MONITORING DRINKING WATER SUPPLIES FOR PROTOZOAN PATHOGENS

Paper Presented by :

Christine Kaucner

Authors:

Joanne O'Toole, *Senior Consultant*
Christine Kaucner, *Microbiologist*

AWT / Water ECOscience

61st Annual Water Industry Engineers and Operators' Conference
Civic Centre - Shepparton
2 and 3 September, 1998

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Joanne O'Toole, *Senior Consultant*, AWT/WATER ECOscience
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ABSTRACT

This paper describes potential sources of protozoan pathogens within a water supply catchment. The need to understand catchment characteristics through both sanitary surveys and a catchment monitoring program is highlighted. The design of a protozoan monitoring program for source water and also waters discharging and impacting on sourcewaters is discussed and recommendations are made. Currently available methods for analysis of waters for both *Giardia* and *Cryptosporidium* are compared and the merits and disadvantages of each detailed. Means of assessing treatment processes for their effectiveness in removing these pathogens are also discussed; including the use of surrogates as challenges to treatment and the role of particle counting in assessing plant performance.

KEYWORDS

Cryptosporidium, *Giardia*, water supply catchment, sanitary surveys, detection methods, monitoring programs

1.0 INTRODUCTION

The protozoan pathogens, *Giardia intestinalis* and *Cryptosporidium parvum* have been recognised as a public health threat in drinking waters and the cause of several waterborne disease outbreaks. The features of these organisms which may facilitate waterborne transmission are the large numbers of cysts and oocysts excreted by hosts and the increased potential for environmental spread and contamination as a consequence of their lack of, or reduced, host specificity. The prevalence and levels of both *Giardia* and *Cryptosporidium* in a water supply depends upon a variety of contributors and their associated activities performed in the catchment area. The presence of these contributors will vary between catchments, highlighting that any monitoring programme will have to take individual catchment characteristics into account. As a consequence, any protozoan monitoring programme will be enhanced by the findings of, a sanitary survey.

This paper seeks to address the issue of the design of a protozoan monitoring programme and the performance of sanitary surveys, with reference to the approaches taken elsewhere. In particular, the aim of this paper is to give water supply personnel an understanding of the factors which influence the design of a protozoan monitoring programme and to make suggestions relating to monitoring frequency. In addition, it presents the currently available options for analysis of source and treated waters for *Giardia* and *Cryptosporidium* and the limitations and advantages of each. Further to this, the use of surrogates as challenges to treatment and the role of particle counting in assessing plant performance is explored.

Based upon overseas approaches and the practicalities and cost of protozoan monitoring, a programme based on 10 monitorings per year is recommended with monitoring taking into account pertinent events such as heavy rainfall, animal birthing and seasonal influences. The use of particle counting as an adjunct to microbiological (indicator and pathogen) and operational (flow rates, turbidity, filter backwash frequency, etc) monitoring is recommended to ensure that the design and operation of treatment plants is optimised for particle (including protozoa) removal.

2.0 DISCUSSION

2.1 Potential Contributors Of *Giardia* Cysts And *Cryptosporidium* Oocysts In Source Water

The nature of surface source waters is such that they are all at risk of contamination, though some source waters may be of higher risk than others. Surface water quality is subject to frequent, dramatic changes in microbial quality as a consequence of a variety of activities within a catchment. These changes are caused by the discharges from sewage treatment plants at specified point source locations into receiving waters or by stormwater run-off into the drainage basin at non-point locations all over the catchment. The potential contributors of *Giardia* cysts and *Cryptosporidium* oocysts in raw water are summarised in a review article by Smith *et al*.(1995). Contributors include municipal sewage, stormwater, domestic and wild animals. Knowledge of such contributors to specific catchments can assist in the determination of sampling locations and analysis strategies.

