (4).

Despite improvements to water treatment and water quality overall, outbreaks of cryptosporidiosis associated with drinking water still occur. Notably, in June 2008 in Northampton UK, *Cryptosporidium* oocysts were detected in the drinking water supply and over 100,000 homes were advised to boil their water for several days (1).

Cryptosporidium and Swimming Pools

The situation with respect to swimming pools is somewhat different from that of the water industry. Swimming pools can generally be considered a closed water system, which brings both advantages and disadvantages. The water within the swimming pool is continuously turned over and treated by techniques including filtration, coagulation, ozonation, UV irradiation and chlorination. Chlorination, as discussed earlier, is insufficient to render oocysts non-infective. Ozonation and UV irradiation may be effective against oocysts as the water quality is generally high. The number of swimming pools using these methods is not known but is likely to be low due to the high costs associated with their implementation and maintenance. A technique, which is effective if used correctly, is filtration coupled with flocculation. If equipment is set up and maintained correctly, a two-log reduction in oocyst numbers can be achieved in a relatively short space of time.

Despite the availability of techniques, which are effective in either physical removal of oocysts or their disinfection, outbreaks of cryptosporidiosis associated with swimming pools still occur. To highlight this, a recent outbreak in New South Wales, Australia, associated with several swimming pools, resulted in reported and confirmed cases of cryptosporidiosis reaching a ten-year high. Investigators assessed 19 pools across the state and indicated that no one pool was responsible and that they had been unable to pinpoint which pools were harbouring the parasite (8).

This indicates a complication in the detection of *Cryptosporidium* in swimming pools. Contamination of a swimming pool is likely to be transient, occurring in response to a gross contamination event. During this contamination, the number of oocysts entering the pool may be many millions and, to pool users, the potential for infection may be significant, particularly if the contamination event is not reported. If water treatment within the pool is properly maintained the number of oocysts may be reduced within several pool turnovers and the infection risk reduced. In this situation, by the time that cases of cryptosporidiosis are noted by public health officials, the source of the original contamination may be impossible to determine. Meanwhile, infected parties may contaminate other pools and the original source may be re-contaminated. This scenario is particularly likely during the peak swimming season, during the summer months, when children will be using the pools more frequently.

Cryptosporidium Analysis Procedures

Detailed procedures commonly used for the analysis of water samples for the presence of *Cryptosporidium* can be found elsewhere (4, 10).

Analysis of water samples for the presence of *Cryptosporidium* involves several steps and although procedures have improved in recent years, the process remains laborious and requires a high degree of technical skill. The analysis can be broken down into several stages.

The first stage is the collection of a water sample. This is commonly achieved by passing the water source through some form of filtration apparatus. Several aspects must be taken into consideration when choosing a water volume to sample. If the sample volume is too small, it may not be representative of the body of water from which it was taken. In general, the larger the sample volume is, the more representative it becomes. However, the quality of the water, particularly in terms of its particulate content, must be taken into account. Some water types have a high particulate content and the sampling of large volumes is simply not practical or possible. Generally, surface water samples are between 10L and 100L in volume. Finished (potable) waters, which on the whole have a lower particulate content, lend themselves to higher sample volumes of 100L-1,000L or greater. In practice, the selection of a sample volume is a trade-off between what is representative and what is practical and both of these must be balanced with the objectives of the required test.

Once the sample is collected, the captured *Cryptosporidium* oocysts must be recovered from the filter. This is achieved by several means and depends on the type of filter being used. Details of specific procedures and equipment used for the elution of filters can be found in the USEPA and DWI procedures mentioned above.

In addition to *Cryptosporidium* oocysts being eluted from the filter matrix, particulates and other extraneous material may also be present in the eluate. In order to separate the oocysts from these particulates it is necessary to perform a purification step. Purification is most commonly achieved using Immunomagnetic separation (IMS). Prior to using this procedure, the eluate must be concentrated from the eluate volume, typically 50mL to 500mL, to a final volume of 10mL. This concentration step is achieved using further filtration, centrifugation or both depending upon the method being employed.