2.2 Occurrence Of *Giardia* And *Cryptosporidium* In Source Waters

Overseas investigations have shown that seasonal factors including run-off of land drainage may affect oocyst concentration by 10 fold with concentrations in drier periods being significantly lower than those in wetter periods. The character and intensity of both human and domestic animal activities in a watershed has been also found to potentially affect *Cryptosporidium* oocyst concentrations in a surface water by as much as 10-15 fold (Hansen & Ongerth 1991). Other investigators have found that in pristine waters indigenous animals may contribute significant numbers of oocysts on occasions. *Cryptoporidium* oocysts are however, more frequently detected in waters from sewage and agricultural discharges (Rose *et al*., 1991). Some investigators have given a general guide regarding *Cryptosporidium* levels and suggest that when estimating likely concentrations of *Cryptosporidium* in water,.less than 0.1 oocyst/L may be assumed for pristine watersheds and boreholes and between 1 and 100 oocysts/L assumed for contaminated water, dependent upon catchment characteristics (Smith *et al*.,1995).

Some Victorian State funded studies have been undertaken, with findings replicating those of overseas investigators. Findings include the detection of protozoan pathogens at sites designated pristine and the association of *Giardia* cyst detections with sites impacted on by drainage from large sewered towns (DCNR 1995-unpublished).

2.3 Overseas Approaches To Protozoan Monitoring

In the United States in July 1997 water utilities serving 100,000 or more people and using surface water began an 18 month monitoring program for *Cryptosporidium* under the Information Collection Rule (ICR). Collection of this data is designed to accumulate a large body of microbial data to support national regulatory impact analysis. The ICR is not intended to provide informational value regarding the occurrence of pathogens or treatment efficiency at any individual sampling site. It is acknowledgement by the US EPA that representation of water quality for a given site is only possible with large numbers of samples (Cryptosporidium Capsule 1998). Removal of the requirement for smaller supplies serving populations less than 100,000, to monitor for *Cryptosporidium*, as required in the earlier drafts of the ICR, highlights the cost restrictions associated with *Cryptosporidium* analysis.

Prior to the implementation of the ICR, comment was made, by Lisle and Rose (1995) that the monitoring requirements were minimum requirements, providing a somewhat crude representation of the distribution of *Cryptosporidium* in waters. These investigators saw the most significant deficiency as being the non-recognition of those areas contributing, or having the potential of contributing, high concentrations of oocysts to the source water during adverse conditions (eg heavy rainfall events). Their recommendation was that not only the water at the inlet pipe to the water supply be monitored, but also , at least initially, each catchment and /or body of water (tributary non-

point source) impacting on the source water be monitored. Further to this, they recommended that monitoring should also reflect the impact of storm events and seasonal factors.

In the UK, the emerging risk of *Cryptosporidium* has not been regulated by the imposition of numerical standards, rather, regulations require that drinking water must not contain any organism at a concentration that would be detrimental to public health. As a consequence of the recommendations made in the First Badenoch Report on *Cryptosporidium* in 1990 , including a recommendation that levels of oocysts in raw and delivered water be monitored, some several thousand samples a year are taken for this purpose across the UK (Taylor *et al*., 1998)

2.4 Sanitary Surveys

Investigators in the US, in relation to understanding protozoa occurrence in their watershed, noted that it is impossible to collect enough samples over a one to two year period to explain occurrences of protozoa under a variety of seasonal and flow conditions in a major catchment (Crockett & Haas 1997). As a consequence, they suggest that to enable interpretation of protozoan occurrence data, other important information, specifically catchment characteristics, must be integrated into a research study. Identifying land uses associated with sources of protozoa and establishing the priority of their effects based on meteorological conditions (ie significant runoff during wet weather) are considered crucial to enable identification of the type of pollution (point or non point), its general location (immediate or upper regions) and its frequency (daily or wet weather only).

One means of collection of catchment characteristics data is through the execution of sanitary surveys. Sanitary surveys involve the thorough inspection of a water supply system, including the catchment area, and documentation of the various sites and activities which pose the biggest risks. There is much scope in the manner that sanitary surveys may be executed and documented, although a standard approach is preferable. Of some interest is the approach taken in relation to the San Francisco watersheds to determine ways of reducing the risks to water quality from waterborne pathogens carried by cattle and other mammals. The plan developed used a Hazard Analysis of Critical Control Points (HACCP) approach and identified the potential sources of contamination, the critical points that needed to be controlled, and the best management practices to address the critical control points. Areas within the catchment considered were livestock and grazing, feral pigs, wildlife, humans and recreation (Cryptosporidium Capsule Aug 1997).

Victorian Health (Quality of Drinking Water) Regulations, 1991 prescribe that sanitary surveys be undertaken at a frequency of at least once every three years. Obviously for operational monitoring purposes and as a consequence of seasonal changes that occur within a catchment, more frequent sanitary surveys are desirable. Victorian Regulations do not prescribe the manner in which sanitary surveys must be undertaken, nor the reporting format.

Audit Victoria (1995) in relation to catchment management, have recommended that water authorities undertake sanitary surveys in accordance with Victorian Health Regulations.

2.5 Design Of A Protozoan Monitoring Programme

The design of a pathogen monitoring programme will be influenced by a number of factors including available funds, the purpose of monitoring (regulatory or operational) and the purpose for which monitoring data are to be used (eg. as part of a catchment management strategy or to demonstrate removal efficiency of a treatment process). Factors for consideration with respect to *Giardia* and *Cryptosporidium* include method sensitivity, the requirement for viability data and sample volume.

With respect to the demonstration of removal efficiency of a particular treatment process, certain limitations of a *Cryptosporidium* monitoring programme must be acknowledged. Researchers cite the primary limitation of the use of naturally occurring pathogens, specifically *Cryptosporidium*, as

being the occurrence and distribution of the pathogen and sampling methodologies (Lisle & Rose 1995). In a review article dealing with the dilemma of source water quality and treatment plant evaluation, the authors emphasise that knowledge of the likely sources of oocysts and cysts which might contaminate a water catchment is desirable when attempting to determine the potential for exposure to oocysts and cysts at a treatment plant (Smith *et al*., 1995). On the basis of the likely risk of oocysts and cysts contaminating raw water used for abstraction, water treatment plants can be categorised as low or high risk with the level of risk driving the protozoan sampling strategy devised for the plant. The identifiable risks are cited as being seasonal ones or as occurring throughout the year. Dependent upon the perceived risk and based upon data from monitoring surveys, water treatment plants may vary in their risk category at different times of the year, with differences being reflected in the protozoan monitoring programme.

Sampling methodologies provide a significant limitation to the establishment of the true level of *Cryptosporidium* and *Giardia* in any water and recognition must be made of this. For example, occurrence data collected for the ICR is acknowledged as overestimating concentrations at some sites and underestimating concentrations at others.

A means by which the density of *Cryptosporidium* and *Giardia* may be determined in water at selected sampling points has been proposed by Ongerth (1996). He recommends that the general density range must be determined either by informed guess based on published data , or by processing trial samples (for example 3 to 5 replicate 20L volumes). An analytical method must be chosen that is appropriate to the sample conditions. In particular, emphasis must be placed on the recovery efficiency achievable as applied to the water to be analysed. Based upon such information the sample volumes required to produce a useful data set can be determined. A useful data set is deemed to be one where there is a reasonably high percentage of positive results.

2.6 Available Methods

Environmental monitoring for protozoan pathogens is confounded by a number of factors, including the small size of cysts and oocysts, the relatively low concentrations of protozoa in water and the difficulty in identifying cysts and oocysts amongst other particles and debris. As a consequence, current methods for the isolation and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts in water concentrates are variable in their performance. Limitations of some methods include poor reliability, high cost and complexity, inability to discriminate living (viable) and dead (non viable) cysts and oocysts and inability to discriminate *Cryptosporidium parvum* from other species of *Cryptosporidium*. The advantages and disadvantages associated with the currently used methods for sample concentration and protozoan detection are given in (Tables 1 and 2).