IMS is a technique that employs small (~5μM) uniform paramagnetic microspheres coated with, in this instance, antibodies specific for *Cryptosporidium*. The microspheres are added, along with specific buffers, to the concentrated water sample, which is subsequently incubated. During incubation, the *Cryptosporidium* oocysts are captured by the antibodies and can be isolated, washed and purified from the residual sample using a magnet. Once purified, the oocysts can be separated from the microspheres using an acidic solution and physical shearing. The purified oocysts can then be applied to a microscope slide for further examination.

Cryptosporidium oocysts are detected in the sample by fluorescence microscopy and other optical techniques. In order to correctly visualise the oocysts they are initially stained using antibodies, specific for the oocyst wall, conjugated to a fluorescent molecule. Most commonly fluorescein isothiocyanate (FITC) is used although other fluorophores are available. Using UV light and correct filters, FITC fluoresces apple green giving the oocysts a distinct apple green halo. A second staining technique used involves the nucleic acid stain diamidino phenylindole (DAPI). DAPI enters the oocyst and stains the nucleus within each sporozoite. Up to four sky blue nuclei may be seen within the *Cryptosporidium* oocyst. A final technique commonly used is Differential Interference Contrast (DIC) microscopy. Correctly used, DIC, which is an optical technique, creates, in essence, a three dimensional image of the oocyst allowing internal structures to be visualised. The presence of oocysts is therefore subjectively determined, by the analyst, based upon staining characteristics, size, shape and the presence of typical internal structures.

Further confirmation is possible using genotyping techniques, although these are not used routinely by the water industry at this time, except in response to an outbreak. These techniques allow the species of *Cryptosporidium* to be determined along with the specific strain. This in turn allows the potential host range and public health significance to be evaluated.

Cryptosporidium Analysis and Swimming Pools

It appears evident that the complete analysis of swimming pools for the presence of *Cryptosporidium* is beyond the capabilities of swimming pool operators. The complexity of the analysis procedures, the technical skill and equipment required make it impractical to implement. However, it is possible for pool operators to sample their water either on a regular basis, or in response to reports of outbreaks of cryptosporidiosis in the region, and expert laboratories are available, which can process samples on a contractual basis.

There are several options available to swimming pools operators for the sampling of water. Portable, battery operated sampling units, which allow for sampling at multiple locations, are currently used by several utilities within the water industry. In addition, there are several fixed sampling units, with varying degrees of complexity, which can be plumbed into a swimming pools existing water system.

The use of these units is straightforward and the logistics of sampling fairly simple. In practical terms, an expert laboratory could send a filter to the pool operator. The pool operator would then take a sample of the recommended volume and at the appropriate location. The filter would subsequently be returned to the expert laboratory for analysis and reporting of results. Typically, an expert laboratory can turn around results within 24–48 hours depending on the urgency of the sampling event and the current situation. The cost of sample processing may however, be several hundred pounds depending on the nature of the analysis required.

Continuous sampling of a pool would allow *Cryptosporidium* to be detected quickly following an unreported contamination event and allow appropriate steps to be taken to remove or treat the contamination. This would quickly restore the pool to a status suitable for public use and allow appropriate steps to be taken to inform public health officials and the public at large.

Alternatively, in response to a known gross contamination event or an outbreak of cryptosporidiosis at a different location, swimming pool operators could perform isolated testing in order to ensure that their pool is not contaminated and that the public is not at risk of infection.

Final Comments

Cryptosporidium monitoring is increasingly important to public health. The appearance of the organism in the media is becoming more frequent and the public are more aware of the associated risks.

The incidence of cryptosporidiosis associated with swimming pools is becoming more common around the globe. This may be due, in part, to improvements in detection methods and increased analysis for the presence of this organism. Methods for the detection of this organism are continually improving and it is only by swift detection that the risk to public health can be minimised.

The situation with respect to swimming pools is complex due to the transient nature of contamination events in properly maintained pools with effective treatment. However, it is possible for analysis to be performed in swimming pools either on a continuous basis, or in response to an outbreak or reported contamination event.

The costs associated with analysis may be prohibitive at this time. However, increased analysis frequency would certainly result in a reduction of costs whilst ensuring that contamination events are caught quickly and dealt with appropriately.

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