Table 1 : ***Advantages and disadvantages associated with Giardia and Cryptosporidium sample concentration methods***

Concentration Method	Advantages	Disadvantages

Concentration Method	Advantages	Disadvantages
Yarn wound filter	♦ large volumes of sample can be processed. ♦ documented method upon which much historical data is based. ♦ can be employed for field sampling (4 L/min). ♦ filter easily transportable to the laboratory.	♦ method in some disrepute as revealed by US round robin studies. ♦ method since superseded by new USEPA procedure. ♦ variable recovery efficiency depending upon water type. ♦ flotation step required for most samples.
Chemical flocculation	♦ procedure relatively simple ♦ sample collection involves 10-20 L carboy ♦ employed in both UK and USA.	♦ sample volumes relatively small. ♦ literature indicates possible impact on cyst/oocyst viability ♦ designed initially for use with flow cytometer. Sample clean up by flotation required for turbid samples prior to flow cytometry and/or microscopy.
Membrane filtration	♦ maintenance of cyst/oocyst viability	♦ small sample volumes in general can be analysed unless filtration set up on site (vacuum required). ♦ capital expenditure for filtration equipment is high. ♦ time consuming for turbid samples.
Diamond filter	♦ good recovery efficiencies. ♦ Maintenance of cysts/oocyst viability. ♦ large volumes of sample can be processed ♦ can be employed for field sampling (up to 40 L/min) ♦ filter easily transportable to the laboratory.	♦ a relatively new method therefore data on recovery efficiencies limited.

Table 2 : ***Advantages and disadvantages of Giardia and Cryptosporidium detection methods***

Concentration Method	Advantages	Disadvantages
Fluorescence Antibody Staining (FIA)	♦ well documented and used worldwide for staining *Giardia* and *Cryptosporidium* in water concentrates. ♦ Allows numbers of cysts/oocysts present to be counted.	♦ often requires sample cleanup (eg. flotation) prior to microscopy or flow cytometry. ♦ non specific staining of algae and other debris ♦ not specific for *C. parvum* and *G. intestinalis*
DAPI/P combined with FIA	♦ used by a number of investigators. ♦ good correlation with *Cryptosporidium* encystation experiments. ♦ well documented and can be incorporated into current UK and US methods .	♦ time consuming (more microscopy). ♦ question if there is any relationship with infectivity
RT-PCR	♦ can be employed directly after the concentration step (ie. no flotation) irrespective of sample turbidity. ♦ detects viable *Cryptosporidium parvum* and *Giardia spp* ♦ represents latest technology and method has been peer reviewed in international scientific journal. ♦ can specifically detect *C. parvum*	♦ presence/absence test only. ♦ not widely used and therefore not yet subjected to trial by numerous investigators ♦ question relationship with infectivity.

2.7 Suggested Protozoan Monitoring Programme

Taking into account cost constraints associated with protozoan monitoring, the following components of a protozoan monitoring programme are suggested:

- monitoring of source waters at least 10 times per year;
- dataset to include monitoring during pertinent events (eg heavy rainfall, animal birthing season);
- scheduled to coincide with known events in the catchment (eg influx of tourists) and seasonal influences;
- where detected in the source, monitor the waters impacting on the source water;
- monitoring to be performed in conjunction with a thorough inspection of catchment area;
- document the various sites and activities which pose the greatest risk.

2.8 Particle Counting

Studies by Edzwald and Kelley (1998), have found that turbidity and particle counts cannot be used

to predict *Cryptosporidium* concentrations in raw or treated waters. Nevertheless, particle counts and turbidities can show when good treatment occurs and when particles pass through treatment processes. Filtered water turbidities of 0.1 NTU and particle counts of size 2 microns and above of 50/mL or less are indicators of good treatment for controlling *Cryptosporidium*. USEPA's anticipated final interim enhanced surface water treatment rule (IESWTR) and longer-term rules are expected to tighten the current turbidity goal of 0.3 NTU or less for filtered systems even further (Pontius 1997)

In the United Kingdom, the current direction with respect to *Cryptosporidium*, is that the emphasis should be on developing overall strategies for treatment optimisation and risk reduction. To this end a wide range of crucial process advice was given in the second Badenoch Report (1995) relating to the design and operation of treatment plants to achieve a cost effective means of particle removal. Included amongst this advice was that continuous turbidity (or particle) monitoring be implemented.

Victorian State funded studies of particle count monitoring have shown particle counting to be more sensitive than turbidity measurements. Furthermore, during poor water quality events, particle counts proved to be a better indicator of the effectiveness of the match between plant operating conditions and the rapidly changing water quality (DNRE 1997-unpublished).

3.0 CONCLUSION

With respect to the design of a protozoan monitoring programme, for drinking waters, a number of factors must be taken into consideration. These include the frequency of sampling, the location(s) at which samples are to be collected, the timing of sampling (eg during wet weather events etc), and the methodology employed. The volume of sample to be collected,whilst another factor, is largely determined by the recovery efficiency of the method employed and the expected numbers of protozoans in the water being analysed. With respect to method selection, the purpose of the monitoring programme must be considered. For example, where removal efficiencies are to be determined, a qualitative result may be inappropriate. Equally, consideration must be given as to whether a viability result is required.

For the interpretation of *Cryptosporidium* and *Giardia* data derived from catchment monitoring, it is necessary to conduct sanitary surveys of the catchment so that the catchment is understood at a fine level of detail. In this way a better understanding will be gained of the sources, fate, and transport of protozoa.

Based upon overseas approaches and the practicalities and cost of protozoan monitoring, a programme based on a minimum of 10 monitorings per year is recommended with monitoring taking into account pertinent events such as heavy rainfall, animal birthing and seasonal influences.

The use of particle counting as an adjunct to microbiological (indicator and pathogen) and operational (flow rates, turbidity, filter backwash frequency , etc) monitoring is recommended to ensure that the design and operation of treatment plants is optimised for particle (including protozoa) removal.

4.0 REFERENCES

Audit Victoria (1995) *Drinking Water Quality Report*, Victorian Auditor General's Office, Melbourne, Victoria.

Badenoch, J (1990) *Cryptosporidium* in Water Supplies - Report of the Group of Experts,

HMSO, London.

Badenoch, J (1995) *Cryptosporidium* in Water Supplies - Second Report of the Group of Experts, HMSO, London.

Crockett, CS and Haas, CN (1997) "Understanding Protozoa in your Watershed", *J. AWWA*, 89, 9, 62-73.

Cryptosporidium Capsule, (1997) "No ban on Grazing in San Franciscan Watersheds" 2, 10, 3-5

Cryptosporidium Capsule, (1998) "US EPA Cautions Utilities on Interpreting ICR Data" 3, 8, 2-3

Department of Conservation and Natural Resources (1995) *Catchment Management to Control Microbial Contamination of Waterways*, Department of Conservation and Natural Resources, Victoria *unpublished*

Department of Natural Resources and Environment (1997) *Particles present in Filtered Water* Department of Natural Resources and Environment, Victoria. *unpublished*

Edzwald, JK and Kelley, MB (1998) "Control of *Cryptosporidium*: from Reservoirs to Clarifiers to Filters", *Wat. Sci. Tech.*, 37, 2, 1-8

Hansen, JS and Ongerth, JE (1991) "Effects of Time and Watershed Characteristics on the Concentration of *Cryptosporidium* Oocysts in River Water" *Appl. Environ. Microbiol.* 57, 10, 2790-2795

Lisle, JT and Rose, JB (1995) "*Cryptosporidium* Contamination of Water in the USA and UK: A Mini-Review", *J.Water SRT-Aqua*, 44, 3, 103-117

Ongerth, JE (1996) "*Cryptosporidium* and *Giardia* in Water - Study Design and Data Interpretation" *Proc. Water Microbiology For The 21st Century*, Stockholm, August, 1996

Pontius, FW (1997) "Communicating ICR Data on *Cryptosporidium*", *J AWWA*, 90, 2,20-26,118

Rose, JB, Gerba, CP and Jakubowski, W (1991) "Survey of Potable Water Supplies for *Cryptosporidium* and *Giardia*" *Environ, Sci, Technol.* 25, 8, 1393-1400

Smith, HV, Robertson, LJ and Ongerth, JE (1995) "Cryptosporidiosis and Giardiasis: The Impact of Waterborne Transmission", *J Water SRT-Aqua* ,44, 6, 258-274

Taylor, GS, Hillis, P and Campbell, AT (1998) "Managing the Risk of Waterborne Cryptosporidiosis - Towards Best Practice in the UK" *AWWA Watertech Conference*, Brisbane, 1998